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Developing and Assessing Different Cordon Establishment Techniques for Long-Term Vineyard Management

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Developing and assessing different cordon establishment techniques for long-term vineyard management

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Abstract

This project investigated the impact of different permanent cordon training techniques on vine performance in the years immediately following cordon establishment. The treatments included cordons that were wrapped very tightly around their supporting wire, a method common in Australia and certain other winegrowing regions, as well as other less constrictive training methods. Measurements of vegetative growth at one site including cordon circumference and pruning weight suggest that the growth of tightly wrapped cordons may have been hindered by the constrictive nature of their training method. Other measurements however, including plant area index did not find this to be the case. A treatment involving the use of two parallel cordon wires around which arms were trained in a loose, s-shaped bend actually showed the greatest reduction in plant tissue area. Differences in harvest parameters between training methods such as yield and grape chemistry were minimal and did not follow a meaningful pattern. Differences in bud fertility and the occurrence of primary bud necrosis were also minimal.

Investigating carbohydrate status, there was a trend at one site where starch concentration was lower in shoots from cordons placed on top of the wire than other treatments in the later seasons. While this suggests the treatment may have been beneficial in promoting the translocation of carbohydrates to the perennial structures of the cordon, the same effect was observed in the proximal, intermediate, and distal sections of the cordon, suggesting the impact of the treatment on the movement of carbohydrates along the cordon arms may have been limited. Using micro-CT, it was discovered that cordons which were wrapped tightly around the cordon wire had significantly smaller xylem conduit volumes relative to total cordon volume, as well as thinner xylem vessels and less connections between vessels per unit volume. Additionally, the theoretical specific hydraulic conductivity (K_s) of tightly wrapped cordons was much lower than other treatments ($p < 0.0001$), suggesting that their capacity for normal hydraulic conductivity was negatively impacted by the treatment.

It was determined that a vigour-based length adjustment performed on new cordon arms during their establishment was beneficial in promoting early vegetative growth, particularly in the middle of cordon arms. The benefits of the method were limited to one season however, as control cordons had no more missing spur positions than length adjusted cordons after the first season of growth. Further research in this area should focus on a lower vigour setting. A survey was conducted on older commercial vineyards to investigate the relationship between cordon strangulation resulting from tight wrapping, dieback, and the expression of fungal trunk disease symptoms. Rather than finding strangulation to be a driving force behind dieback, there was actually a trend observed where the cordons displaying the greatest degree of strangulation

displayed the least amount of dieback. While the results of this survey were unexpected, the quantitative scale presented for the use of the assessment of degree of strangulation is a novel tool that could be useful in future research.

Overall, the results suggest that the newly established cordons trained in the most constrictive fashion (tightly wrapped around the cordon wire) performed the worst over the course of the project. Interestingly, this was true even in the case of breakage caused by mechanical damage, which cordons trained in this manner are purported to be less prone to due to improved canopy stability. Because the project was limited to a timeframe of four years, it is likely that the negative trends developing with the practice of tightly wrapping developing cordon arms around the wire would continue to worsen over time with continued observation. The poorly developed vascular systems seemingly attributable to this training method in particular suggest that its use should potentially be avoided.

Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint award of this degree.

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Patrick O'Brien **Date**

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Abbreviations

%	Percent
µm	Micrometer
3D	Three-dimensional
ANOVA	Analysis of variance
ATA	Arm transversal area
A_w	Sapwood cross-sectional area
C	Control
°C	Degree Celsius
cm	Centimetre
D°	Distal
E. lata	*Eutypa lata*
g	Gram
GTD	Grapevine trunk disease
h	Hour
ha	Hectare
I°	Intermediate
IP	Inflorescence primordia
kg	Kilogram
K_s	Theoretical specific hydraulic conductivity
LA	Length adjusted
LSD	Least significant difference
m	Metre
m^2	Square metre
m^3	Cubic metre
Micro-CT	Micro-computed tomography
ML	Megalitre
mL	Millilitre
mm	Millimetre
mm^2	Square millimetre
mm^3	Cubic millimetre
MPa	Megapascal
n	Number of vessels
nm	Nanometre
no.	Number

ns	Not significant
NSC	Non-structural carbohydrate
P	Placed on top
P°	Proximal
PAI	Plant area index
PBN	Primary bud necrosis
Pc	*Phaeomoniella chlamydospora*
PC	Principal component
PCA	Principal component analysis
p-value	Probability
RNA	Ribonucleic acid
rpm	Revolution per minute
s	Second
S	S-bend
SPAC	Soil-plant-atmosphere continuum
std	Standard deviation
T	Tightly wrapped
TA	Titratable acidity
TSS	Total soluble solids
W	Woven through clips
Xf	*Xylella fastidiosa*
η	Water viscosity
ρ	Water density
Φ	Canopy porosity

Chapter 1. Introduction

1.1. General Introduction

Grapevines grown for the purpose of wine production can be trained in a wide variety of manners, and the decisions regarding which methods are commonly adopted within particular winegrowing regions may be made for a multitude of reasons. Cultivar, soil, climate, demand for regulation of vegetative vigour, use/accessibility of mechanical equipment, and economics are important considerations when planting or reworking any vineyard (Bernizzoni et al., 2009). Historical regional management practices are often resistant to change and development, as although perhaps imperfect, have often been practiced for considerable periods of time, sometimes generations, and are not without their own merit (Carbonneau et al., 2001). Training method is no exception to this, and techniques which are common cultural practices in some regions may be misunderstood by growers in other regions or even dismissed as outright errors. In many locales where permanent cordon training systems are utilised it is common to see developing cordon arms wrapped tightly around their supporting wire during training and establishment. This strategy may lead to an extreme constriction of the cordon which is visually apparent in older cordons, and is often accompanied by signs of decline including dieback and wood decay. This condition typically becomes progressively worse over time as the cordon ages, and could have negative implications on vine health and longevity, resulting in the necessity for premature remedial attention such as reworking or replanting. Tight wrapping is popular with many growers for specific reasons however, as it may increase the speed of and reduce the cost of training, and may impart additional stability to the cordon. It is believed that this increased stability helps to mitigate the risk of cordon rolling and the disruption it may cause to the establishment of spur architecture (Caravia et al., 2015b). It is also purported to alleviate the risk of wind and mechanical damage (Boehm & Coombe, 1992), particularly in systems offering minimal to no canopy support (e.g. sprawl).

Current scientific literature has not investigated the outcome of this training method on cordon health and productivity. By comparing cordons trained in this manner with others trained using alternative, less constrictive methods, it is possible to assess its impact on cordon vitality. The overall aim of this study was to quantify the impacts of different cordon training techniques on indicators of vine well-being including changes to vascular morphology, vegetative growth, grape chemistry and yield components, carbohydrate status, fertility, and expression of fungal trunk disease symptoms. Better understanding the benefits and drawbacks of different training methods may allow growers to make more informed decisions that are less likely to lead to the early degeneration of permanent cordons structures.

1.2. Research Objectives

The objectives of this project were to:

- Investigate whether different cordon establishment techniques have an impact on grape composition or yield and its components in a sprawl canopy system.
- Demonstrate that vegetative growth is negatively affected by the constrictive effects of tight wrapping via measurements of plant area index (PAI), pruning weight, and cordon circumference.
- Determine the impact of training methods on vine physiology and water status via xylem morphological assessment.
- Assess the impact of training methods on carbohydrate status via microplate assay.
- Examine if and to what extent tight wrapping has an impact on susceptibility to fungal trunk diseases and their symptom expression.
- Compare vines with and without a vigour-based cane length adjustment during cordon establishment to determine the benefits of this method.
- Evaluate whether reproductive health is affected by different cordon establishment techniques via microscopic dissection of buds for examination of inflorescense primordia and the occurence of primary bud necrosis (PBN).

1.3. Linking Statement

The research presented in this thesis is ordered into chapters and includes four prepared manuscripts intended for publication.

Chapter 1 is an introduction to the project and its aims.

Chapter 2 is a published literature review providing information relevant to factors which are likely to have an influence on cordon longevity. Covered topics include grapevine physiology and its relationship with water status, selection and maintenance of different training systems, and vascular diseases and their relationship to cordon health status. The results of a small survey are included which investigated the relationship between cordon strangulation and trunk disease.

Chapter 3 presents the results of a survey of commercial vineyard sites investigating the relationship between cordon strangulation, dieback, and the expression of fungal trunk disease (*Eutypa lata*) symptom expression. It provides a novel scale to quantify the severity of cordon strangulation resulting from the constrictive pressure of tightly wrapping the cordon around the cordon wire and an analysis of its impact on the occurrence of other symptoms of decline.

Chapter 4 investigates the question of whether adjusting the length of canes selected for the establishment of permanent cordon arms is a beneficial practice. It presents the results of a trial wherein a treatment was applied where the vigour of new cordon arms was assessed during their training and their retained node number was then limited based on their apparent vigour.

Chapter 5 examines the impact of different cordon training techniques on the vascular morphology of canes from the distal region of cordons and perennial cordon wood. Micro-computed tomography (micro-CT) and optical microscopy (stereo microscope) were used to provide a quantitative assessment of the properties of cane and cordon xylem conduits.

Chapter 6 presents an investigation into the question of whether cordon training technique directly impacts indicators of cordon well-being including vegetative growth, reproductive health, and carbohydrate status in the years following establishment. It also investigates the outcome on harvest parameters including grape chemistry and yield components.

Chapter 7 is a concluding discussion. It considers the significance of the results reported in the different sections of this thesis and highlights their key findings and implications. It also discusses what questions remain and the possible direction that future research could take to achieve further understanding in this area.

Chapter 2. Published Literature Review: A Review of Factors to Consider for Permanent Cordon Establishment and Maintenance

Statement of Authorship

Title of Paper	A Review of Factors to Consider for Permanent Cordon Establishment and Maintenance
Publication Status	☑ Published ☐ Accepted for Publication ☐ Submitted for Publication ☐ Unpublished and Unsubmitted work written in manuscript style
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Name of Principal Author (Candidate)	Patrick O'Brien		
Contribution to the Paper	Conceptualisation, methodology, formal analysis, data curation, writing—original draft preparation, writing—review and editing		
Overall percentage (%)	85		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	25/09/2022

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);
ii. permission is granted for the candidate in include the publication in the thesis; and
iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Cassandra Collins		
Contribution to the Paper	Conceptualisation, methodology, writing—review and editing, supervision		
Signature		Date	26/09/2022

Name of Co-Author	Roberta De Bei		
Contribution to the Paper	Conceptualisation, methodology, writing—review and editing, supervision		
Signature		Date	26/09/2022

Name of Co-Author	Mark Sosnowski		
Contribution to the Paper	Methodology, writing—review and editing		
Signature		Date	26/09/2022

A Review of Factors to Consider for Permanent Cordon Establishment and Maintenance

Abstract: Decisions made during the establishment and reworking of permanent cordon arms may have long-term consequences on vineyard health and longevity. This review aims to summarise several of the important considerations that must be taken into account during cordon establishment and maintenance. Commonly practiced cordon training techniques such as wrapping developing arms tightly around the cordon wire may result in a constriction of the vascular system, becoming worse over time and disrupting the normal flow of water and nutrients. Studies have shown that other factors of cordon decline such as the onset of vascular diseases may be influenced by pre-existing stress conditions. Such conditions could be further exacerbated by water and heat stress events, an important consideration as these scenarios become more common under the influence of climate change. Vineyard sustainability may be improved by adopting cordon training techniques which promote long-term vitality and avoid a reduction in vine defence response and the costly, premature reworking of vines.

2.1. Introduction

Grapevines (*Vitis vinifera* L.) are woody perennial plants, which under the right conditions can sustain impressive longevity, producing fruit over many growing seasons (Grigg et al., 2017). In extreme examples, their lifespan may have the potential to exceed 400 years, as is the case of the Old Vine from Lent (Maribor, Slovenia), regarded as the oldest living example of cultivated grapevine in the world (Vršič et al., 2011). Typically, older vineyards are more highly coveted than their younger counterparts, particularly with red cultivars, with their fruit and resultant wine being perceived as having higher potential quality (Heymann & Noble, 1987; Reynolds et al., 2008). It is not uncommon for commercial vineyards to remain productive for 50–60 years, and in long established regions, commercial vineyards may be found with average vine ages of well over 100 years. It is becoming increasingly rarer, however, to observe vines of this age around the world, and it is not unusual to see vineyards undergo major reworking or replanting after only a few decades, either to be replaced with other cultivars or due to a decline in production relative to crop value (Bou Nader et al., 2019). A number of factors may, on their own or in a cumulative manner, contribute to a vineyard's eventual decline in yield and economic viability including nematodes (Rahman et al., 2008), trunk diseases (Siebert, 2001; Sipiora & Cuellar, 2014; Sosnowski & McCarthy, 2017), viruses (Atallah et al., 2011; Credi & Babini, 1997), phytoplasmas (Ripamonti et al., 2020), and other problems arising from poor management decisions such as improper selection of rootstock, poor pruning practices, mechanical damage, nutrient and irrigation deficiencies, lack of weed control, and compaction of the soil (White, 2015) (Figure 2.1). These issues are further complicated by climate change, the impacts of which are well documented and include increases in global temperature, CO_2 concentration, and solar radiation, as well as extreme changes in weather patterns and increased frequency of drought conditions and water scarcity (Duchêne & Schneider, 2005; Jones et al., 2005; Keller, 2010; Schultz, 2000).

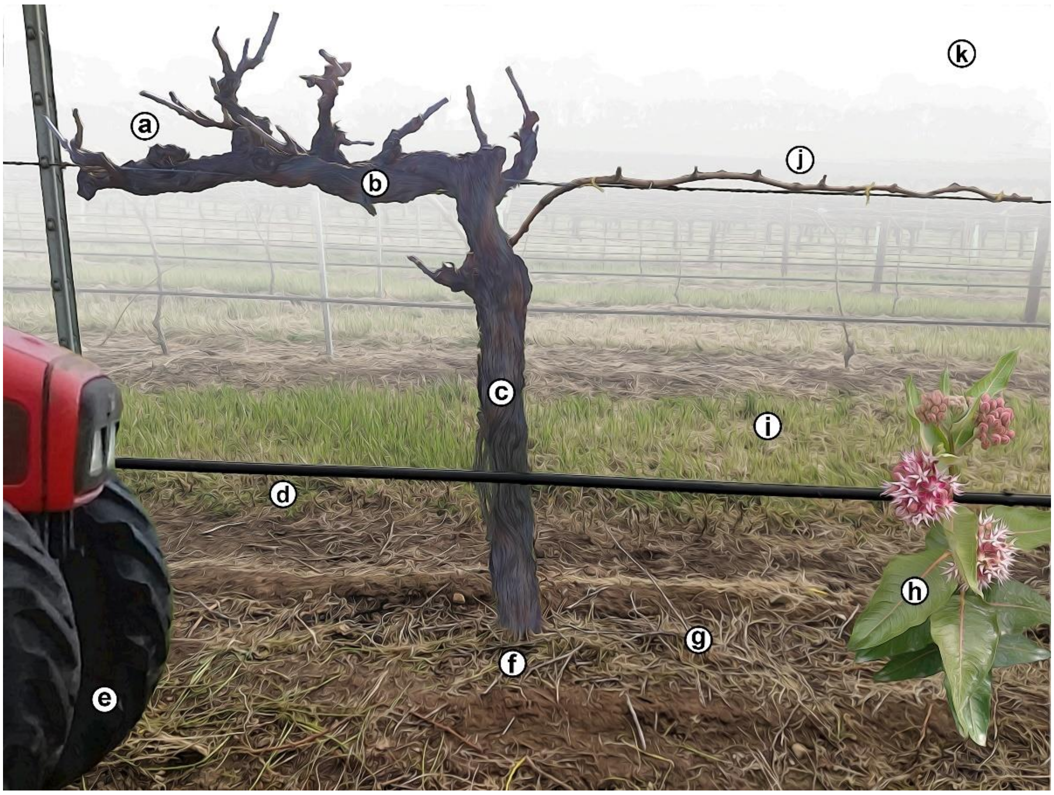

Figure 2.1. Factors which many have an influence on vineyard longevity. (**a**) Loss of spur positions as a result of mechanical damage, improper pruning, or dieback; (**b**) strangulation of the cordon; (**c**) insufficient carbohydrate reserve storage in perennial organs, or reduced functionality from vascular disease, virus infection, phytoplasma infection, or improper selection of rootstock; (**d**) poor soil water content, or irrigation mismanagement; (**e**) soil compaction; (**f**) soil pathogens including nematodes; (**g**) nutrient mismanagement, or poor soil microbial activity; (**h**) inadequate weed control; (**i**) mismanagement of cover crops and/or insect populations; (**j**) seasonal impacts such as frost damage and major heat events; (**k**) changing climate and weather patterns.

Other management decisions made during the establishment or reworking of vineyards may have lasting consequences on vineyard health and longevity as well. In Australia, and in many grape-growing 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. It stands to reason that this wrapping may cause a constriction of the vine's vascular system, becoming worse over time and disrupting the flow of water and nutrients (Caravia et al., 2015b). This is especially apparent in older vineyards, where the wire is often visibly embedded within the wood of the cordon and signs of decline and dead arm symptoms are frequently observed. It is possible that the stress resulting from the constriction of the vines vasculature may reduce the vines natural ability to deal with external factors such as the onset of vascular diseases. This condition could also be further exacerbated by water and heat stress events, an issue of major concern as these scenarios become more and more common under the influence of global warming. While there are many different factors which may play a contributing role in the overall health and longevity of a vineyard, this review will focus primarily on those most likely to directly impact the vitality of permanent cordons arms, as well as the best practices for their establishment and maintenance.

2.2. Physiology of the Grapevine

2.2.1. Water and Nutrient Transport

Grapevines, like all vascular plants, require water to grow and thrive (Sperry, 2003). The movement of this water throughout the vine, along with essential mineral nutrients, sugars, and amino acids, is achieved via the vascular system (Lucas et al., 2013). Along with providing this principal function, the vascular system also provides the vine with mechanical support, important as grapevines in their natural state are climbing plants. Water is transported in the form of sap, the ascent of which is explained by the cohesion-tension theory whereby the evaporation of water molecules at the leaf surface during transpiration pulls water from the soil into the roots and through the xylem conduits towards the leaves (Dixon & Joly, 1895; Pickard, 1981). This process uses no metabolic energy; rather the movement of water is driven by capillary forces, and the xylem conduits that serve to carry a network of broken and more importantly unbroken "threads" of sap are composed of dead cell walls (Brown, 2013; Venturas et al., 2017). The water within the xylem conduits is constantly under tension (i.e., the xylem pressure potential is negative), and this tension increases with a reduction in soil moisture or increase in transpiration rate (Tyree & Sperry, 1988). It is upon this concept that the soil–plant–atmosphere continuum (SPAC) model is based (Choné et al., 2001), as well as the Ohm's law analogy, where water flux through the various parts of the SPAC is treated as a catenary process, comparable to the current in an electric circuit composed of a series of conductances (or inversely, resistances) (Tyree & Ewers, 1991). Using this analogy, the flux of water through a part of the vine can be thought of as being proportional to the product of the hydraulic conductance of that region and the water potential drop across said region (Tyree & Ewers, 1991). Other significant components of the vascular system include the phloem, which is important in the transport of water and nutrients and plays a major role in inter-organ communication, and meristematic tissues, which are regions of unspecialised cells contributing to vital activities including cell multiplication, secretion, photosynthesis, and storage (Evert, 2006).

2.2.2. Xylem Morphology

Healthy cordons require healthy vascular systems for the translocation of water and other important substances. It has been demonstrated that xylem morphology is impacted by water availability (Lovisolo & Schubert, 1998; Munitz et al., 2018), and hydraulic properties of old wood are different from those of young shoots, as vessel length and diameter are correlated with stem diameter and mature trunks and limbs tend to have longer, wider vessels compared to younger stems (Ewers & Fisher, 1989; Jacobsen et al., 2012; Jacobsen et al., 2015). Additionally, the ratio between the width of the xylem and total stem diameter is greater in mature stems (Sun et al., 2006). Xylem conductivity is determined by the structure and size of vessels (Schultz & Matthews, 1993; Tyree & Ewers, 1991) as well as their efficiency, which may be affected by the presence of embolisms (air-filled conduits that are not available for water conduction) (Lovisolo & Schubert, 1998; Tyree & Sperry, 1989). Along with non-living tracheary elements (vessels and tracheids interconnected by lateral pits), the secondary xylem contains other cell types including fibres and living parenchyma cells, the latter of which are in contact and communication with xylem conduits (Sun et al., 2008; Tyree & Ewers, 1991). Although non-living cells cannot respond to wounding or parasite entry, living cells may respond by excreting gels, lignin precursors, and other substances, or by forming tyloses (extensions of adjacent parenchyma cells into vessel lumens) (Bonsen & Kučera, 1990; Esau, 1977). Such responses may be triggered by several factors including frost, flooding, wounding, leaf abscission, and infection by different pathogens, and function as a xylem-sealing mechanism, obstructing the conduction of water through the affected xylem (Pérez-Donoso et al., 2016). These vascular occlusions serve an important function in wound sealing and reducing the spread of pathogens in the xylem (Bonsen & Kučera, 1990; Dute et al., 1999; Saitoh et al., 1993). Working with Chardonnay, Sun et al. (2008) reported that the type of occlusion induced after wounding was dependent on the season in which wounds were inflicted, with tylose formation resulting in permanent xylem obstruction occurring predominantly in the summer and reversible gel formation occurring predominantly in the winter. This study also showed that a higher fraction of vessels developed occlusions in summer and autumn (over 80%) than in winter and spring (about 60%). Of particular interest 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 water transport, particularly in the context of climate change, could have a drastic impact on cordon health and productivity.

2.2.3. Phloem Morphology

While xylematic flow is driven by a tensional gradient along a series of dead cells, in contrast, the phloematic conduit is comprised of a series of living cells forming a sieve tube system (Schulz, 1998). A wide variety of substances are transported via the phloem to distant organs, including sugars, amino acids, micronutrients, lipids, hormones, proteins, and RNAs (Evert, 2006). Some of these materials serve an important role as informational or signalling molecules (Ruiz-Medrano et al., 2001). The phloem of *Vitis vinifera* is somewhat atypical compared to most other plant species in that it has an unusually long life, with its sieve tubes functioning for more than one year, becoming inactive during the winter and resuming activity in the spring (Esau, 1948). This reversible inactivity begins with the sieve tubes developing a provisional callus before the onset of winter dormancy, and terminates in the spring with the sieve tubes acquiring the same characteristics of active elements as when first differentiated from the cambium (Esau, 1948). Once the cambium is activated during the early stages of the growing season, it begins to generate new phloematic tissues that will mature by the end of the season, when the phloem of the previous year will begin a process of loss of functionality and obliteration (Gonzalez Antivilo et al., 2019). Occlusion of dead, non-conducting sieve elements may occur from outgrowths of contiguous parenchyma cells known as tylosoids (tylose-like protrusions which do not grow through pits in secondary walls) (Esau, 1965). Such protrusions may invade the lumina of inactive sieve elements or simply push the sieve element wall to one side, resulting in the collapse of the sieve element (Evert, 2006). The growing season begins and ends with one ring of functional phloem, with the activity of the phloematic tissues generated during two consecutive growing seasons overlapping temporarily mid-season (Esau, 1948; Gonzalez Antivilo et al., 2019). Early spring vegetative growth is driven by the translocation of carbohydrate reserves from perennial organs (Bates et al., 2002; Holzapfel & Smith, 2012; Zapata et al., 2004), which become available for budburst with the generation of auxins, degradation of callose, and reactivation of the phloem (Aloni et al., 1991).

2.2.4. Effects of Water Stress

Although some growers utilise deficit irrigation methods to impose water constraint as a means of improving fruit quality (Dry et al., 1995; Stoll, 2000), there remains a question as to whether this practice may be detrimental to vine longevity owing to the stress imposed. Under the effects of water stress, vines show changes in water flow rate resulting from modifications of the conductivity components of the transpiration pathway (root, shoot and stomata) (Lovisolo & Schubert, 1998). It has been reported that water stress affects shoot conductivity by inducing embolism in the xylem vessels (Lovisolo & Schubert, 1998; Schultz & Matthews, 1988; Tognetti et al., 1996; Tyree & Sperry, 1989). It has also been demonstrated that water deficits during the growing season can inhibit vine vegetative growth and photosynthesis (Dayer et al., 2013; Keller et al., 2016; Rossouw et al., 2017; Schultz & Matthews, 1988) as well as having a negative effect on yield and fruit/wine composition (Marciniak et al., 2013; McCarthy, 1997; Naor et al., 1993; Romero et al., 2015). Such deficits affect the source-sink balance of the vine (Poni et al., 1994), and may therefore affect productivity both in the current growing season as well as in following years given that early spring shoot growth is supported by and reliant on reserves (Bates et al., 2002; Holzapfel & Smith, 2012; Zapata et al., 2004). In their study on Malbec, Dayer et al. (2013) found that severe water stress reduced trunk starch concentration without having an effect on the concentration of total non-structural carbohydrates. This is in agreement with previous studies which found that the seasonal impacts of water deficit could potentially inhibit starch accumulation in peach trees (Lopez et al., 2007) and grapevines (Smith & Holzapfel, 2009). It is therefore reasonable to hypothesise that water stress symptoms caused or exacerbated by the constrictive effects of wrapping developing arms tightly around the cordon wire could lead to a reduction in carbohydrate reserves that could have a long-term impact, hindering growth in future seasons. Likewise any reduction in cordon volume occurring as a result of constriction could directly affect the vine’s capacity to overwinter carbohydrates as the perennial structures of the vine including the roots, trunk, and cordon are the major storage organs for carbohydrates (Bates et al., 2002).

2.2.5. Impact of Cordon Health on Reproduction and Vine Balance

Another issue of concern is whether cordon constriction could have an impact on fruitfulness, measured as the number of inflorescences per node after budburst, or during para-dormancy as the number of inflorescence primordia (IP) within the compound bud with the potential to develop into inflorescences the following season (Dry, 2000). Inflorescence development and shoot growth rely on current season photosynthesis, as well as the translocation of previous season reserves, and carbohydrates are supplied to developing shoots and inflorescences via remobilisation from perennial structures (Bates et al., 2002; Bennett et al., 2005). Vegetative and reproductive growth occur simultaneously, and as such competition for carbohydrate reserves may exist between vegetative and reproductive structures under stress conditions when resources are not sufficient to support potential growth rates (Cox et al., 2012; Nuzzo &

Matthews, 2006; Rossouw et al., 2017). Sustainable viticulture therefore requires a balanced vineyard that via canopy photosynthesis during the growing season is able to provide enough carbohydrates to ripen the target fruit load and support IP development as well as replenish sufficient reserves in the perennial organs to enable budburst and support initial spring shoot growth in the following season (Dayer et al., 2013; Holzapfel et al., 2010). In a study on Shiraz, the number of bunches per shoot and berries per bunch were found to be correlated with water and nitrogen availability during flowering the previous season (Guilpart et al., 2014). If the primary bud dies during initiation due to a physiological disorder known as primary bud necrosis (PBN), then secondary buds, which are less fruitful and form smaller bunches, may grow in compensation for its loss (Collins & Rawnsley, 2004a). Given studies have found that the incidence of PBN may be influenced by reduced bud carbohydrate levels (Vasudevan et al., 1998) and water deficit (Collins & Rawnsley, 2004b), it is possible that any interruption in the vasculature of the cordon induced by wrapping may have an influence on the incidence of PBN as well. IP number and size are also likely to be influenced by such an interruption regardless of whether the primary bud becomes necrotic.

2.3. Cordon Establishment and Maintenance

2.3.1. Selection of Training System

One of the most important decisions during the establishment of a new vineyard is the choice of training system. This decision may be based on a number of factors including cultivar, soil, climate, accessibility of mechanical equipment, demand for regulation of vegetative vigour, cost, and consideration of historic regional management practices (Bernizzoni et al., 2009; Carbonneau et al., 2001). Planting choices including rootstocks, trellis and vine spacing, and the heights and thickness of wires can affect productivity during vine establishment and also in the long term. An important distinction regarding vines that are hedgerow trained is the presence or absence of a permanent cordon. Regardless of other decisions of training system (i.e., unilateral vs. bilateral cordon, divided vs. non-divided canopy, vertically shoot positioned vs. sprawl, etc.), a choice must be made as to whether permanent cordons or canes will be used to supply the nodes from which the fruit-bearing shoots will grow each season. In the case of cane pruning, 1–8 canes (commonly two) of reasonable length from the previous season are selected and retained each year, usually from the crown of the vine and as such no permanent arms are required. These canes are then trained and secured to the cordon wire(s), often wrapped around the cordon wire itself, or brought over top of another parallel wire and back down to the cordon wire to form an arch. Studies have indicated that the selection of thicker canes may be beneficial in increasing yield and profitability. In a study on Sauvignon blanc over two growing seasons, Eltom et al. (2014) determined that average inflorescence number per shoot and the proportion of inflorescences having an outer arm with flowers increased in relation with an increase in cane diameter. Cane microclimate is an important consideration in this instance as inflorescence number per bud is influenced by environmental conditions (primarily light and temperature) during the initiation of inflorescence primordia (Trought, 2005). While cane pruning has the advantage of maintaining the most fruitful nodes (Jackson, 2001), it is more expensive than spur pruning and a tendency towards apical dominance may result in uneven budburst (May et al., 1978). Carbohydrate reserves may also potentially be impacted as 2-year-old wood may be removed (Winkler et al., 1974). In contrast, spur pruning normally involves the retention of spurs of 2–3 nodes located along a permanent cordon, with wood no older than one growing season being removed in the case of healthy vines. Along with cheaper pruning costs, this system has the advantage of having a higher capacity for reserve storage and often more uniform shoot growth (Tassie & Freeman, 1992). It is also more suitable to mechanisation than cane pruning, and may significantly reduce yearly labour demand, even if hand harvesting and pruning are employed (Intrieri & Poni, 1995). A mechanical pre-pruning followed by a light manual pruning is a popular technique among growers, that along with reducing labour costs, may help to maintain cordon vitality over time by promoting proper pruning practices. The ease of operation afforded by such a strategy could, for example, encourage the careful selection of nodes and consideration of the types of cuts being made. Along with increased susceptibility to longevity related issues such as dieback, other important considerations regarding the use of permanent cordons include the selection of cultivars suitable for their basal fertility, as well as the use of canopy management practices which optimise the positioning of foliage and bunches for mechanisation.

2.3.2. Wrapping Canes Tightly around the Cordon Wire

In many wine-producing countries, it is a common practice for canes to be wrapped tightly around the cordon wire during the establishment of permanent cordon arms for several reasons. Wrapping the canes around the cordon wire has the inherent advantage of providing the developing arms with additional support, reducing the risk of rolling and mitigating the need for foliage wires. This added support is especially appreciated in heavily mechanised regions such as Australia, where the stability of the canopy is an important factor in regard to limiting mechanical damage. While some growers have begun to wrap canes more loosely around the cordon wire out of concern for strangulation, it is unclear whether or not this practice is enough to totally eliminate the possibility of the wire becoming embedded within the wood of the cordon and eventually affecting productivity. Other methods including placing the canes on top of the wire and securing them in place in three or four positions on each arm (Caravia et al., 2015b), or utilising a coiled wire through which the canes may be trained (Gasparinetti et al., 1999), can be more costly and time consuming by comparison. Having the cordon trained on top of the cordon wire also requires the presence of at least one foliage wire, as in the absence of any support for developing shoots to latch on to, arms positioned in this fashion have a tendency to roll under the influence of their own weight (Caravia et al., 2015b). This can disrupt the selection and formation of permanent spur positions and potentially overexpose any fruit that may be present. The risk of rolling is also related to cordon length, with longer cordons having a greater susceptibility.

It has been hypothesised that wrapping the cordon tightly around the cordon wire may cause a constriction of the vascular system, becoming worse over time and disrupting the normal flow of water and nutrients through cordon arms (Caravia, 2016; Caravia et al., 2015b). This stress may in turn contribute to a decline in productivity and the occurrence of dead arm symptoms, either on its own or in combination with other factors. While little research has been conducted on grapevines in this regard, it has been reported that constriction applied via plastic straps on olive trees led to a reduction in vegetative growth and canopy volume compared to control trees, as well as a reduction in yield for low vigour cultivars (Tombesi & Farinelli, 2016). This reduction in vegetative growth corresponded to lower stem water potential in constricted trees, likely as a result of reduced xylem girth growth caused by the plastic strap at the constriction point (Tombesi et al., 2016). A similar reduction in vigour was observed after girdling (removing a thin strip of the outer stem layer, i.e., cambium and phloem) in various orchard trees (Dann et al., 1984; Rufato et al., 2014; Sousa et al., 2007; Tombesi et al., 2016), especially when performed early in the season. Such an interruption to the phloem prevents the downward flow of sugars and other organic compounds, with extra damage or even death resulting if the cut is deep enough to damage the xylem or is too slow to heal (Day & DeJong, 1999). Girdling is commonly used in table grape production to increase yield (Jensen et al., 1975), and is sometimes utilised in winegrape production to promote sugar and anthocyanin accumulation (Li et al., 2015). Working with Kyoho grapevines, Li et al. (2015) demonstrated that prolonged disconnection of the phloem after girdling significantly suppressed the length of fruiting shoots and the number of leaves per shoot on girdled arms, possibly due to a disruption of the accumulation of carbohydrate reserves in the trunk and roots. Similar results were obtained by Eltom et al. (2013) who, working with Sauvignon blanc, determined that cane girdling before budburst restricted carbohydrate availability and reduced shoot growth rate and internode number. Based on these results it seems probable that any disruption to the vines vasculature imposed by constriction could inhibit carbohydrate accumulation in the perennial organs, negatively affecting growth and productivity in the following seasons. Investigating the effect of tight wrapping on the developing arms of Shiraz, Caravia (2016) reported pruning mass to be around 20% higher in non-wrapped vines after a single season, suggesting more favourable conditions for growth. This was further supported by measurements of arm transversal area (ATA) which was found to be significantly higher in the distal portion of non-wrapped arms compared to wrapped arms. Additionally, significantly higher concentrations of soluble sugar were observed at the distal portion of non-wrapped arms during dormancy and there was a trend (though not significant) of higher trunk starch concentration in non-wrapped vines. While the reduced vegetative growth observed in the wrapped cordons and the lower concentration of sugars observed in the distal portion of wrapped arms seem to suggest that constriction may have had a negative effect on xylematic flow, the same cannot necessarily be said about phloematic flow. Considering that an interruption to the phloem would be likely to disrupt the translocation of carbohydrates to perennial structures for overwintering, one would expect that the concentration of sugars observed in the distal portion of arms during dormancy would be higher in the case of the tightly wrapped arms had there been such an interruption.

2.3.3. Establishment of Cordon Arms

When establishing permanent cordon arms during the development or reworking of a vineyard, canes are selected based on several factors including their vigour, health, and perhaps most importantly their proximity to the cordon wire. This selection is important as it has a permanent impact on the vine's canopy architecture, and in the case of bilateral cordons, the formation of a "v-zone" of appropriate size at the crown of the vine helps to regulate canopy density, improving light and wind penetration and reducing the risk of future foliar disease pressure. In certain scenarios, a unilateral cordon may be preferable over a bilateral cordon when accounting for factors such as vigour and intra-row vine spacing. The length of cordon arms is often decided based on this spacing, with the distal ends of the arms of two adjacent vines ending in close proximity to each other. Perhaps unsurprisingly, as any gaps along the cordon wire without the presence of productive cordon equates to a loss in potential profit. As such, common practice involves cutting canes intended as future bilateral cordon arms to a length equal to about half the distance between the trunks during establishment, effectively leaving them as long as possible. While this method has the advantage of getting the entirety of the desired length of the new arm into position right away, evidence suggests that it may not always be the best approach. The capacity of newly established arms to grow and develop new shoots, as is the case with all parts of the vine is based on vigour (Winkler et al., 1974). When low vigour-canes with a small diameter are selected for the establishment of new cordons, possibly by necessity due to a lack of better options as the case may be, a length adjustment based on the apparent vigour of the canes may be an advisable consideration (Castaldi, 2008). Canes of small diameter may not have the vigour needed to support uniform budburst and growth of new shoots, especially in the case of the buds located in the middle of the canes (Dry & Loveys, 1998). By adjusting the length of the canes intended as new arms based on their apparent vigour (Figure 2.2), less buds are retained, encouraging their successful growth and the development of shoots that are of greater diameter and are better suited for selection as permanent spur positions (Castaldi, 2016; Winkler et al., 1974).

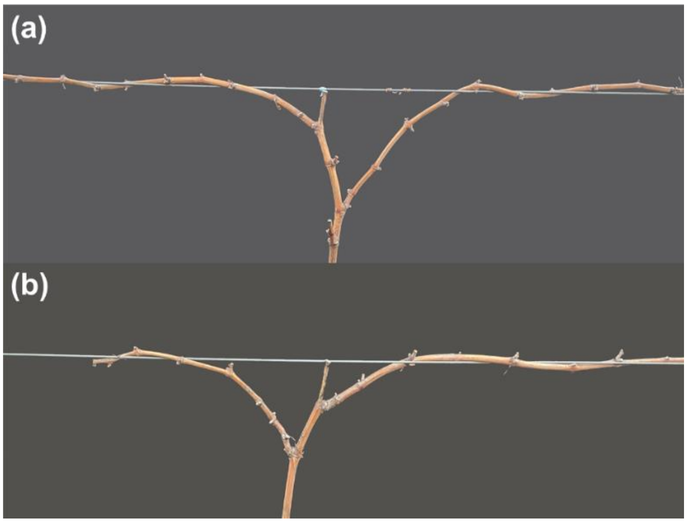

Figure 2.2. Length adjustment of low-vigour canes selected as permanent cordon arms. (**a**) Both canes selected as permanent cordon arms were deemed suitably high vigour and did not receive a length adjustment; (**b**) one cane selected as a permanent cordon arm was deemed to have an apparent vigour too low to support uniform budburst and shoot growth and received a length adjustment based on its apparent vigour.

One of the shoots from the most distal portion of the developing arm, close to the cutting point, may then be selected and trained along the cordon wire horizontally during the growing season to extend the cordon arm to its final length. This process may require attention several times to secure the extension as it grows but the desired result is more numerous, healthier spur positions. Following the same logic, the removal of secondary shoots as well as those growing from locations deemed undesirable for permanent spur positions may be beneficial in encouraging the growth of shoots at more desirable spur positions (Bernizzoni et al., 2011; Castaldi, 2016). An early shoot thinning, performed when newly burst shoots are 5–10 cm, could help to combat the acrotonic tendency of the vine to send sap to the most distal nodes, improving uniformity in the length and diameter of shoots along the cordon (Simonit et al., 2012).

2.3.4. Maintenance of Cordon Arms

A healthy permanent cordon may lose productivity simply as a result of decisions made during the spur pruning process. "Blinding" of spur positions (for example removing one node out of every two along the length of the cordon, or removing all of the nodes within the v-zone) is a process that is sometimes performed intentionally for the purpose of keeping productivity low or maintaining spatial separation between spurs (Castaldi, 2016). This process is ideally performed on 1-year-old canes during establishment by scraping off undesired buds or early developing shoots. Spur positions may also be lost unintentionally, sometimes permanently, due to the overzealous actions of pruners with insufficient experience in selecting which spurs to retain each year. Likewise, any accidental nicking of the cordon or other wood older than 1-year-old, deep enough to damage the vasculature or illicit a wound response, or producing larger cuts than necessary, could be detrimental to vitality (Castaldi, 2008; Simonit & Sirch, 2009b). Cordon length is an important consideration in relation to vine vigour. Cordons of insufficient length may lack the spur positions required to provide an adequate framework for the substantial amount of vegetative growth observed in high-vigour situations. Likewise, excessively long cordons may be unable to support uniform budburst and growth, especially in low-vigour situations, leading to underdeveloped or missing spur positions (Dry & Loveys, 1998). Important pruning practices include limiting the number and size of clean cuts in order to help reduce the susceptibility of the cordon to fungal trunk disease infection, as well as avoiding pruning during wet conditions when spore inoculum is prevalent (Carter, 1957). It has been suggested that training methods involving minimal pruning show less esca disease effect than methods involving regular manual pruning (Lecomte et al., 2021), and techniques which concentrate pruning wounds on the crown of the vine should be avoided, particularly for cultivars sensitive to wood disease. Recent research has indicated that the total surface area of cuts on a cordon rather than the diameter of each cut is the more important factor in regard to incidence of Eutypa dieback (Henderson et al., 2020). Such wounds may expose the vascular system to the environment, dehydrating cells adjacent to cuts and producing desiccation cones (Faúndez-López et al., 2021), susceptible to colonisation by fungal pathogens (Cholet et al., 2021; Travadon et al., 2016). It is also important to be cognizant of the proximity of the cutting point to retained buds when spur pruning, as necrosis will occur near the cutting point which must be allowed for with a desiccation zone. It is recommended to allow for a length between the cutting point and retained buds equal at a minimum to twice the diameter of the wood being pruned (Castaldi, 2016; Simonit et al., 2013b), so as not to compromise the vitality of the wood tissue in close proximity to the bud. A recent study by Faúndez-López et al. (2021) has indicated that the area and depth of wood necrosis induced by pruning is influenced by the distance between the cutting point and the node, but not by the diameter of pruned spurs. Interestingly, this study also found that budburst and shoot development were not affected by the distance between nodes and pruning cuts. A new method being promoted by some practitioners involves retaining the top node position of each spur in a two-node spur system preferentially each year (Simonit et al., 2013a), allowing for a gradual increase in spur position height, purporting to reduce trunk disease incidence. This is in contrast to the classic and widely adopted method of retaining the bottom node each year, which has the benefit of limiting variation in cordon architecture and keeping the height of spur positions in closer proximity to the cordon. While both methods have their advantages, an issue that may arise with unchecked vertical or horizontal elongation of spur positions is an increase in the frequency of breakage via mechanical damage (Castaldi, 2011). Shifting the vegetation further away from the cordon also inevitably results in an unavoidable progressive loss of leaf area, if the trimming/hedging point remains unchanged. Overly tall spur positions may, however, be brought back down to starting height periodically when necessary, with the use of a replacement cane originating in close proximity to the cordon (Simonit et al., 2013a). One added benefit of tall spur positions is an increase in the volume of perennial wood, providing greater capacity for carbohydrate reserve storage. Increased spatial separation of the fruit zone from the cordon may also have positive implications on bunch microclimate and fruit and foliar disease pressure.

2.4. Vascular Diseases of Grapevine

2.4.1. Mechanism of Infection and Implicated Pathogens

Grapevine trunk diseases are caused by a range of phytopathogenic fungi and represent a serious challenge to viticulturists all over the world, having the capacity to drastically reduce vineyard productivity and cause significant loss of income (van Niekerk et al., 2011). In a study examining the impact of grapevine age on water status and productivity of Riesling, Bou Nader et al. (2019) identified wood diseases as the main factor behind the decline of old vines, suggesting that their management is a key component in regard to improving vineyard longevity. Trunk disease pathogens associated with the decline and dieback of grapevines include species of the Diatrypaceae family, most prominently *Eutypa lata* (Moller & Kasimatis, 1978; Trouillas et al., 2010), species of the Botryosphaeriaceae (Pitt et al., 2010; Úrbez-Torres & Gubler, 2009; van Niekerk et al., 2004), *Phomopsis* spp. (van Niekerk et al., 2005), various basidiomycete species (Fischer, 2006), *Phaeomoniella chlamydospora* (Crous et al., 1996), *Phaeoacremonium* spp. and *Cadophora* spp. (Travadon et al., 2015), among others. These pathogens infect the grapevine primarily through pruning wounds, with spores dispersed by wind and rain splash germinating in exposed xylem vessels upon contact and colonising woody tissue (Gramaje et al., 2018). The same grapevine can by infected multiple times with one or more fungal pathogens, with different pathogens causing similar symptoms of decline, supporting the idea that trunk diseases may best be regarded as a complex of infections (Larignon & Dubos, 1997; Rolshausen et al., 2004; Úrbez-Torres et al., 2006). Occlusion of the xylem and phloem elements may occur in infected vines, along with wood decay, impairing the translocation of water and nutrients (Rolshausen et al., 2010). Visible symptoms of decline often do not develop until 10 or more years after planting and may include uneven periderm maturation, stunted shoots, necrotic and marginally scorched or distorted leaves, reduced bunch size, uneven ripening, fruit wilting, and eventually vine death (Chatelet et al., 2006; Sosnowski et al., 2013). Studies have suggested that variations in the severity of symptoms from year to year may be more influenced by climatic factors such as rainfall and temperature than by vineyard practices (Sosnowski et al., 2007; van Niekerk et al., 2011).

Pierce's disease, also a vascular disease of the grapevine, is caused by the xylem-limited bacterium *Xylella fastidiosa* (Xf) and is transferred from vine to vine by sap-feeding insect vectors (Hopkins, 1989). Expression of symptoms occur as a result of the systemic colonisation of the xylem by Xf and the progressive occlusion of xylem conduits. The disease is fatal to grapevines, as the development of tyloses, gels, and embolisms in xylem vessels cause vascular transport to become increasingly impaired, resulting in water deficits and their associated consequences (Sun et al., 2013; Thorne et al., 2006). Drought conditions and water shortage issues are becoming more and more common under the influence of global warming. Accordingly, such impediments to the movement of water and other substances through the vasculature of the cordon and other vine tissues, as well as their interaction with environmental conditions, are becoming of increasing concern.

2.4.2. Relationship between Stress and Disease Symptom Expression

Vines may be infected by fungal and bacterial pathogens without displaying any symptoms. Several studies have yielded results indicating that infected vines may be more likely to express symptoms when their health is compromised by stress. Water stress has been shown to exacerbate disease symptoms associated with *Phaeomoniella chlamydospora* (Pc), a pathogen implicated in esca and Petri disease (Edwards et al., 2007a, 2007b). Leaf water potentials were determined to be lower in infected Cabernet Sauvignon and Zinfandel vines when subjected to water stress, indicating that infection inhibited the ability of the vines to transport water. In a greenhouse trial, Ferreira et al. (1999) observed that significantly more Chenin blanc vines inoculated with Pc exhibited dieback symptoms when subjected to water stress. Similarly, working with Müller-Thurgau and Riesling, Fischer and Kassemeyer (2012) observed a greater number of symptomatic xylem vessels in vines infected with Pc when subjected to water stress.

Investigating the effect of water and temperature stress on grapevines inoculated with *Eutypa lata* (Eutypa dieback), Sosnowski et al. (2011a) demonstrated that Red Grenache vines in pots subjected to a combination of extreme heat or cold plus low or high soil moisture displayed more severe foliar symptoms than those in moderate conditions; however, severe symptoms were not produced with either of these factors alone. In a subsequent water-deficit trial, the extent of colonisation of *E. lata* and *Diplodia seriata* (Botryosphaeria dieback) did not increase under water stress, with the progress of *E. lata* actually showing a reduction in water stressed vines (Sosnowski et al., 2021). Further examining the same vines and irrigation treatments, Oswald (2017) reported that smaller xylem vessel area and narrower cane diameter were correlated with less colonisation of *E. lata* in the water stressed vines, contradicting the theory that restricting vascular tissue might lead to increased progression of Eutypa dieback. Additionally, Pouzoulet et al. (2017) reported that increased xylem vessel diameter was correlated with greater susceptibility to Pc, due to less efficient vessel compartmentalisation.

Pierce's disease produces symptoms which have been found to correlate with low leaf water potential and turgor, impaired hydraulic conductance, and higher stomatal resistance (Goodwin et al., 1988). In their study on Cabernet Sauvignon, Choi et al. (2013) reported that potted vines infected with Xf exhibited a significantly stronger transcriptional response of pathogen-induced genes when exposed to water deficit, as well as increased severity of disease symptoms and extent of pathogen colonisation. This agrees with the results of Thorne et al. (2006) who found that vines inoculated with Xf and exposed to water deficit developed more extensive symptoms than when well-watered, as well as Choat et al. (2009) who found a positive relationship between Xf concentration and symptom formation in deficit-irrigated vines. If, as some of these results indicate, stressed vines are more likely to express symptoms of vascular disease, then adopting a training method which avoids constriction of the vasculature of the cordon may help to limit the onset of disease symptoms by avoiding a reduction in vine defence response. As of the present, however, the exact nature of the relationship between these factors of decline remains unclear.

2.4.3. Relationship between Strangulation and Trunk Disease

There is currently no published literature regarding the relationship if any that exists between constriction of the cordon from tight wrapping, dieback, and incidence of vascular disease. A small survey was conducted in the Waite Campus vineyard, University of Adelaide, South Australia in the spring of 2020 to investigate the relationship between cordon strangulation and fungal trunk disease expression. Fifteen-year-old Cabernet Sauvignon vines were assessed for the presence of characteristic Eutypa dieback foliar symptoms, comprising stunted shoots with chlorotic (yellow) leaves, often cupped and with tattered margins (Carter, 1991), as well as cordon dieback. From those assessed, two vines were selected which displayed cordon dieback but no foliar symptoms of Eutypa dieback, two vines were selected which displayed cordon dieback as well as foliar symptoms, and two vines were selected which were healthy in appearance and displayed no visible signs of dieback or foliar symptoms (Figure 2.3).

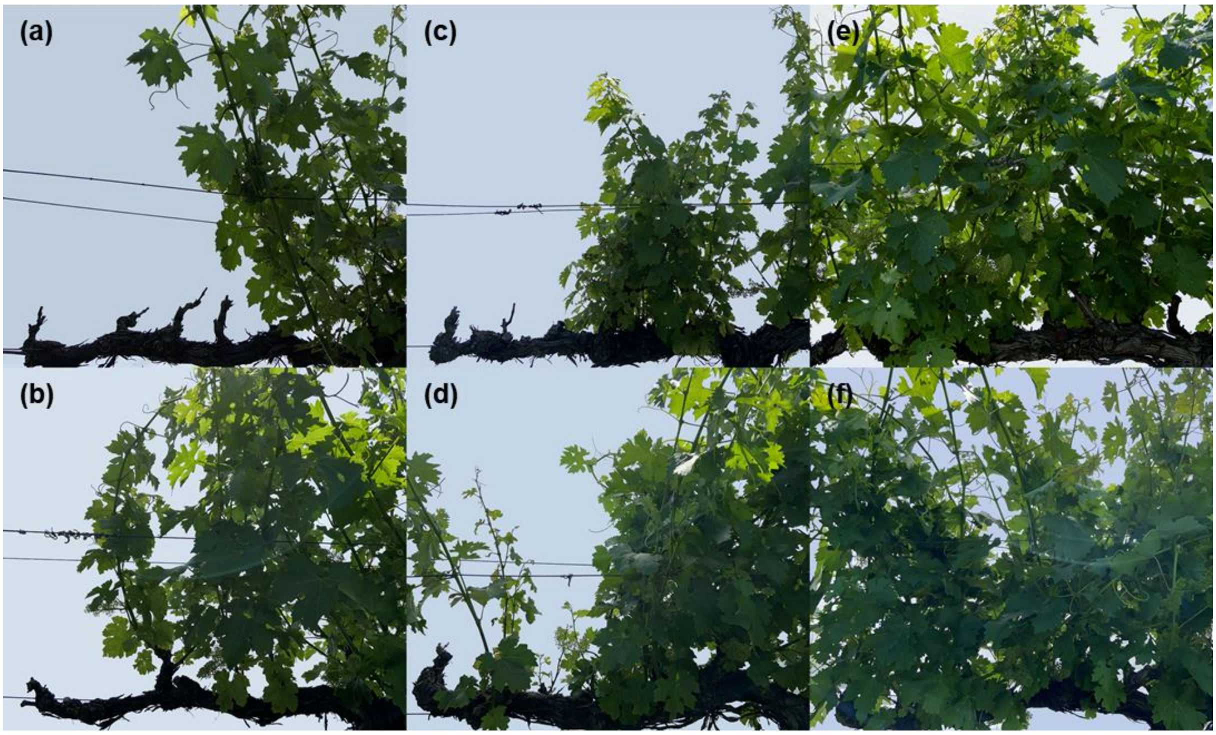

Figure 2.3. Presence of cordon dieback and foliar symptoms of Eutypa dieback. (**a**,**b**) Cordon dieback but no foliar symptoms; (**c**,**d**) cordon dieback and foliar symptoms (stunted shoots); (**e**,**f**) symptomless.

Based on visual examination, the cordons of all six vines appeared to have been established by tightly wrapping developing canes around the cordon wire, with the wire now visibly embedded within the wood of all cordons. Samples of approximately 15 cm length were cut from the cordons for cross section examination and for collection of samples for laboratory diagnosis by isolation. Upon direct examination of the cross sections, wedge patterns of staining were observed in the samples collected from the cordons displaying signs of dieback both in the presence and absence of characteristic Eutypa dieback foliar symptoms but were not observed in the samples collected from the cordons which were healthy in appearance (Figure 2.4).

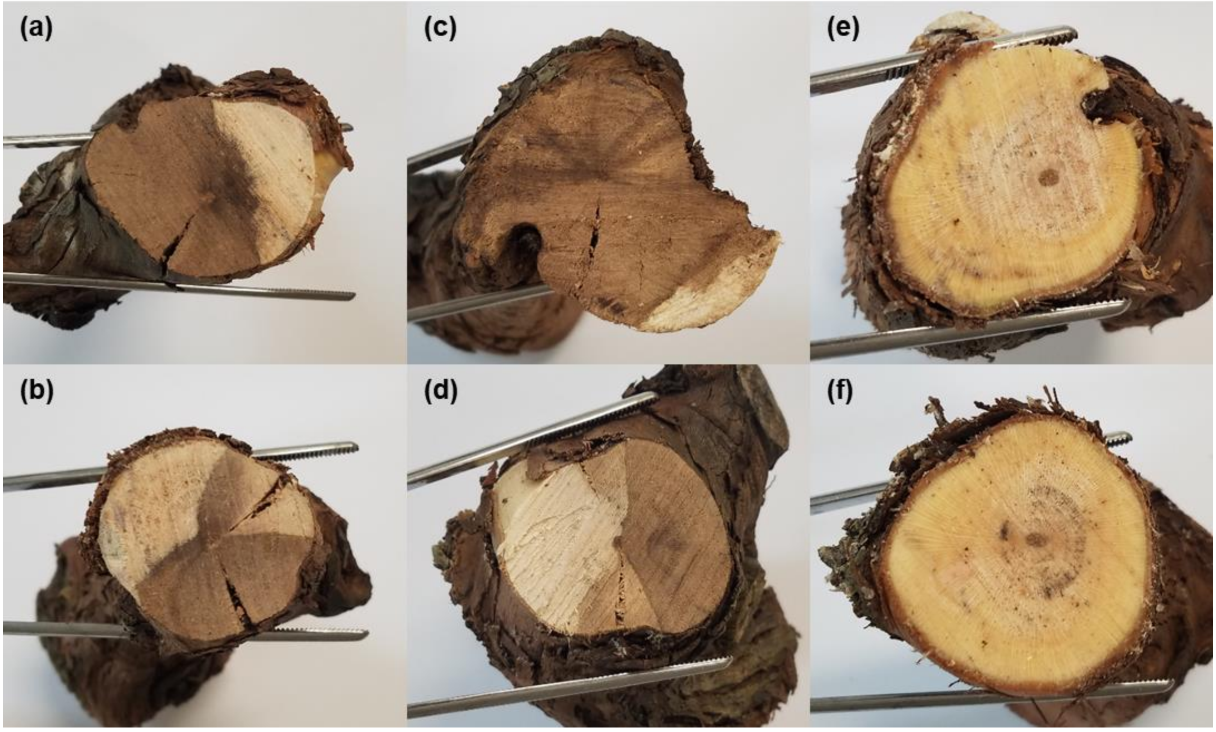

Figure 2.4. Examination of cross sections. (**a**,**b**) Cordon dieback but no foliar symptoms; (**c**,**d**) cordon dieback and foliar symptoms; (**e**,**f**) symptomless.

This is logical when one considers that regardless of the cause of dieback (pathogen or strangulation) it is expected that dead wood will be observed in the cross section given that the wood is dying back. To diagnose if pathogens were present in the symptomatic wood, the cordon samples were surface disinfested in bleach before a drill was used to collect shavings made along the margin between live and dead wood. For the asymptomatic samples wood shavings were collected solely from wood which was healthy in appearance. Small sections of the shavings were then placed on potato dextrose agar plates and incubated for a week at 25 ℃ with a 12 h photoperiod before examination for the presence of fungal growth (Sosnowski et al., 2021). *Eutypa lata* was positively identified in plates from wood samples collected from cordons displaying dieback both in the presence and absence of foliar symptoms as well as from symptomless cordons. Additionally, other Diatrypaceous and Botryosphaeriaceous species were identified only in samples from cordons displaying Eutypa dieback foliar and dieback symptoms. These findings confirm the inherent difficulty in relying solely on the expression of foliar symptoms when investigating for the presence of fungal pathogens (Sosnowski et al., 2011a; Sosnowski et al., 2007), as they may be present in symptomless vines, which are otherwise healthy in appearance. There remains a question as to what extent constriction of the cordon may influence the speed and severity at which symptoms of decline resulting from the presence of these pathogens are expressed, as such, more research is required.

2.5. Conclusions

A simple visual assessment of the cordon of a mature vine whose arms are wrapped so tightly that the cordon wire has long become embedded within the wood of the cordon is enough evidence for most observers to conclude that there has been a negative impact on the health of the vine. This situation is typically accompanied by various states of decay and dieback along with an oftentimes severe decline in productivity. In such a scenario, the constrictive effects of cordon strangulation may be working in conjunction with other factors such as the onset of vascular disease to drastically reduce yield and economic viability. It is for this reason that careful consideration must be given when planning and implementing cordon training techniques, both during the initial establishment of young vines as well as the reworking of older vineyards. Climate change is also an important consideration in this scenario, as heat and water stress may further exacerbate the factors driving cordon decline. Understanding the potential benefits of adopting cordon establishment techniques, which avoid constriction of the vine's vasculature, could provide vineyard managers with a strategy aimed at improving vineyard sustainability with little to no added consideration, input or cost after the initial period of establishment. Further research is required to investigate the impacts of wrapping developing arms tightly around the cordon wire in comparison to other cordon training techniques. This research would quantify the impacts of different cordon establishment techniques on vine health and longevity. Other considerations such as the benefits of adjusting the length of canes selected as permanent arms based on their apparent vigour before extending them to final length could also be investigated. By gaining a better understanding of the long-term advantages and disadvantages of different cordon establishment methods, growers in the future may be better equipped to avoid management decisions likely to lead to cordon decline and the costly, untimely reworking of vineyards.

2.6. Acknowledgements

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Chapter 3. Published Manuscript 1: Research Note: Assessing the relationship between cordon strangulation, dieback, and fungal trunk disease symptom expression in grapevine

Statement of Authorship

Title of Paper	Research Note: Assessing the relationship between cordon strangulation, dieback, and fungal trunk disease symptom expression in grapevine
Publication Status	☐ Published ☐ Accepted for Publication ☑ Submitted for Publication ☐ Unpublished and Unsubmitted work written in manuscript style
Publication Details	OENO One

Principal Author

Name of Principal Author (Candidate)			
Contribution to the Paper	Conceptualisation, methodology, formal analysis, data curation, writing—original draft preparation, writing—review and editing		
Overall percentage (%)	90		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	25/09/2022

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);
ii. permission is granted for the candidate in include the publication in the thesis; and
iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Cassandra Collins		
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Signature		Date	26/09/2022

Research Note: Assessing the relationship between cordon strangulation, dieback, and fungal trunk disease symptom expression in grapevine

Abstract: Grapevine cordons wrapped tightly around the cordon wire during establishment may be susceptible to an early occurrence of decay and dieback symptoms. A loss of productivity is often observed in older permanent cordons that have been constricted in this fashion but is not unique to them. Other factors of decline, such as fungal trunk disease infection, also play a significant role in perennial wood decline. This study aimed to quantify the impact of different contributors to cordon decline, including cordon strangulation and incidence of *Eutypa lata* infection and to investigate their relationship to each other. A survey was conducted over two seasons at ten vineyard sites to visually assess and capture images for algorithmic analysis of vines displaying varying degrees of cordon strangulation, cordon dieback, and characteristic foliar symptoms of Eutypa dieback. Rather than finding evidence of cordon strangulation being a driving force behind cordon decline, there was actually a trend of lower severity of dieback observed with cordons displaying the greatest degree of strangulation. There was also a trend for increased foliar symptoms in relation to an increase in the degree of strangulation, but it is difficult to assign causality in this regard, as the occurrence of foliar symptoms may be influenced by a multitude of factors, including climatic conditions. Further research quantifying the extent to which xylem morphology and functionality are compromised by constrictive pressure in severely strangled cordons could provide further insight into this condition and the effect it could have on vine health and defence response.

3.1 Introduction

Wrapping canes tightly around the cordon wire during the establishment of permanent cordon arms is a method used by many grape growers to reduce costs, provide additional canopy stability, and aid in mechanisation (Caravia et al., 2015a; O'Brien et al., 2021). While not observed in all wine regions, it is an extremely common practice in heavily mechanised areas such as Australia, where permanent cordon training methods are prevalent. It may reduce the risk of cordon rolling, especially in the seasons immediately following establishment, thereby helping to avoid fruit overexposure and the accompanying risk of sunburn damage (Chorti et al., 2010). It may also aid in the establishment of cordon architecture by mitigating disruption to the selection and establishment of permanent spur positions. One key drawback of this method however, is that wrapped cordons may become tightly constricted over time, with the cordon wire often becoming visibly embedded within the wood of the cordon, potentially disrupting the flow of water and nutrients through vascular conduits (Caravia et al., 2015b). In the context of climate change, any disruption to vine water movement is an issue of growing concern, as it is likely that such a condition may be exacerbated by increasingly common water and heat stress events (Keller, 2010; Schultz, 2000). Strangulation of the cordon as a result of tight wrapping is a condition that visibly worsens over time as the cordon grows and thickens, with arms fashioned in this manner often displaying signs of decay and dieback after as little as 15-20 years. These declining cordons may also be suffering from the negative effects of fungal trunk disease infection, caused by a range of phytopathogenic species (Fontaine et al., 2016; Mondello et al., 2018; van Niekerk et al., 2011), among the most economically impactful for growers in Australia being Eutypa dieback, caused by *Eutypa lata* (Siebert, 2001; Sosnowski et al., 2013) and Botryosphaeria dieback, caused by a range of Botryosphaeriaceous species (Bénard-Gellon et al., 2015). Grapevine trunk diseases (GTDs) are not a problem that is unique to Australia however, with winegrowing regions all over the world subject to their damaging effects (Bois et al., 2017). While cordons may die back themselves over time without the presence of any such infections, possibly in part or in whole as a consequence of severe strangulation, there may be a complex relationship between such dieback and the susceptibility of the vine to infection and symptom expression. This symptom expression may include visible dieback of the cordon itself, as well as in the case of *E. lata*, foliar symptoms occurring as a result of translocated toxins released by the advancing fungus (Tey-Rulh et al., 1991). The colonisation of woody tissue by one or more fungal pathogens typically leads to the occlusion of cordon xylem and phloem elements and, eventually, decay and dieback (Rolshausen et al., 2010). Vines may, however be infected by fungal pathogens without displaying any symptoms, and the results of several studies have indicated that vines may be more likely to express non-foliar symptoms if their health is already compromised by stress (Claverie et al., 2020; Edwards et al., 2007a, 2007b; Ferreira et al., 1999; Fischer & Kassemeyer, 2012; Songy et al., 2019). In a trial on potted vines, those subjected to a combination of extreme heat or cold plus low or high soil moisture displayed more severe foliar symptoms of Eutypa dieback than those in moderate conditions; however, severe symptoms were not produced with either factor alone (Sosnowski et al., 2011a). This conflicts with the results of a subsequent water-deficit trial, where the extent of colonisation of *E. lata* and *Diplodia seriata* (Botryosphaeria dieback) didn't increase under water stress, with the progress of *E. lata* showing a reduction in water-stressed vines (Sosnowski et al., 2021). Previous research has investigated the relationship between certain cultural practices and GTDs. Numerous studies have indicated that the incidence and severity of GTDs may be influenced by pruning practices (Henderson et al., 2020; Sosnowski

& Mundy, 2019; Travadon et al., 2016). The pruning technique has also been reported to impact the area and depth of wood necrosis in the absence of GTDs (Faúndez-López et al., 2021). Training methods involving minimal pruning reportedly show less esca disease effect than methods involving regular manual pruning (Lecomte et al., 2021), and surgically removing necrotic wood has been successful in the recovery of esca-diseased grapevines (Cholet et al., 2021). If stressed vines are more likely to express symptoms of *E. lata* and other fungal trunk diseases (Songy et al., 2019), then it is possible that the use of training methods which avoid strangulation of the cordon may help to limit the onset of symptoms by avoiding a reduction in vine defence response. There is currently limited research in this area, and the exact nature of the relationship between these factors of decline (cordon strangulation, dieback, and incidence of GTDs and their symptom expression, including Eutypa dieback foliar symptom expression) remains unclear. This research aimed to investigate the nature of the relationship between these factors by assessing their incidence in commercial vineyards displaying varying degrees of cordon decline.

3.2 Materials and Methods

3.2.1. Vineyard site selection and trial design

Ten vineyard sites across the Barossa Valley and the Adelaide Hills wine regions in South Australia were selected for this study. These sites displayed varying degrees of cordon strangulation and were selected based on their age, use of a permanent cordon training system, and willingness of vineyard managers to participate in the trial. Site selection took place in the spring of 2020 and each site was surveyed and imaged twice, once in the spring of 2020 and again in the spring of 2021. At each site 10 rows were randomly selected, and depending on the block, 20 or 21 consecutive vines were selected from each of these rows for a total of 200 or 210 vines surveyed in each vineyard. However, some vines were missing from selected panels, and were removed from the trial resulting in fewer vines assessed at these sites. Additionally, three rows of vines were removed from the trial when Site A was partially reworked during the winter of 2020. Site information is displayed in Table 3.1.

Table 3.1. Site details of vineyards selected for assessment.

Site	Variety	Region	Block Size (ha)	Year Planted	# Vines Assessed	Cordon Length (m)	Rootstock	Irrigation Applied (ML/ha)	Soil Type
A	Shiraz	Barossa Valley	3.1	2007	146	2.0	Own Roots	0.88	Red brown earth over ironstone
B	Shiraz	Barossa Valley	1.4	2003	183	1.8	Own Roots	0.4	Sand over ironstone
C	Shiraz	Barossa Valley	3.7	1988*	191	2.0	Riesling	0.2	Red brown earth over ironstone
D	Shiraz	Barossa Valley	2.1	2000**	207	1.8	Merlot	1.2	Sandy loam over red clay
E	Shiraz	Barossa Valley	1.8	1998	200	2.5	Own Roots	1.2	Sandy loam over red clay
F	Shiraz	Barossa Valley	2.0	1998	209	1.8	Own Roots	0.5	Sand over clay
G	Chardonnay	Adelaide Hills	0.9	2001	200	1.5	Own Roots	0.74	Sandy loam over red clay
H	Shiraz	Barossa Valley	2.2	1997	209	1.5	Own Roots	0.7	Loamy sand over granite
I	Shiraz	Barossa Valley	3.0	1998	204	1.5	Own Roots	0.7	Loamy sand over granite
J	Cabernet Sauvignon	Barossa Valley	2.9	1998	206	1.4	Own Roots	0.7	Loamy sand over granite

*grafted 2003, **grafted 2004.

The climatic conditions for the sites were sourced from the nearest Australian Bureau of Meteorology (http://www.bom.gov.au/, accessed on 11 May 2022) weather stations: Nuriootpa PIRSA (station number 23,373), Woodside Wicks Estate (station number 23,920), and Mount Barker (station number 23,733). Mean average temperature and rainfall were calculated for surveyed years (2020 and 2021) as well as the year preceding the survey (2019). Accumulated degree-days (base 0) were calculated for the spring of each survey year (1 October–30 November) using monthly averages.

3.2.2. Visual assessments

All sites were surveyed in the spring of two consecutive growing seasons (2020 and 2021) when symptomless developing shoots were 50–100 cm long (late October to early December). Each living vine was visually assessed as follows: (i) Cordon dieback was assessed on a 0–100 % scale as a proportion of entire cordon length which had died back and where the canopy was no longer present (i.e., no remaining foliage = 100 % dieback). This assessment did not take into account the cause of dieback, but rather quantified loss of productive cordon indiscriminately. (ii) The presence of Eutypa dieback foliar symptoms was assessed on a 0–100 % scale as a proportion of the canopy present which displayed characteristic Eutypa dieback foliar symptoms (stunted shoots with chlorotic and yellow leaves, often cupped and with tattered margins) (Carter, 1991) (Figure 3.1).

Figure 3.1. Typical Eutypa die back foliar symptoms (stunted shoots with chlorotic and yellow leaves, often cupped and with tattered margins).

This assessment did not include areas of the cordon where foliage was not present and in the case of vines displaying dieback, was expressed as a percentage of all remaining foliage displaying symptoms. (iii) Vines were assessed for degree of cordon strangulation only once, during the second survey (as the degree of strangulation remained relatively constant between the two seasons). The degree of cordon strangulation was assessed on a 0–4 scale according to the criteria presented in Table 3.2.

Table 3.2. Visual scale for degree of cordon strangulation caused by tight wrapping. Arrows indicate areas where more severe strangulation is apparent.

0	no strangulation	Cordon positioned entirely on top of wire or otherwise lacking significant wire contact. Minimal to no pressure applied from wire to cordon.	
1	minimal strangulation	Cordon wrapped loosely around wire with much of cordon directly in contact with wire. Minimal pressure applied from wire to cordon and though cordon slightly coiled, minimal to no embedding of wire in wood of cordon.	
2	moderate strangulation	Cordon wrapped moderately tightly around wire with almost all of cordon in direct contact with wire. Moderate pressure applied from wire to cordon, with wire slightly embedded within cordon wood in some areas. Cordon coiled in shape and wire also potentially slightly coiled as a result of contact with cordon.	
3	severe strangulation	Cordon wrapped tightly around wire with almost all of cordon in direct contact with wire. Large amount of pressure applied from wire to cordon and in many areas wire visibly embedded deeply in cordon wood. Cordon coiled in shape and wire likely coiled as well in line with shape of cordon.	
4	very severe strangulation	Cordon wrapped extremely tightly around wire with almost all of cordon in direct contact with wire. Large amount of pressure applied from wire to cordon and in many areas wire no longer visible as so deeply embedded in cordon wood. Cordon tightly coiled in shape with creases visible in cordon wood.	

3.2.3. Canopy assessment with VitiCanopy

Images were taken with the front camera of an iPhone (Apple, Cupertino, CA) at the time of visual assessment for the purpose of measuring canopy architecture using the VitiCanopy App (De Bei et al., 2016). One upwards facing image was taken from about 80 cm below the cordon of each vine and used for the determination of the plant area index (PAI).

3.2.4. Statistical analysis

Principal component analysis (PCA) was used to identify the dominant patterns in spectral data using The Unscrambler X Version 10.2 (CAMO Software, Oslo, Norway). The Hotelling T^2 test was computed on PCA scores, and spectral outliers were removed (defined as any samples falling outside the associated critical limit of a *p*-value of 5 %). Additional PCA and ANOVA were performed using XLSTAT Version 2021.2.2 (Addinsoft SARL, Paris, France). Means were assessed across all sites and were separated using Fisher's LSD test at a significance level of $p \leq 0.05$ for all data. Arithmetic means within each site were used to calculate site averages.

3.3 Results and Discussion

The spring of 2020 was warmer than the spring of 2021, with 1061-degree days compared to 913-degree days in the Barossa Valley, and 986 compared to 876 in the Adelaide Hills (Figure 3.2).

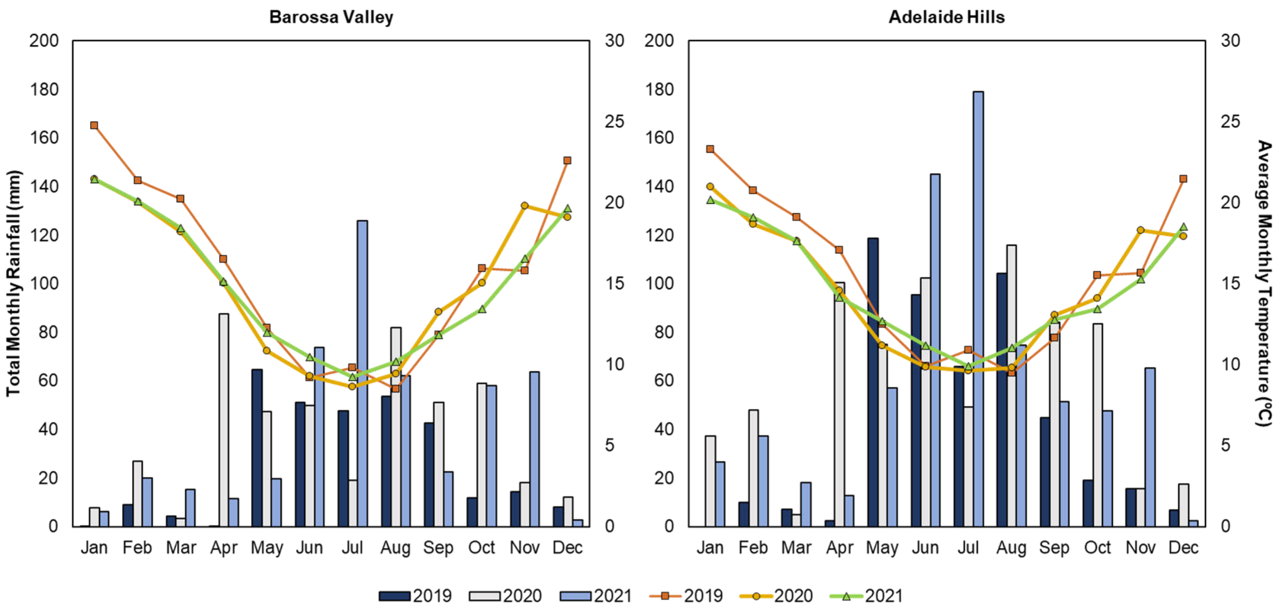

Figure 3.2. Average monthly temperature and rainfall calculated for 2019, 2020, and 2021. Climatic data were sourced from the nearest Australian Bureau of Meteorology (http://www.bom.gov.au/) weather stations.

The first two principal components (PCs) in the PCA in Figure 3.3 explain 70 % of the variation in the dataset. PC1 separates dieback from PAI, degree of strangulation, and cordon age. There was a strong correlation observed between the degree of strangulation and cordon age. Sites with younger cordons, such as A, B, and C, are separated from older sites including G and J. PC2, which explains over 30 % of the variability in the data set, mostly separates site E from the other sites and in particular from sites B, D, and J, and is related to higher foliar symptoms for site E in both seasons, particularly 2021.

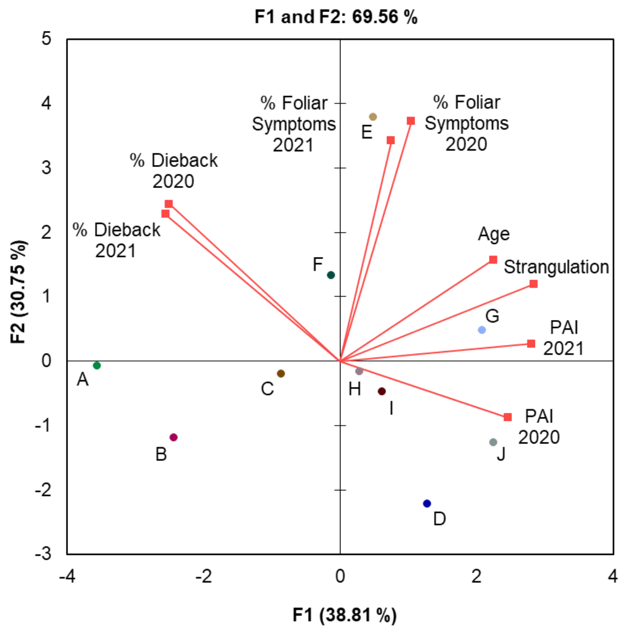

Figure 3.3. Principal component analysis biplot of % cordon dieback, % foliage displaying symptoms characteristic of Eutypa dieback, plant area index (PAI), degree of cordon strangulation, and cordon age observed across the 10 surveyed vineyard sites (A-J).

Both the percentage of cordon displaying dieback and the percentage of foliage displaying signs of characteristic symptoms of Eutypa dieback increased at all sites between assessed seasons (Table 3.3). The percentage of cordon with visible dieback symptoms increased from an average of 12.7 % across all sites in 2020, to an average of 18.8 %. Cordon dieback is an irreversible condition that cannot be rectified in the absence of major reworking and as such the increase in dieback observed between assessed seasons at all sites is in line with what one would expect from such a survey. It was decided to conduct the survey across ten vineyard sites to capture as wide a range of degrees of strangulation as possible under different vineyard conditions. Some sites had a greater within-site variability of observed strangulation degrees. Others such as sites E and G were comprised almost entirely of very severely strangled vines. Assessed vines were selected to be representative of the sites, and these differences came down to the directions given to the practitioners who were responsible for the cordon training. Considering each site individually, it was apparent that not every site provided the same response to strangulation according to their own growing conditions. However, several sites which were highly variable in their range of observed strangulation degrees displayed surprising trends where the most severely strangled cordons displayed less dieback than their less strangled counterparts. When comparing the means of data collected at all sites, a trend was observed where cordons suffering from the greatest degree of strangulation displayed the least amount of dieback (Figure 3.4). Vines with the most cordon strangulation (4) showed significantly less dieback than those that displayed little (1) or no (0) signs of strangulation in both 2020 and 2021. In 2020, very severely strangled (4) cordons actually showed significantly less dieback than all other degrees of strangulation, up to and including vines which were severely strangled (3). This is surprising given that the dieback is a condition which is normally associated with advanced cordon age, and a correlation was observed between the degree of strangulation and cordon age (Figure 3.3). This correlation is logical when one considers how the condition of cordon arms wrapped tightly around the cordon wire typically worsens over time. Arms positioned in this fashion may not display any signs of constriction during the seasons immediately following establishment. However, with normal growth, the pressure applied by the wire onto the cordon and the extent to which it may become embedded within the cordon wood can become progressively worse over each passing year. One might also suspect that cordons displaying very severe strangulation could be more susceptible to dieback given the possibility that the normal functionality of their vascular conduits could be compromised by constrictive pressure. Any decrease in the number or diameter of xylem conduits would likely reduce the capacity of the cordon for water and nutrient transport, as xylem conductivity is determined by vessel structure, size, and efficiency (Schultz & Matthews, 1993; Tyree & Ewers, 1991). It is also very common to observe dieback in tightly wrapped arms in older vineyards, both in the presence and (apparent) absence of grapevine trunk diseases. One possible explanation for the negative correlation observed between strangulation and dieback in this trial is that three of the sites with the youngest cordons (A, B, and C) had a high occurrence of dieback but did not have a very high average degree of strangulation, pulling the results towards a negative correlation between the degree of strangulation and dieback. Site B, in particular, had by far the lowest average degree of strangulation (1.60) and among the highest rates of dieback for both 2020 and 2021 (16.2 % and 20.3 % respectively) (Table 3.3). While the exact causes of the dieback at this site were not investigated outside of the factors assessed in the survey, it is likely that Eutypa dieback was a significant contributing factor as the presence of foliar symptoms in both seasons of assessment confirmed its presence. Additionally, some sites, such as D and G, had very high average degrees of strangulation (3.87 and 3.96) and relatively low incidence of dieback (6.9 % and 6.0 % in 2020).

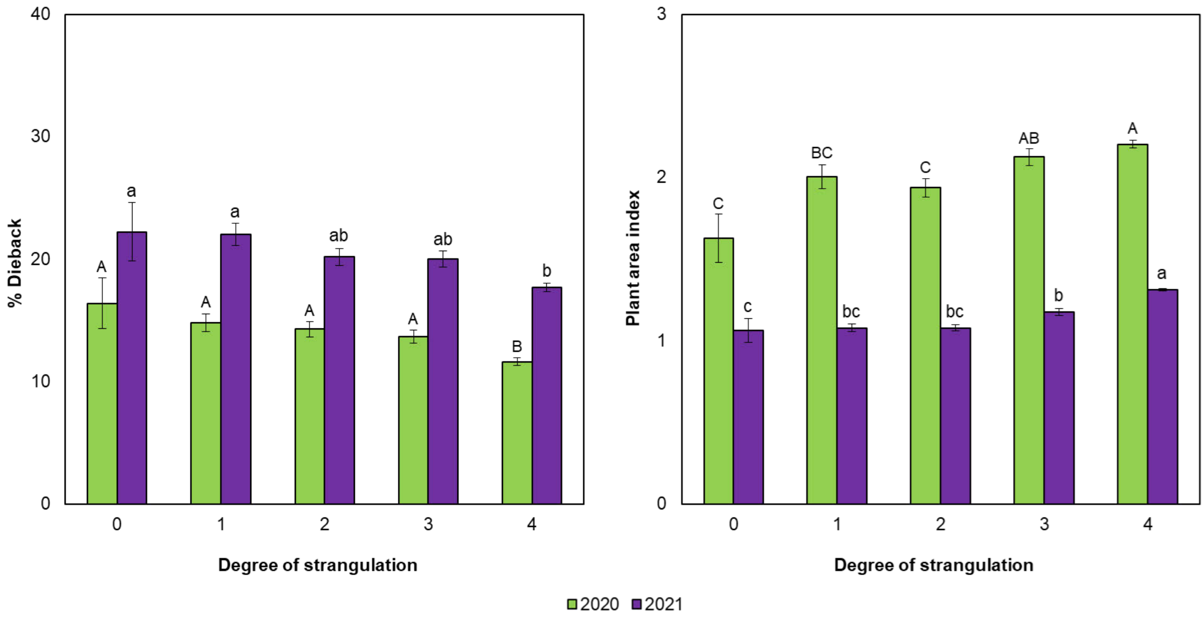

Figure 3.4. Effect of cordon strangulation on cordon dieback and plant area index, PAI. Means of data across sites were separated by ANOVA using Fisher's LSD test at a significance level of $p \leq 0.05$. Bars indicate the standard error. Upper case letters indicate significant differences between degrees of strangulation in 2020. Lower case letters indicate significant differences between degrees of strangulation in 2021.

PAI decreased at all sites between seasons, with an average observed decrease of 0.90 or 42 %. Very severely strangled cordons generated surprisingly high PAI values. Because of the nature by which strangulation may constrict the vasculature of the cordon and interrupt water flow, one would not expect that strangulation would favour growth, especially in the most distal areas of the cordon. In both 2020 and 2021, vines displaying the least amount of strangulation (0, 1, 2) had a significantly lower PAI than those displaying the greatest average degree of strangulation (4) (Figure 3.4). While the assessment of cordon dieback in this trial was based on visual observation and is, therefore, to some extent subjective, measurement of PAI was undertaken with the use of the VitiCanopy app. This app uses upward-facing digital images of canopies captured by a smartphone to implement image analysis algorithms automatically, calculating canopy architecture parameters and thereby providing an objective measurement of PAI. In the results of this trial, high cordon dieback correlated with low PAI, helping to eliminate the possibility of human error during the visual assessments used for the determination of dieback severity. This negative correlation between dieback and PAI is expected given that PAI describes the total one-sided area of plant tissue per unit ground surface area (De Bei et al., 2016), and more dieback equates to the less remaining productive cordon. The positive correlation between the degree of strangulation and PAI is difficult to explain; however, one would typically imagine that vines of advanced age and displaying severe strangulation would be more prone to increased canopy porosity (light penetration through the canopy) and reduced PAI.

The percentage of foliage displaying signs of characteristic symptoms of Eutypa dieback increased at all sites between assessed seasons. In both 2020 and 2021 a trend of an increase in visible Eutypa dieback foliar symptoms was observed with an increase in the degree of strangulation (Figure 3.5). While the presence of characteristic foliar symptoms of Eutypa dieback indicates the presence of the pathogen *Eutypa lata,* the absence of such symptoms does not necessarily mean that a vine is free of infection from *Eutypa lata* or other fungal wood pathogens, as vines may be infected by such pathogens without displaying any symptoms (Sosnowski et al., 2011b). One possible explanation for the increased presence of foliar symptoms observed with an increase in the degree of cordon strangulation is that the effects of strangulation could negatively impact the vines' ability to counter the advance of fungal disease infection. In this case, it is possible that cordons which are already under increased stress from the constrictive effects of tight wrapping may be more likely to express symptoms of infection than those which are trained in a less constrictive manner. It is difficult to assign causality here however, as many factors may be involved in the propensity of infected vines to express visual symptoms (Claverie et al., 2020; Fischer & Ashnaei, 2019; Songy et al., 2019). It has been reported that foliar symptoms of Eutypa dieback are influenced by climatic factors including winter rainfall and spring temperature. Sosnowski et al. (2007) stated that increased symptom expression may be related to increased rainfall during the winter of the year prior to the growing season of interest. Our results differ from this in that winter rainfall (June–July) in the Barossa valley, where most of the sites of this trial were located, was 98.8 mm in 2019 and 69.1 mm in 2020 (Figure 3.2). While in the Adelaide hills, the winter rainfall for 2019 and 2020 was 161.4 mm and 151.8 mm respectively. The percentage of foliage displaying foliar symptoms in this trial increased from an average of 1.46 % in 2020 to an average of 3.24 % in 2021 (Figure 3.5). The same 2007 study suggested that decreased disease incidence may be related to increased spring temperature. Our results agree with this as spring degree days (base 0) were higher in both the Barossa valley and the Adelaide hills in 2020 (1061 and 986) than 2021 (913 and 876). While it is interesting that in this trial foliar symptoms seemed to increase in relation to the degree of strangulation, it is curious that this effect was observed in the absence of increased cordon dieback. This could be related to poor pruning practices, as the number and size of pruning cuts may influence the susceptibility of the cordon to fungal trunk disease infection (Henderson et al., 2020), as well as pruning during wet conditions when spore inoculum is prevalent (Carter, 1957). Additionally, poor pruning decisions may lead to an associated loss of spur positions in the absence of infection (Castaldi, 2016), resulting in a redistribution of reserves and, therefore, a bigger canopy in areas of the cordon that remain productive.

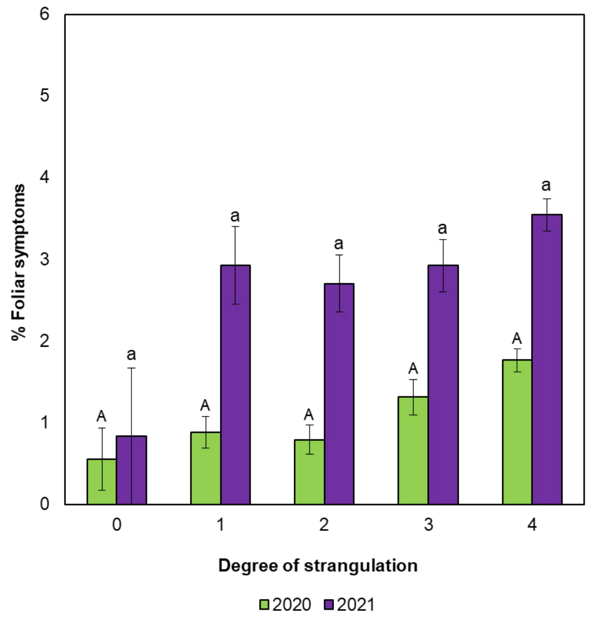

Figure 3.5. Effect of cordon strangulation on incidence of foliage displaying characteristic foliar symptoms of Eutypa dieback. Means of data across sites were separated by ANOVA using Fisher's LSD test at a significance level of $p \leq 0.05$. Bars indicate the standard error. Upper case letters indicate significant differences between degrees of strangulation in 2020. Lower case letters indicate significant differences between degrees of strangulation in 2021.

The argument that increased stress owing to tight wrapping could reduce vine defence response and result in more symptoms of wood disease is to some extent based on the idea that this stress is linked to an increased prevalence of dieback. Other stress-imposing conditions including water deficit have been demonstrated as potentially having such an impact on vine defence response (Songy et al., 2019). In this trial, this did not seem to be the case as there was actually a trend for less dieback observed in cordons displaying the greatest degree of strangulation, an unexpected result that may be at least in part related to pruning practices. Vigour would have also likely played a role in the vine's response to strangulation. Whether tightly wrapping cordons around the cordon wire could influence the susceptibility of vines to fungal trunk disease infection or the likelihood of infected vines expressing symptoms is not immediately clear. Moreover, unclear is the question of whether such an effect could be observed in advance of or absence of visual cordon dieback, a possibility if strangulation was impeding the regular functionality of vascular conduits without being so severe as to cause perennial wood dieback. Further research quantifying the extent to which xylem morphology and functionality are compromised by constrictive pressure in severely strangled cordons could provide further insight into this condition and the effect it could have on vine health and defence response. Micro-CT could be a valuable tool in this regard as it has recently been used to model grapevine graft unions (Milien et al., 2012), the spatial distribution of xylem network connections in cane internodes (Wason et al., 2021), and defective wood suffering from symptoms of black rot, necrosis, and decay (Vaz et al., 2020). Examining esca-diseased Cabernet Sauvignon, Ouadi et al. (2021) reported sap flow measurements were significantly lower in infected plants several weeks before changes in the expression of stress and defence-genes were observed, as well as the appearance of any foliar symptoms. While the restriction of vascular tissue reduces the capacity for sap flow, it may not lead to increased progression of Eutypa dieback as one might expect. Conversely, a study on the effect of water stress on *E. lata* colonisation found that smaller xylem vessel areas and narrower cane diameter were correlated with less colonisation of *E. lata* in water stressed vines (Oswald, 2017). Increased xylem vessel diameter has also been reported to be correlated with greater susceptibility to *Phaeomoniella chlamydospora*, due to less efficient vessel compartmentalisation (Pouzoulet et al., 2017). These results highlight the complexity of the relationship between these different factors of cordon decline and the challenge presented in elucidating their interactions with one another in regard to their impact on vine health. While a negative trend between the degree of strangulation and dieback was observed in this survey, it was not a strong trend. Dieback is a common problem in many older vineyards displaying signs of severe strangulation, and it is logical that such a constriction may be having a negative impact on health and productivity. If vineyard management decisions such as the selection of cordon training method could reduce the likelihood of early cordon decline, then a quantitative analysis of different cordon establishment techniques could provide valuable understanding in regard to this decision-making. A limitation of this study was the absence of additional physiological measurements to attest to the disruptive effect the observed strangulation had on vine function. Future projects could include additional variables, such as water conductivity, as part of the investigation to improve the scope of the research.

Table 3.3. Individual site details for average measurements of degree of strangulation, % of died back cordon, % of canopy displaying typical Eutypa dieback foliar symptoms, and plant area index (PAI) (mean ± std).

Site	Degree of Strangulation	% Dieback 2020	% Dieback 2021	% Foliar Symptoms 2020	% Foliar Symptoms 2021	PAI 2020	PAI 2021
A	2.42 ± 0.95	16.3 ± 7.5	23.5 ± 10.5	1.01 ± 2.69	3.53 ± 6.25	1.14 ± 0.18	0.63 + 0.11
B	1.60 ± 0.84	16.2 ± 9.1	20.3 ± 9.8	0.31 ± 1.53	1.82 ± 4.67	1.46 ± 0.30	1.10 ± 0.21
C	3.55 ± 0.69	15.9 ± 10.7	21.1 ± 12.0	0.99 ± 3.02	2.01 ± 4.88	1.40 ± 0.26	1.30 ± 0.27
D	3.87 ± 0.42	6.9 ± 7.3	13.1 ± 9.1	0.44 ± 2.62	1.48 ± 4.06	1.99 ± 0.33	1.45 ± 0.27
E	3.99 ± 0.08	19.1 ± 11.8	23.7 ± 13.5	3.95 ± 6.49	6.56 ± 9.19	1.91 ± 0.45	1.33 ± 0.28
F	3.29 ± 0.85	17.5 ± 10.7	21.6 ± 12.1	2.46 ± 6.01	2.86 ± 5.65	1.52 ± 0.30	1.40 ± 0.27
G	3.96 ± 0.19	6.0 ± 7.0	12.9 ± 9.7	2.81 ± 5.80	5.41 ± 7.10	2.00 ± 0.29	1.29 ± 0.29
H	2.74 ± 1.12	11.6 ± 8.8	22.1 ± 12.6	0.64 ± 1.82	3.67 ± 6.15	3.11 ± 0.52	1.17 ± 0.22
I	3.02 ± 1.10	11.8 ± 8.7	17.7 ± 11.2	1.25 ± 2.94	2.92 ± 6.04	2.96 ± 0.70	1.12 ± 0.26
J	3.73 ± 0.53	8.1 ± 9.3	14.8 ± 12.3	0.88 ± 3.23	2.43 ± 5.80	3.36 ± 0.67	1.37 ± 0.29

3.4 Acknowledgements

The authors would like to thank the University of Adelaide, as well as Wine Australia, who invest in and manage research, development and extension on behalf of Australia's grape growers and winemakers and the Australian Government. We would also like to express our gratitude to the growers who allowed us access to their vineyards for the conduction of our surveys.

Chapter 4. Prepared Manuscript 1: Is vigour-based length adjustment during permanent cordon establishment a beneficial practice?

Statement of Authorship

Title of Paper	Is vigour-based length adjustment during permanent cordon establishment a beneficial practice?
Publication Status	☐ Published ☐ Accepted for Publication ☐ Submitted for Publication ☑ Unpublished and Unsubmitted work written in manuscript style
Publication Details	

Principal Author

Name of Principal Author (Candidate)			
Contribution to the Paper	Conceptualisation, methodology, formal analysis, data curation, writing—original draft preparation, writing—review and editing		
Overall percentage (%)	90		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	25/09/2022

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);
ii. permission is granted for the candidate in include the publication in the thesis; and
iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Cassandra Collins		
Contribution to the Paper	Conceptualisation, methodology, writing—review and editing, supervision		
Signature		Date	26/09/2022

Name of Co-Author	Roberta De Bei		
Contribution to the Paper	Conceptualisation, methodology, writing—review and editing, supervision		
Signature		Date	26/09/2022

Is vigour-based length adjustment during permanent cordon establishment a beneficial practice?

Abstract: Low vigour canes selected for the establishment of permanent cordon arms may lack the sufficient vigour required for uniform budburst and growth of new shoots following cordon training. This may lead to stunted or missing spur positions, particularly in the middle of new cordon arms where the effect is most pronounced due to the prioritisation tendencies of the vine including apical dominance and acrotony. A trial was performed to investigate the benefits of adjusting the length of newly trained canes intended as permanent cordon arms during their establishment to limit their bud number and guide new growth. This length adjustment was based on an assessment of the apparent vigour of selected canes and was performed at the start of the first season of cordon growth, with cordons then extended to their final length. The trial did not yield results indicating a long-term beneficial response to this practice, with physiological measurements including pruning weight showing no difference between length adjusted and control vines in the later seasons of the trial. There was also a lower plant area index (PAI), and higher canopy porosity (Φ) observed in length adjusted vines compared to control vines at several points. There was no difference observed in circumference measures of the distal portion of arms which had undergone a length adjustment, suggesting that the exercise did not have an adverse impact on their capacity for transport and reserve storage. Harvest yield components did not vary with treatment; however, a significantly lower pH was observed in length adjusted vines compared to control vines in the trials final season. Further research could help to provide more insight into the benefits of this practice, as some results from the trial, including a significantly higher pruning weight, cane number, and cane weight observed in the intermediate sections of cordon arms during the first season of growth suggest that it may have been of some aid to the cordons on which it was implemented.

4.1 Introduction

The management of grapevine vegetative growth is a key component of modern viticulture and the concept of vegetative 'vigour' is now well understood by most growers. Loosely recognised as the quality or condition inherent in the expression of rapid growth of vegetation biomass (Winkler et al., 1974), it can be measured in terms of both the speed and quantity of said growth. There are many factors which may influence vine vigour including soil profile (Hubbard et al., 2021), nutrient availability (Balachandra et al., 2009), ground cover (Muscas et al., 2017), water availability and/or irrigation regime (Dry & Loveys, 1998), temperature and sunlight exposure

(Hugalde et al., 2020), and pruning technique (Wang et al., 2019). It is a crucial element in maintaining vineyard balance, i.e. the sought-after equilibrium between desired vegetative and reproductive seasonal growth. While in this context it is important in the ongoing management of long-established vineyards, its control is arguably even more important in the early stages of vineyard establishment. Many practitioners believe that thin and short shoots do not make a very good framework for newly trained vines as their growth is likely to be weak and lacking in uniformity (Simonit, 2019). On the other hand, overtly long and excessively thick internodes may not be a better alternative as they can impede the selection and formation of evenly spaced spur positions and general cordon architecture. Careful selection of canes is paramount during the establishment process as those which are retained will grow to become the permanent structures of the vine including the trunk and cordon arms. Logically, cane health is an important consideration in this regard as the utilisation of unhealthy canes may compromise productivity and the health and longevity of perennial wood (Boehm & Coombe, 1992). Node location and directionality is another important factor driving this decision making (Simonit & Sirch, 2009a). A ‘v-zone’ may be formed at the head region of bilaterally trained permanent cordons by originating the cordon arms from a node position located at a desirable distance below the cordon wire. This may help to alleviate foliar disease pressure in the area, especially in high vigour situations where the canopy may be particularly dense. As such, canes intended as permanent cordon arms are often selected on the basis of their proximity to the cordon wire and their direction of growth. During the establishment process, it is common to trim back the growth of one or two canes selected as suitable candidates to develop the trunk to just below cordon wire height after the first season of growth (Simonit & Sirch, 2009a). There is a risk however with this method of obtaining shoots with very long internodes, and it may be advisable to allow several shoots to develop from the top of the new trunk to limit vigour and internode length (Castaldi, 2016). Another common strategy, as the grower may prefer, is to trim all growth right back to ground level, retaining as little as two nodes, with the goal of increasing uniformity across vines during the next growing season.

The desire for between vine uniformity extends to the establishment of cordon arms. Whether using a unilateral or bilateral training system, after canes have been selected as new arms, they must be trained and secured to the cordon wire. In some heavily mechanised regions, this training may involve wrapping the cordon tightly around the cordon wire to increase stability or for expense reduction (Caravia et al., 2015b). The length of cordon arms is typically based on intra-row vine spacing, as any gaps without productive cordon equate to loss in potential yield and profit (Van Zoeren et al., 2020). When securing new arms to the cordon wire, technicians will often cut canes to a length of approximately half of the distance between trunks in the case of bilaterally trained cordons and close to the whole distance between trunks in the case of unilaterally trained cordons. This leaves the distal end of each cordon in close proximity to the cordon of the next vine in the hedgerow sequence, avoiding costly gaps with no productive cordon. The advantage of this method is that it gets the entirety of the desired length of the new cordon into place immediately, and in the case of healthy vines, normally requires minimal oversight after this initial training. An issue that may arise with this method however is that if canes selected as permanent cordon arms are weak and small in diameter, they may lack the vigour needed to support uniform budburst and growth of new shoots (Klodd & Clark, 2020; O'Brien et al., 2021). The impact of this is especially evident in the node positions in the centre of low vigour arms as grapevine shoots display apical dominance (Fournioux, 1998), as well as having an acrotonic tendency to prioritise branching in the shoot distal zone (Torregrosa et al., 2021). Sometimes less than ideal canes may be selected for establishment by necessity due to a lack of more suitable alternatives. In this case, due to a lack of sufficient vigour, some buds may fail to burst at all, even if the process of blinding is undertaken (removing buds at undesirable node positions to guide new growth). On a weaker cane, internodes can appear shorter, and as such additional nodes may need to be de-budded to ensure evenly, well-spaced spur positions. An early shoot thinning is one strategy which can help to improve uniformity in the length and diameter of new shoots along cordons suffering from low vigour in this scenario (Simonit et al., 2012), as the rate of shoot growth tends to increase as the number of shoots per plant decreases (Keller et al., 2015).

A technique promoted by some practitioners entails adjusting the length of low vigour canes during their establishment as permanent cordon arms, by cutting them back to a shorter length and thereby limiting retained node number (Klodd & Clark, 2020; O'Brien et al., 2021). The decision of whether this length adjustment is warranted is based on visual assessment, taking into account cane length and diameter, as well as node number. Low vigour canes are then cut back in length, with the number of nodes retained based on the apparent vigour of the cane. In this case, canes deemed to have sufficient vigour to support uniform growth are cut to full length (i.e. ending in close proximity to the next cordon). The hope is that by adjusting the length of canes intended as new arms and limiting the number of nodes retained, successful budburst may be encouraged as well as the growth and the development of shoots that are of greater diameter and are better suited for selection as permanent spur positions (Castaldi, 2016). Canes that receive a length adjustment may then be brought to full length during the course of the growing season, by selecting an ideally downward facing shoot from the distal portion of the cane, near the cutting point, and training it horizontally along the cordon wire. This selection and extension could also take place during winter pruning. While this method may require some attention to secure the extension as it grows to full length, the desired outcome is a healthier, more productive cordon (O'Brien et al., 2021). This study aimed to quantify the impact of such a length adjustment on cordon arms in the seasons immediately following establishment.

4.2 Materials and Methods

4.2.1. Experimental site

The trial was set up at a newly planted (3-year-old) vineyard site in Williamstown, South Australia (34°40'21.4"S 138°53'27.0"E) in the spring of 2018. The cultivar was Cabernet Sauvignon (WA Cape Selection), grafted onto 1103 Paulsen, and planted at 3 m x 2 m inter-row and intra-row spacing with a north–south row orientation. Each row had a single foliage wire located 12 cm above the cordon wire, for the use of a sprawl canopy system. The total amount of irrigation discharged was ~1.0 ML/ha during each growing season of the trial (2018-2022). The climatic conditions for the site were sourced from the nearest Australian Bureau of Meteorology (http://www.bom.gov.au/, accessed on 28 May 2022) weather stations located in Williamstown (station number 023752) and Mount Crawford (station number 023878).

4.2.2. Experimental design

The trial consisted of 78 randomly selected vines which were low-to-medium vigour. Thirty-nine of these vines received a length adjustment (LA), where the vigour of canes selected as permanent cordon arms was assessed and then the lengths of these canes were adjusted based on their apparent vigour. Thirty-nine control vines (C), received no length adjustment. The canes of control vines were left as long as possible, training them to full length immediately at the time of their initial training if their existing length and health allowed for it (Figure 4.1). The assessment of how many nodes to retain during the adjustment of LA vines was based on a visual assessment of apparent vigour. The criteria for this approximation of vigour was cane length, diameter and node number. Where possible, for canes which received a length adjustment, one of the shoots from the most distal portion of the developing arm was selected and trained along the cordon wire horizontally during the growing season to extend arms to their final length.

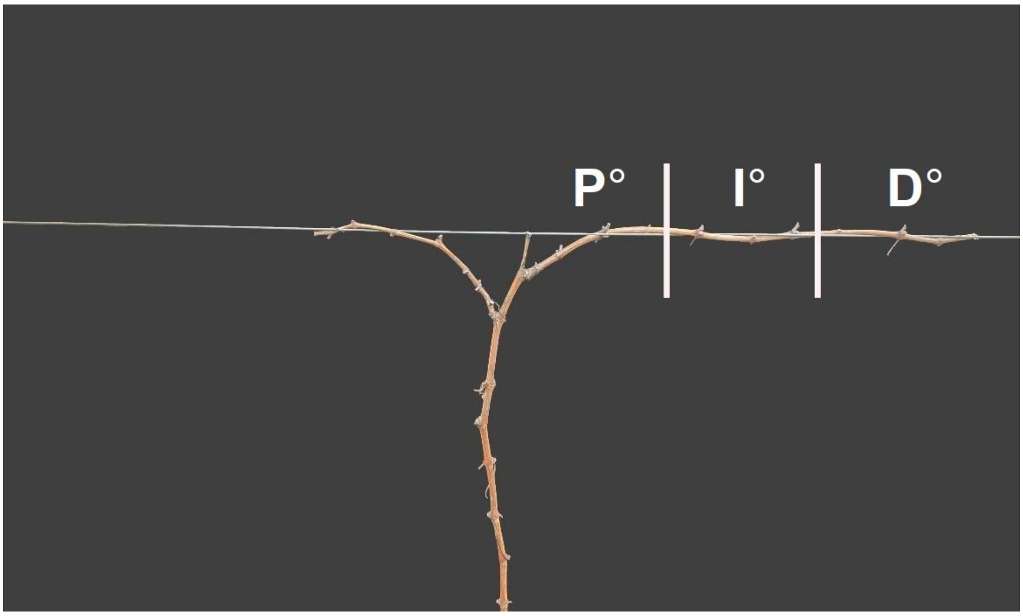

Figure 4.1. An example of a newly trained vine which received a length adjustment. The cane on the left was deemed to be of insufficient vigour to support uniform growth and was cut back to a more limited retained node number. The cane on the right was deemed to be of sufficient vigour, and was cut to full length. The delineation between arms sections Proximal (P°), Intermediate (I°), and Distal (D°) are indicated on the right arm.

Full length cordons comprised approximately 10 spur positions on each arm, pruned to typical 2-node spurs over the following seasons. The final step of the cordon extension process for length adjusted vines took place at pruning, when selected canes were cut to final length and secured to their training system. In the case of vines which received a length adjustment on a single arm (as in Figure 4.1), only the arm which had been adjusted was considered for analysis. Shoot thinning was performed in the spring of 2018-2020 when newly burst shoots were 5-10 cm, but was not performed in 2021. During this process, shoots originating from the base of spurs, multiple shoots originating from the same node, and shoots originating from non-spur positions including the v-zone or trunk were cleanly removed.

4.2.3. Vegetative growth

Images were taken with the front camera of an iPhone (Apple, Cupertino, CA) twice during the 2018-2019 growing season and three times during each following growing season for the purpose of measuring canopy architecture using the VitiCanopy App (De Bei et al., 2016). One image was taken of each cordon arm from about 80 cm below the vine cordon for the algorithmic assessment of plant area index (PAI).

Arms were split into three sections, proximal (P°), intermediate (I°) and distal (D°) (Figure 4.1), and pruning weights were measured for each individual arm section. Section boundaries were determined according to expected total arm length, rather than existing arm length, and comprised 3-4 spur positions each. Cordon circumference was measured at three points (P°, I°, D°) along the arms of each vine using a flexible measuring tape at the time of pruning.

4.2.4. Harvest maturity/yield components

Vines were hand harvested each year and bunch number and total yield per vine were recorded. Total yield was measured with a digital scale. Cordon length was measured at the time of harvest so that yield and its components could be determined on a per metre basis. From yield and bunch number, the average bunch weight was calculated. Five bunches were collected from each vine and were stored at 4 °C for further lab analysis in the days immediately following harvest. 50 berries were randomly collected from these bunch samples and weighed for determination of average berry weight. Bunch samples were then hand crushed in plastic bags with the juice then being collected in 50 mL tubes and centrifuged at 5000 rpm for 5 min (Hettich Universal, Tuttlingen, Germany) before total soluble solids (TSS), pH and titratable acidity (TA) were measured according to (Iland et al., 2004), using an automatic titrator (G20S Compact Titrator, Mettler Toledo, Thebarton, Australia) and a digital refractometer (BRX-242 Erma Inc. Tokyo, Japan).

4.2.5. Physiological

One-year-old cane samples were collected from distal cordon arm sections during pruning for analysis of carbohydrate concentration. Samples consisting of nodes three and four and their interjoining internode were collected from canes originating from the basal node of each 2-node spur from the previous growing season. All samples were stored at 4 °C until further processing. Sections approximately 1 cm long were cut from the centre of each internode sample using secateurs and then combined and powderised using a mechanical grinder (A11 basic, IKA, Germany). A commercial enzyme assay kit (Total starch assay kit, Megazyme, Ireland) was used to analyse starch levels following the method described in Edwards et al. (2010) based on colorimetric assay. Using a spectrophotometer (Multiskan Spectrum, model 00300011, Thermo Electron Corporation, Vantaa, Finland) absorbances were read at 505 nm and starch content was determined using a glucose standard curve. For the analysis of sugar concentration, an anthrone assay was performed with absorbances then read at 600 nm and concentration determined using a fructose standard curve.

4.2.6. Reproductive

Cane samples were collected during winter dormancy in 2020 and 2021 for the purpose of microscopically dissecting buds for examination of bud fertility and the incidence of primary bud necrosis (PBN). Samples were collected from the proximal and distal portions of cordon arms and consisted of nodes 1-4 of canes originating from node two of a 2-node spur. 18 samples were considered per treatment. A razor blade was used to slice buds transversally using the methods described by (Rawnsley & Collins, 2005). A light microscope at 25x magnification (Model EZ4W, Leica, Heerbrugg, Switzerland) was used during this process to assess the number of inflorescence primordia (IP) in the primary bud of each compound bud. If the primary bud was necrotic, the largest secondary bud was assessed in its place based on the assumption that it would grow in compensation for its loss.

4.2.7. 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.

4.3 Results

The average November monthly temperature of 2020 was hotter than the other growing seasons, followed by an average December monthly temperature that was cooler than the other growing seasons (Figure 4.2). 2021 was the wettest year of the trial, with July in particular being a very wet month having 167.2 mm of rain.

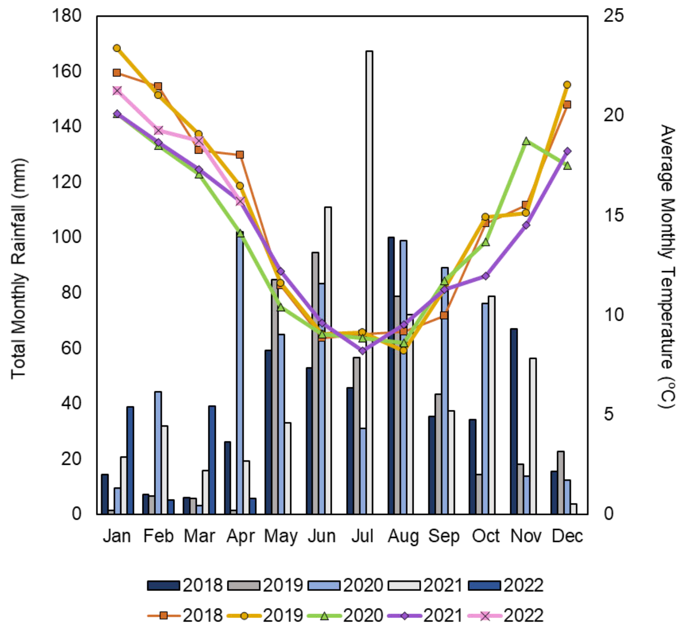

Figure 4.2. Average monthly temperature and rainfall calculated from 2018–2022 at the trial site. Climate data were sourced from the nearest Australian Bureau of Meteorology (http://www.bom.gov.au/ accessed on 11/07/2022) weather stations located in Williamstown (station number 023752) and Mount Crawford (station number 023878).

No difference was observed in plant area index (PAI) between length adjusted and control vines in the 2018-2019 growing season (Figure 4.3). Length adjusted cordons had significantly lower PAI values than control cordons on two imaging dates in the 2019-2020 growing season, one date in the 2020-2021 season, and two dates in the 2021-2022 season.

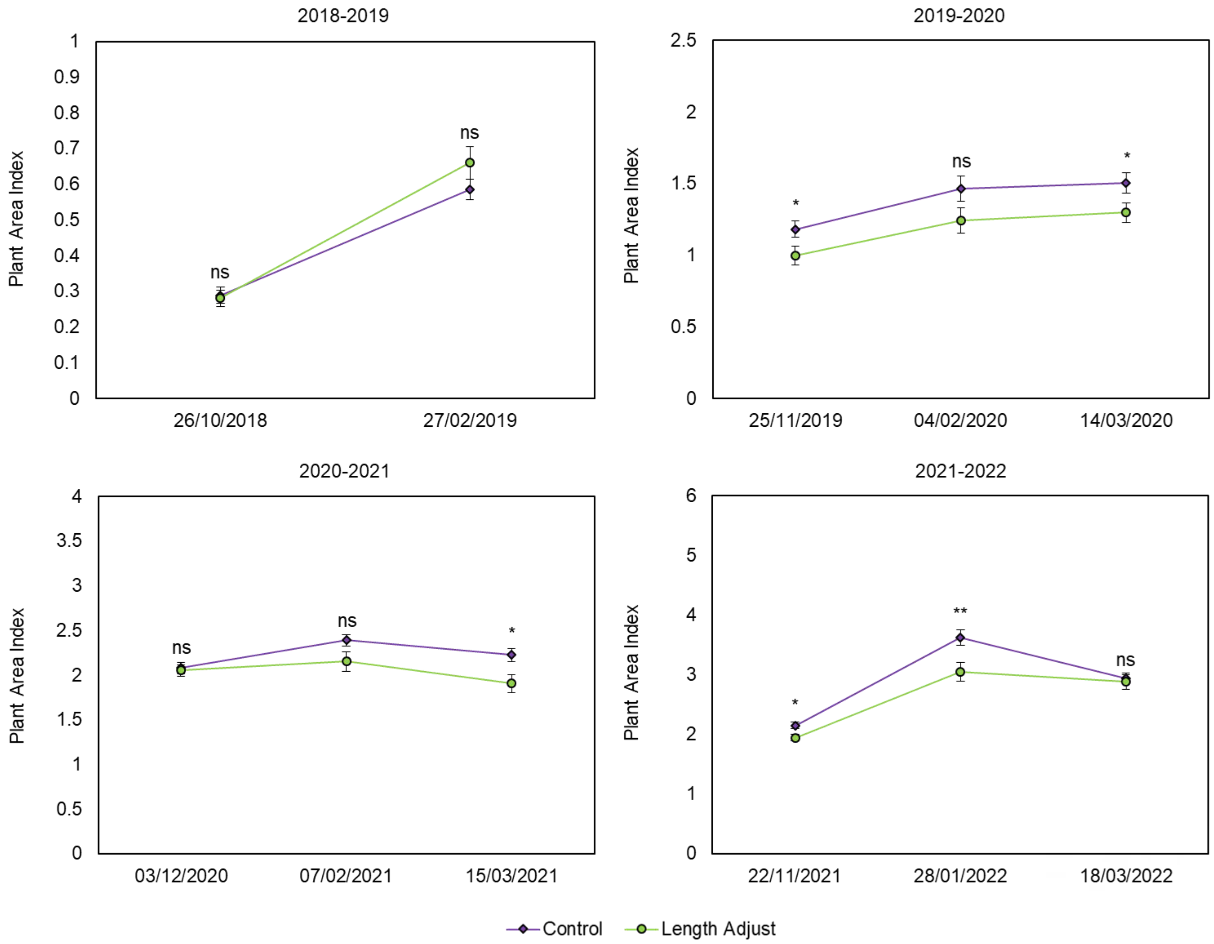

Figure 4.3. Plant area index (PAI) measured periodically throughout the growing seasons from 2018-2022 using the VitiCanopy app. Means were assessed using ANOVA. *, ** indicate significant differences at $p \leq 0.05$ and 0.01. ns = not significant.

Near the end of the first growing season, canopy porosity was found to be significantly lower in vines that had been length adjusted than those that had not (Figure 4.4). In the second year, no difference was observed in porosity on any imaging date. In the third year, canopy porosity was higher in vines that had been length adjusted at both veraison and harvest, but not at the start of the growing season. In the fourth year, porosity was higher in length adjusted vines at veraison only.

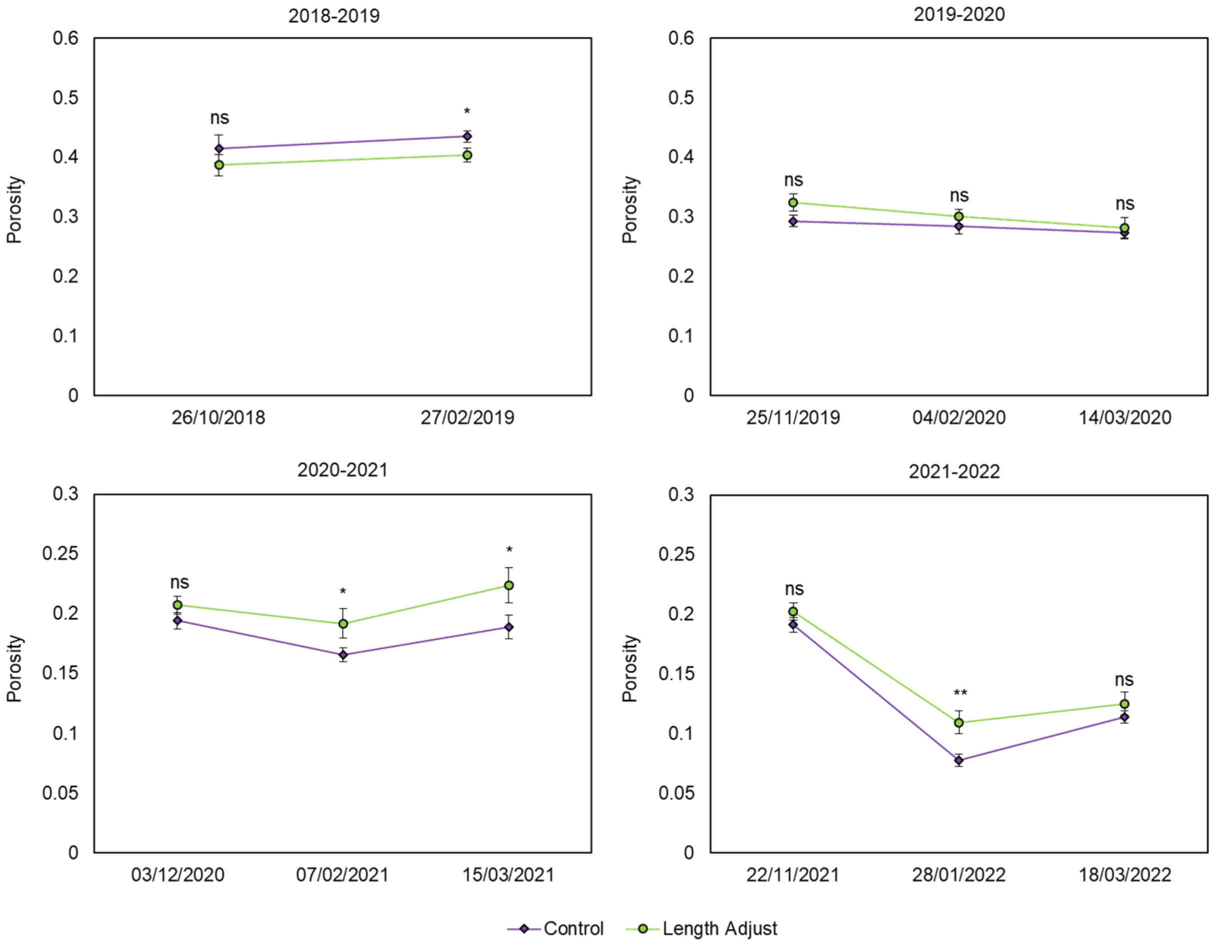

Figure 4.4. Canopy porosity (Φ) measured throughout the growing seasons from 2018-2022 using the VitiCanopy app. Means were assessed using ANOVA. *, ** indicate significant differences at $p \leq 0.05$ and 0.01. ns = not significant.

No significant difference was observed between the circumferences of the proximal, intermediate, or distal arm sections of cordons which received a length adjustment and control cordons in any year (Figure 4.5).

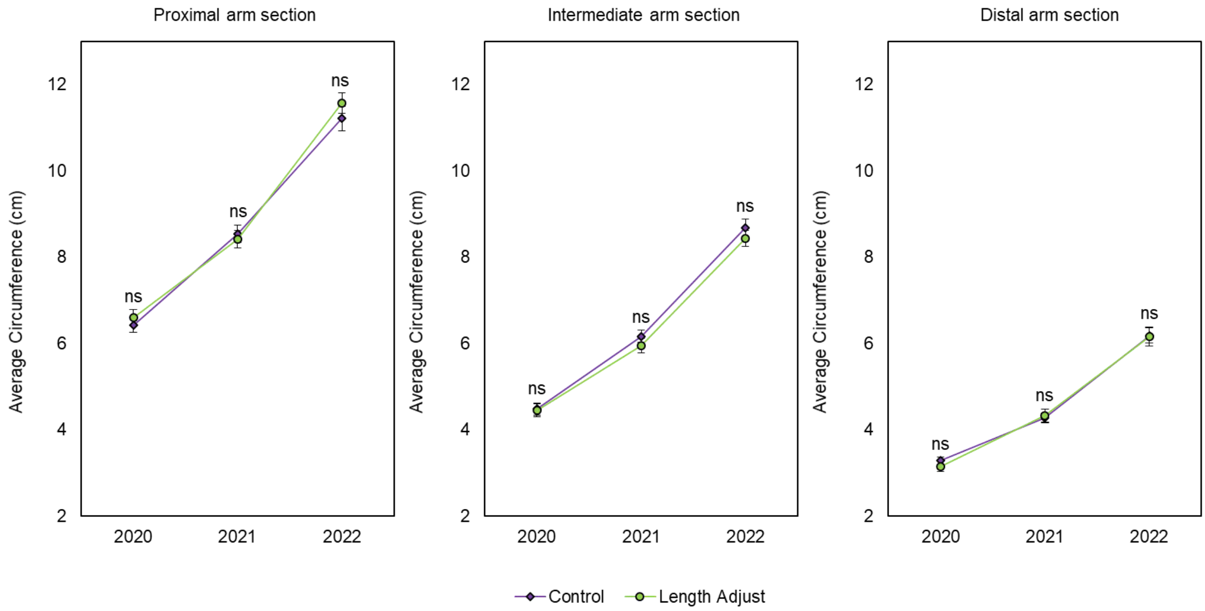

Figure 4.5. Average circumference measurements of proximal, intermediate, and distal arm sections from 2020-2022. Means were assessed using ANOVA ($p \leq 0.05$). ns = not significant.

In 2019, the intermediate arm section of cordons which had undergone the length adjustment had significantly higher pruning weights than control cordons when considered on a per metre basis (Table 4.1). No difference was observed between the pruning weights of the intermediate arm sections in any following year. No difference was observed between the pruning weights of the proximal or distal arm sections in any year. No difference was observed in pruning weights in any year on a whole vine basis.

Table 4.1. Average pruning weight from each individual arm section and from total vines (mean ± std).

Year	Treatment	Proximal Pruning Weight (kg/m)	Intermediate Pruning Weight (kg/m)	Distal Pruning Weight (kg/m)	Total Pruning Weight (kg/m)
2019	Control	0.20 ± 0.10	0.15 ± 0.08	0.31 ± 0.27	0.26 ± 0.16
	Length Adjust	0.20 ± 0.08	0.38 ± 0.16		0.38 ± 0.16
	p value	ns	<0.0001		ns
2020	Control	0.84 ± 0.30	0.45 ± 0.24	0.56 ± 0.25	0.63 ± 0.21
	Length Adjust	0.69 ± 0.31	0.47 ± 0.20	0.55 ± 0.25	0.57 ± 0.17
	p value	ns	ns	ns	ns
2021	Control	1.07 ± 0.42	1.01 ± 0.49	1.28 ± 0.54	1.12 ± 0.37
	Length Adjust	0.97 ± 0.33	0.83 ± 0.23	1.17 ± 0.40	0.99 ± 0.20
	p value	ns	ns	ns	ns
2022	Control	2.09 ± 1.20	1.70 ± 0.82	2.51 ± 1.38	2.11 ± 1.00
	Length Adjust	1.84 ± 1.31	1.58 ± 1.04	2.17 ± 1.58	1.88 ± 1.19
	p value	ns	ns	ns	ns

Means were separated by ANOVA ($p \leq 0.05$). ns = not significant.

No difference was observed in the number or weight of canes for the proximal arm section in any year (Table 4.2). In 2019, the intermediate arm sections of cordons which had received a length adjustment had significantly more canes per metre than control cordons. The average weight of these canes was also higher than that of the control. No difference was observed in the number or weight of the canes of the intermediate arm section in any following year. In 2020, the distal sections of control cordons had significantly more canes per metre than control cordons. No difference was observed in the number of distal canes in any following year, or in cane weight in any year. On a whole vine basis, length adjusted vines had significantly (p = 0.040) more canes per metre compared to control vines in 2019; 14.0 and 12.0 respectively. No difference was observed in the number of canes per metre on a whole vine basis in any following season. No difference was observed in cane weight considered on a whole vine basis in any season.

Table 4.2. Average number of canes and cane weights from each individual arm section (mean ± std).

		Proximal		Intermediate		Distal	
Year	Treatment	Cane no. (no./m)	Cane Weight (g)	Cane no. (no./m)	Cane Weight (g)	Cane no. (no./m)	Cane Weight (g)
2019	Control	10.5 ± 2.8	20.9 ± 12.6	10.8 ± 3.6	16.4 ± 11.8	12.4 ± 3.5	24.2 ± 16.5
	Length Adjust	10.8 ± 3.8	22.6 ± 17.1	13.9 ± 3.2	27.7 ± 10.6		
	p value	ns	ns	0.027	0.014		
2020	Control	16.3 ± 2.6	51.7 ± 16.0	12.4 ± 3.7	35.1 ± 10.6	14.4 ± 2.9	37.9 ± 14.7
	Length Adjust	15.9 ± 3.6	42.3 ± 15.3	13.5 ± 3.8	34.6 ± 10.6	11.0 ± 2.9	49.1 ± 16.7
	p value	ns	ns	ns	ns	0.004	ns
2021	Control	18.3 ± 4.3	58.3 ± 15.4	20.0 ± 3.9	49.6 ± 19.1	22.0 ± 4.3	56.9 ± 19.1
	Length Adjust	18.0 ± 3.2	54.2 ± 17.6	20.5 ± 4.2	40.8 ± 9.8	21.9 ± 2.9	53.0 ± 16.5
	p value	ns	ns	ns	ns	ns	ns
2022	Control	26.3 ± 8.5	84.2 ± 55.8	23.3 ± 4.7	74.4 ± 36.4	30.4 ± 5.4	83.3 ± 46.5
	Length Adjust	23.7 ± 6.5	77.8 ± 57.7	24.8 ± 6.3	64.4 ± 41.7	28.9 ± 4.9	71.0 ± 44.0
	p value	ns	ns	ns	ns	ns	ns

Means were separated by ANOVA ($p \leq 0.05$). ns = not significant.

The concentration of sugar in cane samples collected from the distal portion of cordons which received a length adjustment was lower than control vines in 2021, but not in 2020 (Figure 4.6). No difference was observed in the amount of starch or total non-structural carbohydrates in any year of the study.

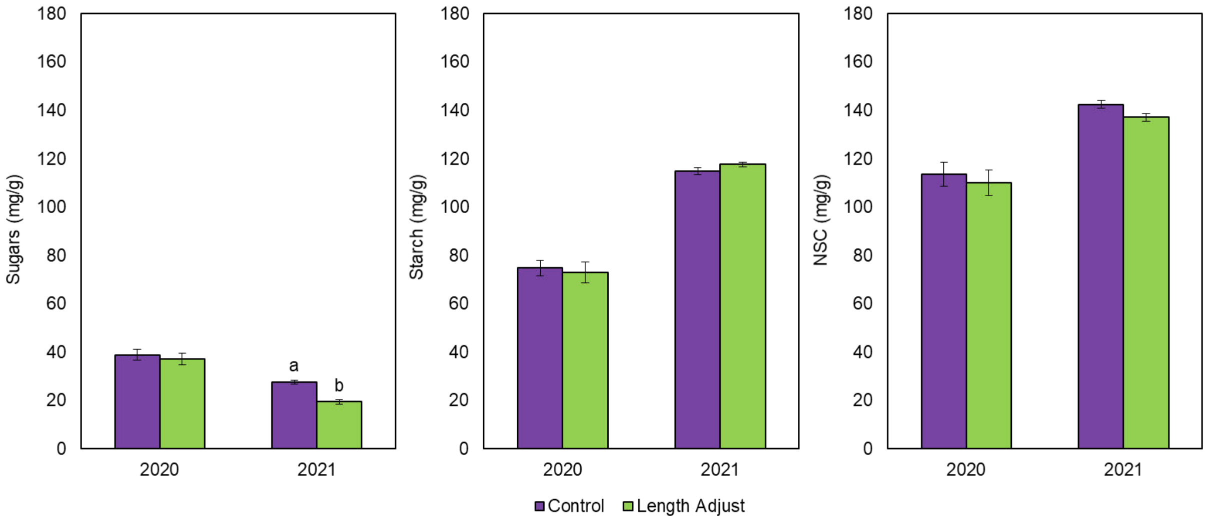

Figure 4.6. Average concentration of sugars, starch, and total non-structural carbohydrates (NSC) in cane samples collected from the distal portion of new cordon arms. Means were separated by ANOVA ($p \leq 0.05$), and different letters indicate significant differences between concentrations within each season of assessment.

No difference was observed in the number of inflorescence primordia in compound buds collected from both the proximal and distal sections of cordon arms in either 2020 or 2021 (Figure 4.7). No difference was observed in the number of buds with primary bud necrosis (PBN).

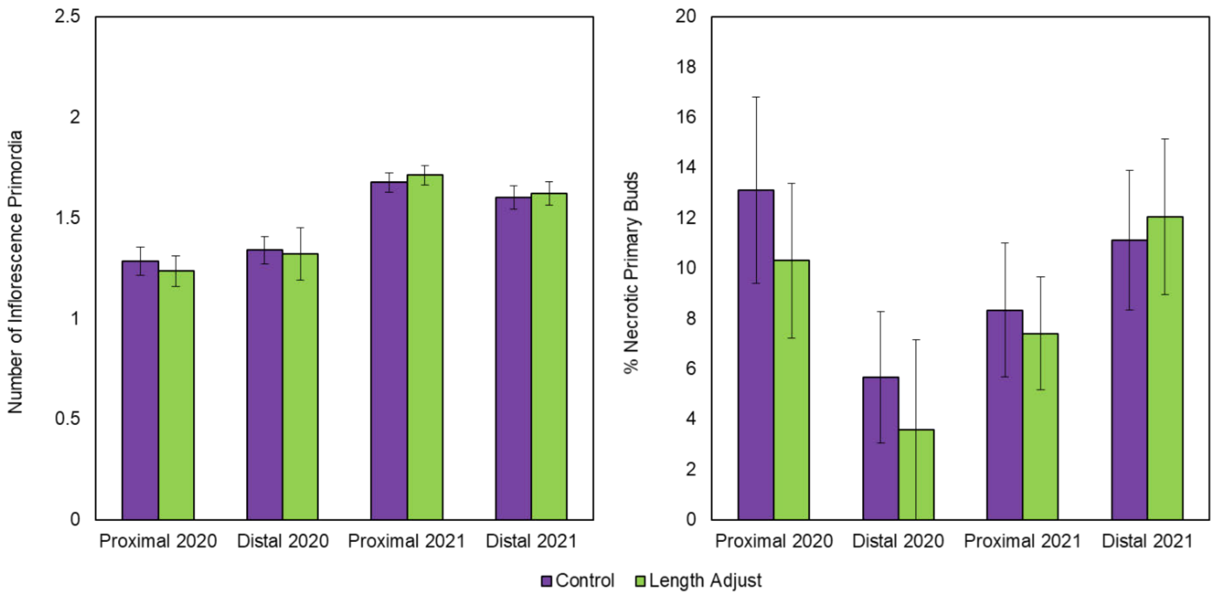

Figure 4.7. Average number of inflorescence primordia within the compound bud and % of compound buds with a necrotic primary bud. Means were separated by ANOVA ($p \leq 0.05$) and differences were found to be non-significant.

Yield and yield components did not vary significantly between control vines and length adjusted vines in any year (Table 4.3).

Table 4.3. Effect of length adjustment on yield and yield components (mean ± std).

Year	Treatment	Yield (kg/m)	Bunch no. (no./m)	Bunch Weight (g)	Berry Weight (g)
2020	Control	1.4 ± 0.4	23 ± 7.8	65.1 ± 28.8	0.65 ± 0.07
	Length Adjust	1.4 ± 0.4	21 ± 6.3	70.1 ± 26.6	0.70 ± 0.19
	p value	ns	ns	ns	ns
2021	Control	3.7 ± 0.8	39 ± 7.1	95.0 ± 16.1	0.80 ± 0.08
	Length Adjust	3.4 ± 0.9	37 ± 7.6	94.6 ± 16.4	0.89 ± 0.27
	p value	ns	ns	ns	ns
2022	Control	3.9 ± 1.5	47 ± 13.7	81.7 ± 14.2	0.95 ± 0.06
	Length Adjust	3.5 ± 1.0	46 ± 10.3	76.2 ± 22.3	0.97 ± 0.22
	p value	ns	ns	ns	ns

Means were separated by ANOVA ($p \leq 0.05$). ns = not significant.

No difference was observed in total soluble solids or TA between treatments in any year (Figure 4.8). pH was significantly lower in grapes harvested from length adjusted cordons in 2022.

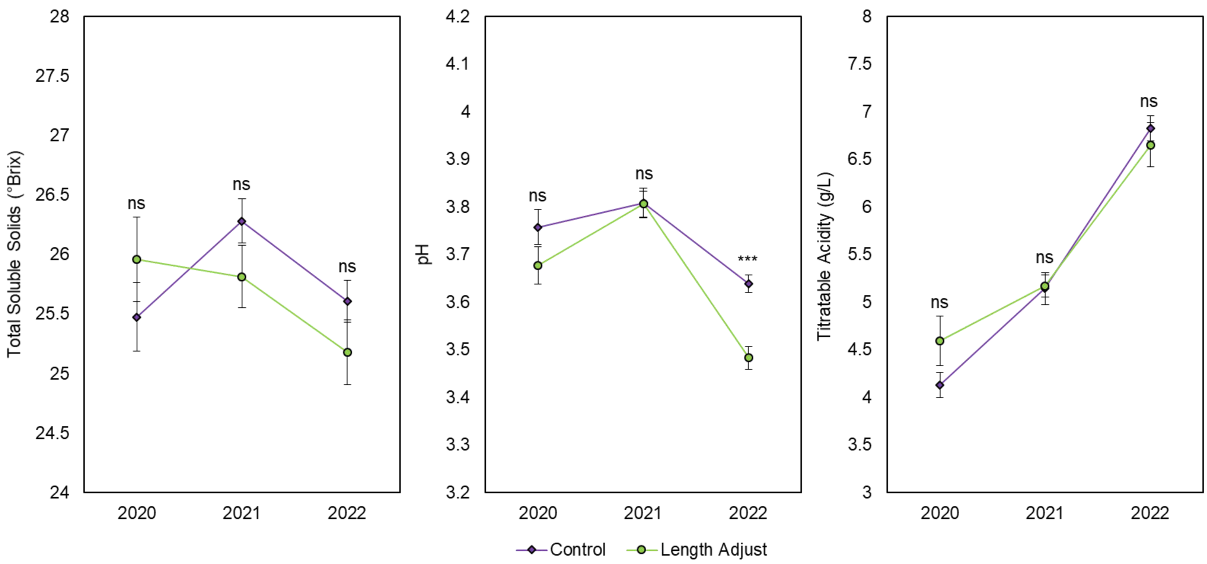

Figure 4.8. Harvest measurements including total soluble solids (TSS), pH, and titratable acidity (TA). Means were assessed using ANOVA. *** indicates significance at $p \leq 0.0001$. ns = not significant.

4.4 Discussion

Physiological measures of vegetative growth were used as a means of assessing the impact of the length adjustment treatment on the health and productivity of new cordon arms. Plant area index (PAI), describing the total one-sided area of plant tissue per unit ground surface area (De Bei et al., 2016), was significantly lower for length adjusted vines than control vines on five out of 11 imaging dates over the course of the 4-year study. While this suggests that length adjusted vines may have suffered from a reduction in vegetative growth rate compared to control vines, other measures indicated that this may have not been the case. The PAI of length adjusted vines was no lower than control vines on either of the imaging dates during the first season of growth, a crucial period of the cordon establishment process. Canopy porosity (Φ) was also lower in length adjusted vines than control vines when imaged near the end of the first season. No difference was observed in average circumference measurements of the proximal, intermediate, or distal arm sections in any year. This includes the winter of 2020, where one might have reasonably expected the distal arm sections of adjusted cordons to be of smaller circumference than control cordons, as they were comprised strictly of 2-year-old wood rather than 3-year-old wood as was the case with medium vigour control cordons which were trained to full length immediately.

After one growing season, there was a significantly higher number of canes observed originating from the intermediate portion of arms which had undergone a length adjustment (13.9 canes/m) compared to those which had not undergone a length adjustment (10.8 canes/m). The increased number of intermediate canes observed as a result of the length adjustment is an indicator of the success of the treatment in providing many suitable candidate canes for selection for the use of extending the cordons to their final length. It also shows that many of the intermediate nodes burst and grew successfully during the first growing season, likely as a consequence of the reduced node count of adjusted arms (Keller et al., 2015). Canes originating from this arm section were also found to be heavier on average in cordons which had received a length adjustment (27.7 g) compared to control cordons (16.4 g) after the first season. This resulted in an average pruning weight of intermediate arms sections which was 153.3% higher for length adjusted (0.38 kg/m) than control (0.15 kg/m) cordons. This increase in the pruning weight of length adjusted vines correlated with the lower porosity observed in length adjusted vines at the end of the first growing season, suggesting that the treatment was successful in encouraging vegetative growth in the early stages of development. No other differences were observed in pruning weights on an individual arm section or whole vine basis in any year of the study.

After the second growing season, significantly less canes were observed in the distal portion of length adjusted canes (11.0 canes/m) compared to control canes (14.4 canes/m). This is logical when one considers that the distal portion of newly extended arms did not yet have fully formed spur positions. In this case, fruit bearing canes originated directly from the nodes of the extended cordon rather than spurs and therefore typically had one cane per spur position rather than two canes. Many of the medium vigour control vines had the entirety of the length of their arms in place during the initial training and as such had distal 2-node spurs at the time of this measure. Lower vigour control vines, while left as long as possible during the initial training, required an extension themselves to be brought to full length, albeit an extension of less node length than had they undergone the length adjustment treatment. Despite LA cordons having less distal shoots, there was no difference observed in the pruning weights of the distal arm sections at the end of the second year. By the end of the third year, 2-node spurs were present in the distal section of all cordons and no difference was observed in the number of distal canes in 2021 or 2022. There was also no difference observed in the number of canes, or the weight of canes originating from proximal or intermediate arm sections in 2021 or 2022. This correlated with pruning weight, where no difference was observed from 2020-2022 for any arm section or for whole vines. On a whole vine basis, length adjusted vines had significantly more canes per metre compared to control vines in 2019 but not in the following seasons. This was driven by the large number of intermediate canes observed in length adjusted vines. In the case of low vigour cordons, it is the intermediate (middle) arm section where one would expect to see the greatest detrimental effects including missing or stunted growth. As such, it makes sense that the pruning weight of the intermediate arm sections (0.15 kg/m) of control cordons was lighter than proximal (0.20 kg/m) and distal arm sections (0.31 kg/m) in 2019.

Yield and yield components did not vary between control vines and length adjusted vines in any year of the trial, suggesting that the treatment did not have a noticeably positive or negative effect on vine productivity. Bunch number and bunch weight varied between years, with no apparent pattern observed between length adjusted and control vines. Bearer number was similar between length adjusted and control cordons, as there was no difference in cane numbers from 2020-2022. The differences observed in PAI and Φ between LA and C cordons on several imaging dates could have had implications on canopy shading and the light environment of the fruit zone, an important parameter for fruit ripening (Jackson & Lombard, 1993; Kliewer & Smart, 1989; Sun et al., 2017). However, no difference was observed in TSS in any year of the study. This suggests that although length adjusted vines had apparently lower PAI and higher Φ values during the ripening periods of 2021 and 2022, this did not have an impact on grape sugar accumulation. In addition to affecting the light environment of the fruit zone, leaf area also determines the capacity of the vine for photosynthesis (Poni et al., 2006). The implication of this is that differences in PAI may indicate differences in source/sink balance both in terms of carbon available for the accumulation of grape sugar and competitory vegetative growth (Kliewer & Dokoozlian, 2005; Ollat & Gaudillere, 1998). While no difference was observed in TA in any season, pH was much lower in grapes from length adjusted vines compared to control vines in 2022, but not in previous years.

Very minimal differences were observed in the reserve status of cane samples collected from the distal portion of cordon arms. There was lower sugar found in samples from length adjusted cordons in 2021, but when considered with starch, no difference in the amount of total non-structural carbohydrates (NSC). In 2020, there was no difference observed in the levels of sugars, starch, or NSC in distal canes. Cordon volume may come into play here, as the volume of the cordon, along with the other perennial structures of the vine including the trunk and roots, impact the capacity of the vine for overwintering reserve storage (Bates et al., 2002). The lack of difference observed between circumference measurements taken from the proximal, intermediate, and distal arm sections indicates that length adjusted cordons had a comparable volume to non-adjusted cordons and also a similar capacity for reserve storage. In terms of bud fertility, neither the proximal nor distal portions of cordon arms displayed differences in the number of inflorescence primordia or % of necrotic primary buds. This is despite the differences observed in PAI and Φ, suggesting the possibility of differences in leaf area and light conditions at the renewal zone. Canopy shading (Perez & Kliewer, 1990) and pruning level (Collins & Rawnsley, 2004a) have previously been implicated in the occurrence of PBN. The lack of differences observed in distal cane internode carbohydrate levels in this trial may have had an impact here as reductions in bud carbohydrate levels are also associated with the occurrence of PBN (Vasudevan et al., 1998).

4.5 Conclusion

While the results of this trial do not indicate a long-term beneficial response as a consequence of adjusting the length of new cordon arms during their establishment via an assessment of cane vigour, this does not mean that the vines which received such an adjustment did not benefit from the practice. During the first growing season, the treatment was seemingly successful in encouraging vegetative growth, with length adjusted vines having more shoots and a lower canopy porosity than control vines. This effect was especially pronounced in the intermediate sections of new cordon arms, which had a greater pruning weight, cane number, and average cane weight. This is critical, as in situations where there is insufficient vigour for unhindered bud burst and shoot development, the effect would usually be most pronounced in this arm section. While the length adjustment did seem to encourage the growth of canes in the middle of the cordon that were both bigger and more numerous, the benefits of this may have been limited to the first growing season. There was an immediate beneficial impact of a higher number of canes to utilise for extending LA arms to their final length, but the low vigour C vines were not lacking in options for their own extensions if required. The supposed benefit of the length adjustment method is that by encouraging growth at struggling node positions, particularly those in the centre of cordon arms, the end result may be fewer missing spur positions. This did not seem to be the case in this trial, as no difference was observed in the number of canes of length adjusted or control vines in any of the three years succeeding the first growing season, including when shoot thinning was (2019-2020 and 2020-2021 seasons) and was not (2021-2022 season) performed. This was true both when considering the entire vine as well as individual arm sections, including the intermediate arm section. Reduced PAI and increased Φ observed in length adjusted cordons during these seasons further supported that there was no benefit in terms of vegetative growth after the first season, and perhaps even a penalty. The perennial structure of the cordon itself did not seem to suffer from the treatment however, with similar circumferences observed between length adjusted and control vines for all arm sections. It is possible that this treatment could have a stronger beneficial response in other cultivars, particularly those in low vigour environments which struggle with apical dominance. Further research focusing on such an environment where the control vines are more likely to suffer severe detrimental effects arising from a high retained node number could better illustrate the comparative beneficial response of the treatment over a longer term.

4.6 Acknowledgements

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Chapter 5. Prepared Manuscript 2: Micro-CT constitutes a valuable tool in assessing the impact of cordon constriction on the vascular morphology of grapevines

Statement of Authorship

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

Name of Principal Author (Candidate)			
Contribution to the Paper	Conceptualisation, methodology, formal analysis, data curation, writing—original draft preparation, writing—review and editing		
Overall percentage (%)	90		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	25/09/2022

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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Contribution to the Paper	Conceptualisation, methodology, writing—review and editing, supervision		
Signature		Date	26/09/2022

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

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 4-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 less connections per unit volume between vessels than other treatments at this site, as well as a lower theoretical specific hydraulic conductivity (K_s). 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 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.

5.1. 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 & 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 & 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 & 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 & Schubert, 1998; Munitz et al., 2018), and hydraulic conductivity (Schultz & Matthews, 1993; Tyree & Ewers, 1991).

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., 2015b). 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 vines ability to transport water to its most distal regions could have a severe outcome on cordon health and productivity.

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.

5.2. Materials and Methods

5.2.1. 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 x 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 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 5.1). 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 three panels of three vines each (27 vines per treatment) were assessed at this site.

Figure 5.1. Applied cordon training methods. (**A**) Cordon wrapped tightly 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.

5.2.2. Measurements

5.2.2.1. 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 Image J version 1.53e (NIH, USA) software (Figure 5.2). 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.

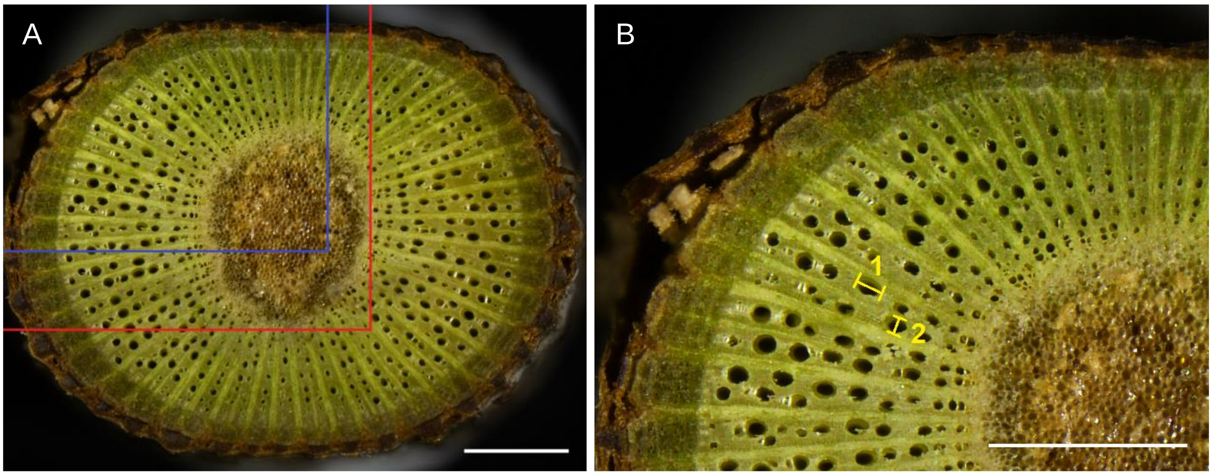

Figure 5.2. Cane samples as imaged by optical microscopy. (**A**) Full cross-section. Red lines indicate where quartering cuts were made. Blue lines indicate edge of region of interest. (**B**) Region of interest showing example measures of vessel length (1) and width (2). Scale bars = 1000 µm.

5.2.2.2. 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 5.3). 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 & 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.

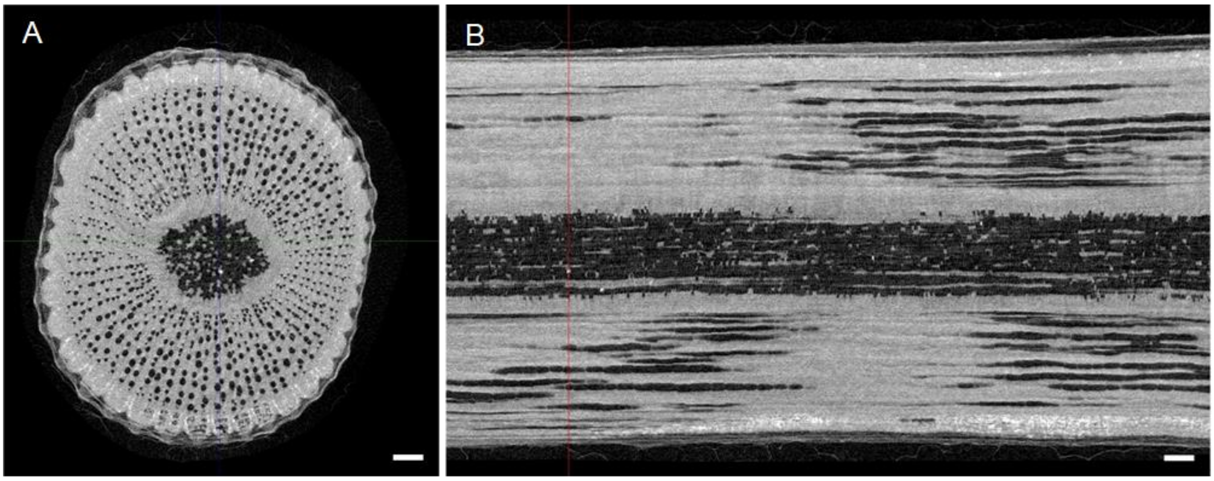

Figure 5.3. X-ray microtomography (micro-CT) of cane sample sourced from the distal portion of a cordon arm. (**A**) Cross-section (**B**) Longitudinal section. Resolution = 5.5 µm. Scale bars = 600 µm.

5.2.2.3. 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 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 5.4). 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 (5.2.2.2).

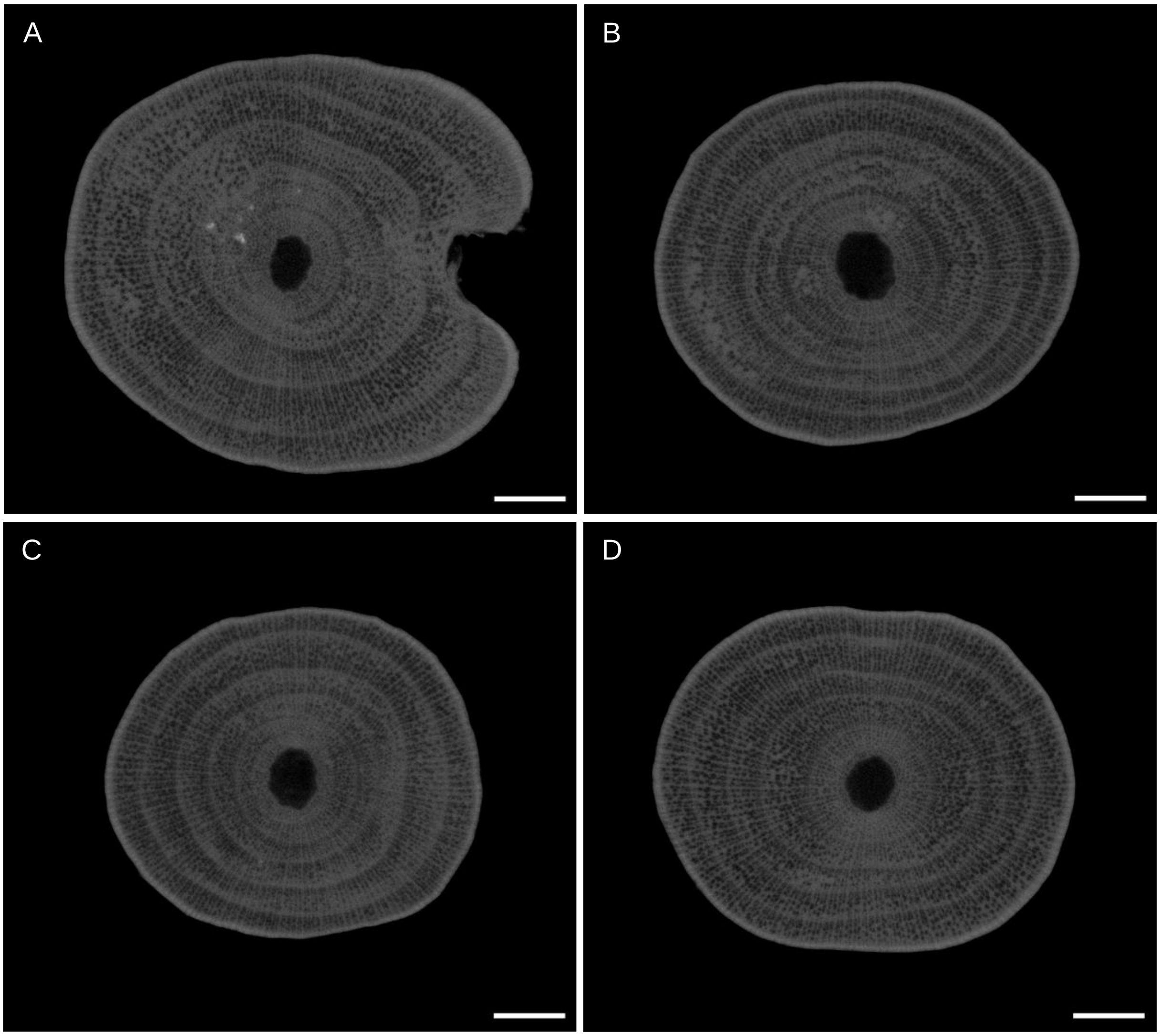

Figure 5.4. X-ray microtomography (micro-CT) of cordon samples, in a cross-sectional view. (**A**) Cordon wrapped tightly around cordon wire, T (**B**) Cordon placed on top of cordon wire, P (**C**) Cordon woven through clip system, W (**D**) Cordon trained in s-bend, S. Resolution = 35 µm. Scale bars = 4 mm.

5.2.2.4. Determination of theoretical specific conductivity

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

$$K_s = \left(\frac{\pi\rho}{128\eta A_w}\right)\sum_{i=1}^{n}(d_i^4)$$

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 (m), and n is the number of vessels.

5.2.2.5. 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.

5.2.3. 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.

5.3. Results

5.3.1. Analysis of 1-year-old canes

At site A in 2019, based on observation by 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 5.1). 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 less 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.

Table 5.1. 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 $\boldsymbol{p} \leq 0.05$. ns = not significant.

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 total cane volume between treatments in 2019 or 2020 at site A (Table 5.1). 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 had significantly more connections per volume than other treatments, while in 2021 they had less 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.

5.3.2. 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 other treatments (Figure 5.5). 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.

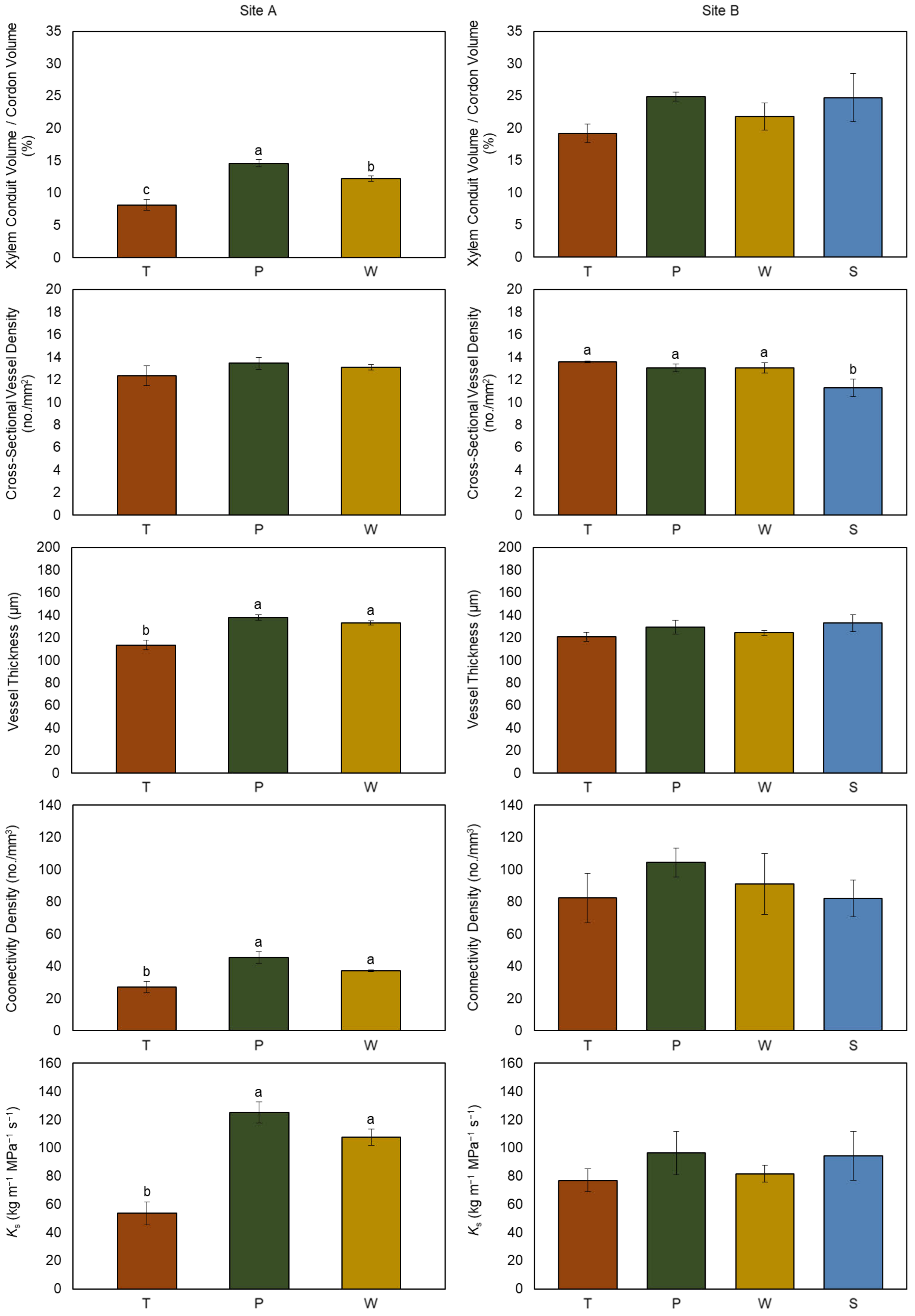

Figure 5.5. Anatomical measurements of cordon samples obtained by micro-CT. T = tightly wrapped, P = placed on top, W = woven through clips, S = s-bend. Means were separated by ANOVA using Fisher's LSD test at a significance level of $p \leq 0.05$, and different letters indicate significant differences between treatments at each site.

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 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 treatment. 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.

5.3.3. 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 5.2). 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.

Table 5.2. 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.

5.4. Discussion

Cane samples in this trial were collected from the distal portion of 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 & Fisher, 1989; Olson & 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 less 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 site 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 & 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 less 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 a 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 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 & Matthews, 1993; Tyree & 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 & 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 & 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 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 5.4). 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 to 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 of newly trained cordons that were only 4-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.

5.5. Acknowledgements

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Chapter 6. Prepared Manuscript 3: Training technique may have an impact on permanent cordon longevity

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Contribution to the Paper	Conceptualisation, methodology, formal analysis, data curation, writing—original draft preparation, writing—review and editing		
Overall percentage (%)	90		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	25/09/2022

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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Training technique may have an impact on permanent cordon longevity

Abstract: A trial was set up at two vineyard sites to quantify the impacts of different permanent cordon training techniques on indicators of vine health and productivity over the seasons immediately following cordon establishment. One of these treatments involved wrapping developing cordons arms tightly around the cordon wire, a practice common in some winegrowing regions. Several results suggest that the constriction applied by the tight wrapping had a negative impact on vegetative growth. These include reduced circumferences of the proximal, intermediate, and distal arm sections of tightly wrapped cordons in the later seasons of the trial at one site, as well as lower intermediate and distal pruning weights. Other measurements including PAI do not indicate that there was a reduction in the vegetative growth of tightly wrapped cordons and suggest that it was cordons trained around two parallel cordon wires in a loose, s-shaped bend that suffered from the greatest reduction in plant tissue area. Measurements of fertility, carbohydrate status, grape chemistry, and yield components did not indicate major differences between the applied training methods. It is likely that the differences observed between the applied treatments would continue to become more pronounced with further observation, as cordon constriction is a condition that becomes progressively worse over time. One of the purported benefits of tightly wrapping arms around the cordon wire is a minimisation of the risk of mechanical damage by means of increased canopy stability. This trial did not find this to be the case, as in one year more mechanical damage was observed in cordons which were wrapped tightly around the wire than other training methods.

6.1 Introduction

Most modern grapevine spur pruned training systems involve the use of some form of permanent cordons; structures of perennial wood extending from the crown or head area of the vine that provide many renewal points from which the fruit bearing shoots will grow each season. These systems typically involve the use of a single cordon wire along which the cordons are trained and secured. This is the case for both unilaterally and bilaterally trained cordons, as well as those with a divided canopy, which although utilise more than one cordon wire, still typically have each cordon arm trained to a single wire (Reynolds & Vanden Heuvel, 2009). In some wine growing regions it is a common practice for growers to wrap canes selected for the use as permanent cordon arms tightly around the cordon wire during the establishment process (O'Brien et al., 2021). This decision can be made for a multitude of reasons including preventing wind damage during early establishment (Boehm & Coombe, 1992), or reducing labour costs, but is primarily chosen with the desired outcome of reducing the risk of canopy rolling by providing additional stability (Caravia et al., 2015b). While some growers may unwind the wrapped cordons after the first season of growth, most leave them in their tightly wrapped manner permanently. The additional stability that is seemingly afforded by the wrapping is appreciated in heavily mechanised regions where machine harvesters and other tractor implements are routinely operated throughout the growing season and where mechanical breakage/damage may be an issue of concern. The technique is not without its drawbacks however, as arms positioned in the fashion are at risk of becoming constricted over time, with the cordon wire often visibly embedded within the wood of the cordon, seemingly at the expense of cordon health and productivity. This is an irreversible condition that may become progressively worse over time as the cordon grows, with major reworking being the only restorative response. Severe decline and dieback are a common sight in older vineyards suffering from such visible cordon constriction. It is likely in this circumstance that the normal movement of water and nutrients via the vascular conduits of the cordon may be compromised, negatively affecting vine vitality, particularly at the most distal points of cordon arms (O'Brien et al., unpublished). This is an issue of particular concern in the context of climate change, where any interruption to ordinary water flow could be exacerbated by increasingly common water or heat stress events (Jones et al., 2005; Keller, 2010). There is also likely a complicated relationship between the strangulation caused by cordon constriction and the onset of vascular diseases (O'Brien et al., 2023), caused by a range of different pathogens. These maladies contribute to the occlusion of the xylem themselves (Chatelet et al., 2006; Rudelle et al., 2005), and vines may be more likely to express symptoms of infection if the health of the cordon is already compromised by stress (Edwards et al., 2007a, 2007b; Ferreira et al., 1999; Fischer & Kassemeyer, 2012; Sosnowski et al., 2011a).

While the tight wrapping method has been more or less abandoned in some regions many years ago, more recently, in areas where the practice is still common, some growers have begun to explore other less constrictive methods of permanent cordon training as an alternative to tight wrapping. Placing the cordon on top of the cordon wire and securing it by tying it in place in several locations is one such method. Cordon rolling can be a serious problem with this technique however, especially in the seasons immediately following establishment. With insufficient anchoring to the cordon wire, arms positioned in this fashion have a tendency to roll under the influence of their own canopy weight, disrupting the selection and formation of permanent spur positions and exposing fruit to sunburn risk (Chorti et al., 2010). This makes the presence of at least a single foliage wire all but a necessity when practicing this training method. Other options being explored involve the use of additional trellis infrastructure such as parallel cordon wires and plastic clips. The drawback with these methods however is that they may require additional time and expenditure to implement, and to date there has been no quantitative comparison of the benefits of each different permanent cordon training technique. This study aimed to quantify the benefits and/or detriments of different permanent cordon training techniques on vine health and productivity over the seasons immediately following cordon establishment. We hypothesise that alternative training methods which are less constrictive in nature than wrapping cordon arms tightly around the wire may provide the developing arms with an environment that is more conducive to their vitality in both the short and particularly the long term.

6.2 Materials and Methods

6.2.1. Experimental design

6.2.1.1. Site A

The first of two trial sites was set up at a newly planted (3-year-old) vineyard site in Williamstown, South Australia (34°40'21.4"S 138°53'27.0"E) in the spring of 2018. The cultivar was Cabernet Sauvignon (WA Cape Selection), grafted onto 1103 Paulsen, and planted at 3 m x 2 m inter-row and intra-row spacing with a north–south row orientation. Each row had a single foliage wire located 12 cm above the cordon wire, for the use of a sprawl canopy system. Cordon wire height was 1 m above the ground. The total amount of irrigation discharged was ~1.0 ML/ha during each growing season of the trial (2018-2022). The climatic conditions for the site were sourced from the nearest Australian Bureau of Meteorology (http://www.bom.gov.au/, accessed on 28 May 2022) weather stations located in Williamstown (station number 023752) and Mount Crawford AWS (station number 023878).

Three permanent cordon training 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; 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 6.1). An early shoot thinning was performed in the spring of 2018-2020 when shoots were 5-10 cm long as a method to improve uniformity in the length and diameter of shoots along all new cordons (Simonit et al., 2012). This shoot thinning targeted shoots originating from spur bases, multiple shoots originating from the same node, and shoots originating from non-spur positions.

Treatments were randomly allocated to different panels. Six sets of three panels of three vines each (54 vines per treatment) were assessed for ongoing analysis. For certain measurements, cordon arms were divided into three segments: proximal (P°), intermediate (I°), and distal (D°), to gain a more localised understanding of the impacts of the different training methods. Each of these arm sections encompassed 3-4 nodes, progressing from the crown area of the arm (P°) to its most apical point (D°).

6.2.1.2. Site B

The second trial site was located in Eden Valley, South Australia (34°38'32.1"S 139°05'53.0"E) and was set up in the spring of 2018 on a newly reworked 20-year-old planting of Shiraz (clone BVRC12), on own roots, planted at 2.8 m × 1.8 m inter-row and intra-row with a north-south row orientation. Each row had a single foliage wire located 30 cm above the cordon wire, for the use of a sprawl canopy system. Cordon wire height was 1 m above the ground. The total amount of irrigation discharged was ~1.0 ML/ha during each growing season of the trial (2018-2022). The climatic conditions for the site were sourced from the nearest Australian Bureau of Meteorology (http://www.bom.gov.au/, accessed on 28 May 2022) weather stations located in Keyneton (station number 023725) and Mount Crawford AWS (station number 023878).

At this site four permanent cordon training techniques were applied and included the same three treatments as site A, as well as an additional treatment (iv) s-bend (S), where canes selected as permanent cordon arms were wrapped around two parallel cordon wires in a loose, s-shaped bend. Treatments were randomly allocated to different panels. Three sets of three panels of three vines each (27 vines per treatment) were assessed.

Figure 6.1. Applied cordon training methods. (**A**) Cordon wrapped tightly 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.

6.2.2. Vegetative growth and canopy architecture

Images were taken with the front camera of an iPhone (Apple, Cupertino, CA) twice during the 2018-2019 growing season and three times during each following growing season for the purpose of measuring canopy architecture using the VitiCanopy App (De Bei et al., 2016). One image was taken on each side of the middle vine of each panel from about 80 cm below the vine cordon for the algorithmic assessment of plant area index (PAI).

Cordon circumference was measured at three points (P°, I°, D°) along the arms of each vine using a flexible measuring tape at the time of pruning. The pruning weight of each arm section (P°, I°, D°) was measured from the middle vine of each panel.

6.2.3. Harvest maturity/yield components

The middle vines of each panel were hand harvested each year and bunch number and total yield per vine were recorded. Arm sections (P°, I°, D°) were harvested separately at the Williamstown site in 2022 and the Eden Valley site in 2021 and 2022. Total yield was measured with a digital scale. Cordon length was measured at the time of harvest so that yield and its components could be determined on a per metre basis. From yield and bunch number, the average bunch weight was calculated. Five bunches were collected from each harvested vine and were stored at 4 °C for further lab analysis in the days immediately following harvest. Berries (n=50) were randomly collected from these bunch samples and weighed for determination of average berry weight. Bunch samples were then hand crushed in plastic bags with the juice then being collected in 50 mL tubes and centrifuged at 5000 rpm for 5 min (Hettich Universal, Tuttlingen, Germany) before total soluble solids (TSS), pH and titratable acidity (TA) were measured according to (Iland et al., 2004), using an automatic titrator (G20S Compact Titrator, Mettler Toledo, Thebarton, Australia) and a digital refractometer (BRX-242 Erma Inc. Tokyo, Japan).

6.2.4. Physiological measures

One-year-old cane samples were collected from proximal, intermediate, and distal cordon arm sections during pruning in the winter of 2019, 2020, and 2021 for analysis of carbohydrate concentration. Samples consisting of nodes three and four and their interjoining internode were collected from canes originating from the first node of each two-node spur from the previous growing season. All samples were stored at 4 °C until further processing. Sections approximately 1 cm long were cut from the centre of each internode sample using secateurs and then combined and ground using a mechanical grinder (A11 basic, IKA, Germany). A commercial enzyme assay kit (Total starch assay kit, Megazyme, Ireland) was used to analyse starch levels following the method described in (Edwards et al., 2010) based on colorimetric assay. Using a spectrophotometer (Multiskan Spectrum, model 00300011, Thermo Electron Corporation, Vantaa, Finland) absorbances were read at 505 nm and the starch content determined using a glucose standard curve. For the analysis of sugar concentration, an anthrone assay was performed with absorbances then read at 600 nm and concentration determined using a fructose standard curve.

6.2.5. Fertility assessments

Cane samples were collected from every panel of each treatment during winter dormancy in 2020 and 2021 for the purpose of microscopically dissecting buds for examination of the incidence of primary bud necrosis (PBN). Samples were collected from the proximal and distal portions of cordon arms and consisted of nodes 1-4 of a cane originating from node 2 of a 2-node spur. A razor blade was used to slice buds transversally using the methods described by (Rawnsley & Collins, 2005). A light microscope at 25x magnification (Model EZ4W, Leica, Heerbrugg, Switzerland) was used during this process to assess the number of inflorescence primordia (IP) in the primary bud of each compound bud. If the primary bud was necrotic, the largest secondary bud was assessed in its place based on the assumption that it would grow in compensation for its loss.

6.2.6. Mechanical damage

Vines were visually surveyed in the weeks following harvest for signs of physical damage from mechanical implements. The assessment was based on a 0-to-100% scale as a proportion of the total number of spur positions which were damaged. Assessment included spurs which had severe damage to only one out of two nodes, as well as spur positions which were damaged enough to be unusable as productive spur positions in following seasons (e.g. one lost spur position out of 20 total on vine = 5% damage).

6.2.7. 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.

6.3 Results

6.3.1. Climatic conditions

At both sites the average November monthly temperature of 2020 was hotter than the other growing seasons, followed by an average December monthly temperature that was cooler than the other growing seasons (Figure 6.2). The 2018 and 2019 seasons were notably drier than 2020 and 2021 at both sites. Site A received 36.1% more rainfall over the course of the trial than site B.

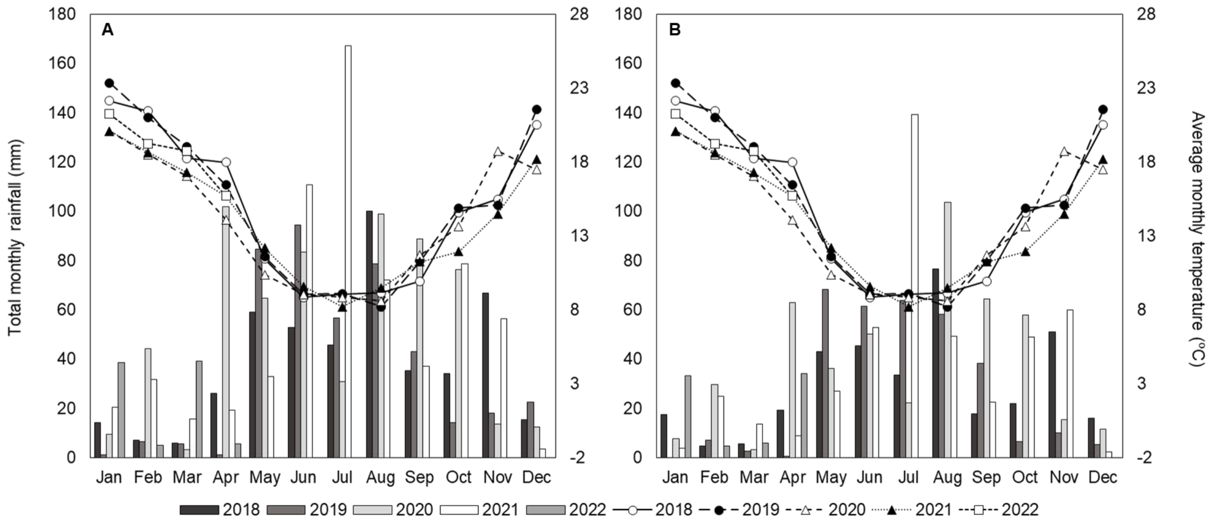

Figure 6.2. Weather conditions at site A (**A**) and site B (**B**) throughout the course of the trial. Data were sourced from the nearest Australian Bureau of Meteorology (http://www.bom.gov.au/ accessed on 11/07/2022) weather stations located in Williamstown (station number 023752), Mount Crawford (station number 023878), and Keyneton (station number 023725).

6.3.2. Canopy architecture

At site A, at the end of the first growing season, cordons which were placed on top of the cordon wire and secured in place had a higher plant area index (PAI) than those that were wrapped tightly around the wire or woven through the plastic clip system centred between parallel wires (Figure 6.3). No difference was observed in PAI on any other imaging date in any year of the study.

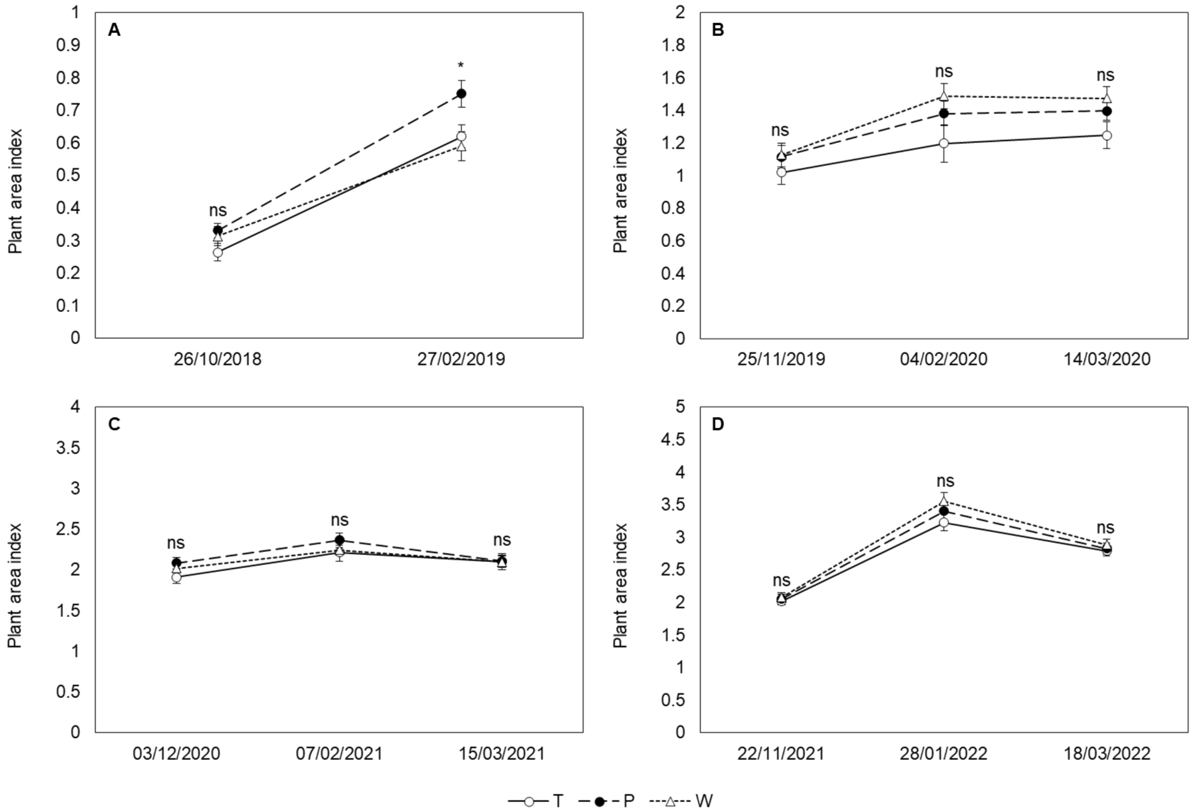

Figure 6.3. Plant area index (PAI) measures taken at site A during the (**A**) 2018-2019 (**B**) 2019-2020 (**C**) 2020-2021 and (**D**) 2021-2022 growing seasons. T = tightly wrapped, P = placed on top, and W = woven. Means were separated by ANOVA. * indicates significant difference at $p \leq 0.05$. ns = not significant.

At site B, no difference was observed in PAI during the first growing season. From the second growing season a trend developed where the cordons which were wrapped around parallel wires in the s-bend had a significantly lower PAI than other treatments on many imaging dates (Figure 6.4). In the final growing season of the trial this was true on all three assessed dates. On the date of harvest in 2022, cordons which were trained on top of the wire had a lower PAI than those which were woven through the plastic clip system.

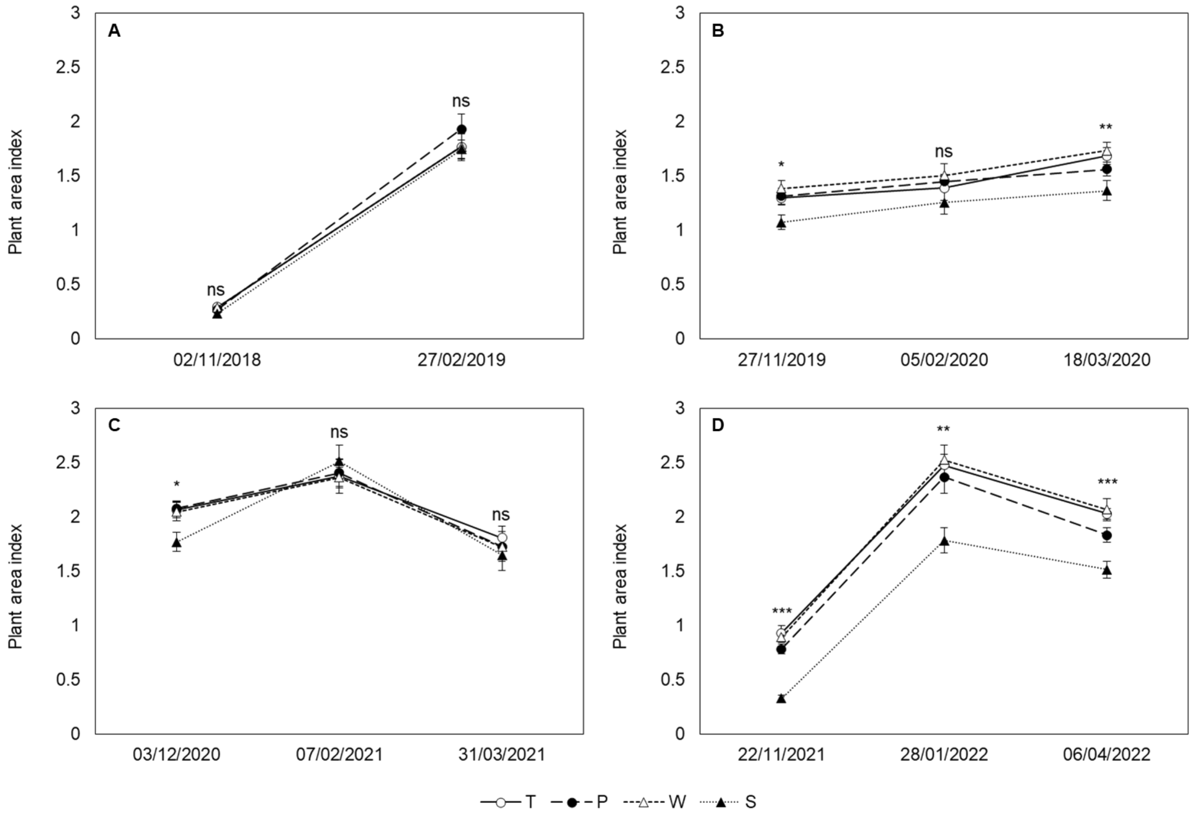

Figure 6.4. Plant area index (PAI) measures taken at site B during the (**A**) 2018-2019 (**B**) 2019-2020 (**C**) 2020-2021 and (**D**) 2021-2022 growing seasons. T = tightly wrapped, P = placed on top, W = woven, and S = s-bend. Means were separated by ANOVA. *, **, *** indicate significant differences at $p \leq 0.05$, 0.01, and 0.0001 respectively. ns = not significant.

6.3.3. Vegetative growth

At site A, the circumference of the proximal arm section of cordons which were wrapped tightly around the cordon wire was significantly lower than those which were woven through the plastic clip system in 2020 and 2021 (Figure 6.5). The circumference of the intermediate arm section of tightly wrapped cordons was lower than both other treatments in 2020, 2021, and 2022. The circumference of the distal arm section of tightly wrapped cordons was lower than both other treatments in 2021, and lower than cordons woven through the plastic clips in 2022.

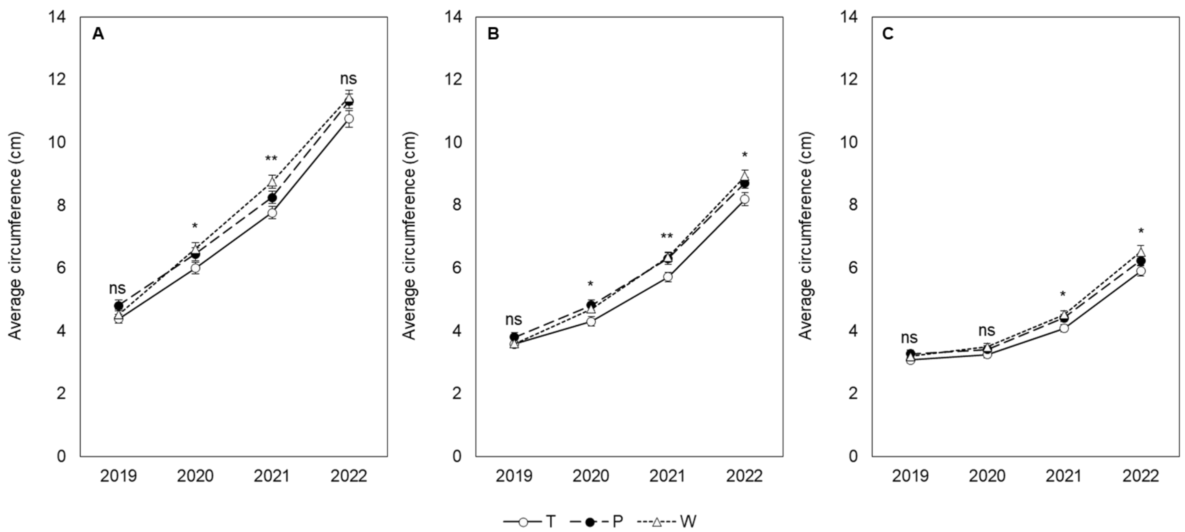

Figure 6.5. Average circumference measurements of the proximal (**A**) intermediate (**B**) and distal (**C**) arm sections at site A. T = tightly wrapped, P = placed on top, and W = woven. Means were separated by ANOVA. *, ** indicate significant differences at $p \leq 0.05$ and 0.01. ns = not significant.

At site B, the average circumference of the proximal section of cordons which were placed on top of the cordon wire was higher than other treatments in 2019 and 2020 (Figure 6.6). The circumference of the intermediate section of cordons placed on top of the wire was higher than other treatments in 2019 only. No difference was observed in the circumference of the distal arm sections in any year.

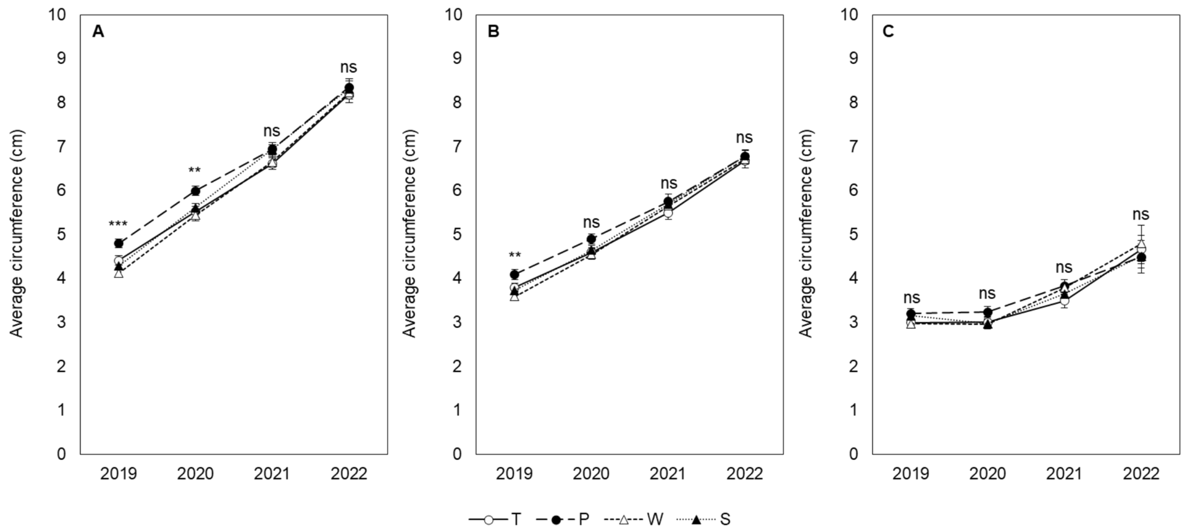

Figure 6.6. Average circumference measurements of the proximal (**A**) intermediate (**B**) and distal (**C**) arm sections at site B. T = tightly wrapped, P = placed on top, W = woven, and S = s-bend. Means were separated by ANOVA. **, *** indicate significant differences at $p \leq 0.01$, and 0.0001 respectively. ns = not significant.

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 6.1). In 2020 and 2021, cordons which were wrapped tightly around the wire had a lower distal pruning weight than both other treatments. Tightly wrapped cordons also had less canes per metre in the intermediate arm section than other treatments in 2021. In 2022 cordons woven through the clip system had much higher average pruning weights than other treatments on a whole vine basis. W cordons had significantly higher distal pruning weights than both other treatments, and higher intermediate pruning weights than cordons which were trained on top of the wire.

Table 6.1. Average pruning weights taken from the different arms sections at site A. T = tightly wrapped, P = placed on top, and W = woven.

		Proximal			Intermediate			Distal			Total		
	Treatment	Cane no. (no./m)	Cane Weight (g)	Pruning Weight (kg/m)	Cane no. (no./m)	Cane Weight (g)	Pruning Weight (kg/m)	Cane no. (no./m)	Cane Weight (g)	Pruning Weight (kg/m)	Cane no. (no./m)	Cane Weight (g)	Pruning Weight (kg/m)
2019	T	10.9	22.9	0.20	12.4	15.3	0.19	14.3	29.9	0.45	13.6 a	24.4	0.34
	P	10.1	23.9	0.22	10.1	21.4	0.19	14.4	32.3	0.47	12.5 ab	28.7	0.35
	W	9.8	18.1	0.17	10.3	17.4	0.17	12.1	20.1	0.24	11.1 b	18.8	0.21
	p value	ns	ns	ns	ns	ns	ns	ns	ns	ns	0.047	ns	ns
2020	T	15.4	46.1	0.74	12.6	35.1	0.46	11.9	37.5	0.46 b	13.4	40.2	0.56
	P	13.7	44.2	0.62	12.9	34.1	0.44	14.2	46.5	0.68 a	13.8	41.7	0.59
	W	14.1	44.1	0.63	13.0	39.2	0.51	14.7	49.7	0.73 a	14.1	44.7	0.64
	p value	ns	ns	ns	ns	ns	ns	ns	ns	0.035	ns	ns	ns
2021	T	17.9	58.8	1.06	20.3	46.1	0.97	19.4 b	50.3	1.01 b	19.3	51.6	1.01
	P	16.9	47.2	0.78	20.1	43.3	0.85	23.0 a	57.6	1.34 a	20.1	49.7	0.99
	W	18.8	48.8	0.91	21.0	48.6	1.01	22.5 a	63.0	1.39 a	20.8	53.5	1.10
	p value	ns	ns	ns	ns	ns	ns	0.020	ns	0.048	ns	ns	ns
2022	T	24.4	94.0	2.15	23.9	71.9 b	1.72 ab	27.8 b	60.0 b	1.68 b	25.5	73.7 b	1.86 b
	P	23.5	66.0	1.51	26.0	61.6 b	1.55 b	29.5 ab	75.0 b	2.23 b	26.4	68.3 b	1.76 b
	W	24.6	93.7	2.31	23.4	101.5 a	2.45 a	32.4 a	112.8 a	3.65 a	26.9	103.7 a	2.81 a
	p value	ns	ns	ns	ns	0.013	0.041	0.028	0.0004	<0.0001	ns	0.022	0.006

Means were separated by ANOVA ($p \leq 0.05$). Different letters indicate differences between treatments within each season. ns = not significant.

At site B, no difference was observed in pruning weights or other weight components including cane number and cane weight for any arm section in any year of the trial (Table 6.2).

Table 6.2. Average pruning weights taken from the different arms sections at site B. T = tightly wrapped, P = placed on top, W = woven, and S = s-bend.

		Proximal			Intermediate			Distal			Total		
	Treatment	Cane no. (no./m)	Cane Weight (g)	Pruning Weight (kg/m)	Cane no. (no./m)	Cane Weight (g)	Pruning Weight (kg/m)	Cane no. (no./m)	Cane Weight (g)	Pruning Weight (kg/m)	Cane no. (no./m)	Cane Weight (g)	Pruning Weight (kg/m)
2019	T	11.4	38.1	0.42	10.4	27.5	0.27	9.9	38.4	0.40	17.9	42.5	0.75
	P	9.5	62.3	0.48	9.4	26.3	0.25	10.7	27.1	0.29	17.8	42.4	0.73
	W	12.6	40.9	0.51	10.2	22.8	0.23	12.4	30.3	0.35	18.3	44.9	0.81
	S	10.9	42.7	0.49	8.8	26.7	0.23	12.8	31.7	0.41	18.4	40.9	0.74
	p value	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
2020	T	17.2	32.1	0.58	19.2	24.8	0.48	20.9	27.6	0.56	24.3	31.2	0.76
	P	16.3	34.6	0.50	18.3	22.8	0.43	17.6	24.9	0.44	22.8	28.5	0.65
	W	17.0	29.5	0.50	18.2	23.3	0.42	17.5	22.8	0.39	22.3	26.2	0.59
	S	17.2	32.9	0.56	17.5	20.8	0.35	19.8	22.8	0.45	21.7	28.5	0.61
	p value	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
2021	T	19.7	30.7	0.64	20.4	20.6	0.40	27.2	23.7	0.66	26.0	25.7	0.66
	P	24.0	27.7	0.67	17.9	19.6	0.35	25.0	15.4	0.39	26.5	20.9	0.56
	W	21.0	24.1	0.50	23.7	18.8	0.45	25.6	23.1	0.59	27.5	21.8	0.60
	S	25.5	28.6	0.74	20.2	21.9	0.44	25.8	20.8	0.52	26.7	24.2	0.64
	p value	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
2022	T	25.5	30.4	0.74	24.0	23.9	0.55	26.3	30.6	0.81	26.6	28.0	0.73
	P	23.5	26.6	0.64	21.9	18.3	0.40	25.6	21.7	0.55	24.9	22.1	0.55
	W	23.3	22.7	0.52	27.4	17.3	0.48	26.3	22.7	0.58	26.7	20.6	0.55
	S	27.1	26.2	0.72	25.3	20.9	0.55	31.6	23.0	0.73	28.8	23.5	0.69
	p value	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

Means were separated by ANOVA ($p \leq 0.05$). Different letters indicate differences between treatments within each season. ns = not significant.

6.3.4. Carbohydrate status

In 2019 at site A, the concentration of total non-structural carbohydrates (NSC) was significantly lower in cane samples collected from cordons which were woven through the plastic clip system than other treatments (Table 6.3). This was true of samples collected from the intermediate and distal arm sections. Concentrations of NSC in canes from the proximal section of woven cordons were also lower than tightly wrapped cordons. This difference observed in NSC in the first growing season was driven by cane sugars levels, with woven cordons having significantly less sugars than both other treatments in all three arm sections. 2020 saw less variability between treatments, with the only difference observed being that cordons placed on top of the wire had less starch than other treatments in canes from the proximal and intermediate arm sections. This same effect was observed again in 2021, with the canes of proximal, intermediate, and distal sections of cordons placed on top of the wire all having less starch than other treatments. Canes from the intermediate section of cordons placed on top of the wire also had significantly less sugar than other treatments. These differences were enough that total NSC concentrations of canes from cordons which were placed on top of the wire were lower than both other treatments for all three arm sections in 2021.

Table 6.3. Average concentrations of sugars, starch, and total non-structural carbohydrates (NSC) in cane samples collected from the different arms sections at site A. T = tightly wrapped, P = placed on top, and W = woven.

		Proximal			Intermediate			Distal		
	Treatment	Sugars (mg/g)	Starch (mg/g)	NSC (mg/g)	Sugars (mg/g)	Starch (mg/g)	NSC (mg/g)	Sugars (mg/g)	Starch (mg/g)	NSC (mg/g)
2019	T	58.8 a	143.8 a	202.6 a	65.5 a	140.1 a	205.7 a	61.1 b	139.0	200.1 a
	P	59.6 a	126.0 b	185.6 ab	60.4 a	135.0 ab	195.4 a	67.4 a	132.7	200.1 a
	W	43.6 b	135.4 ab	179.0 b	47.5 b	126.0 b	173.4 b	48.3 c	128.2	176.4 b
	p value	<0.0001	0.036	0.030	<0.0001	ns	0.0003	<0.0001	ns	0.008
2020	T	36.6	73.9 a	110.5	36.2	75.2 a	111.5	37.1	74.5	111.6
	P	35.3	70.5 b	105.8	36.6	70.1 b	106.7	37.2	72.7	109.9
	W	36.2	75.4 a	111.6	33.4	73.6 a	106.9	39.9	74.4	114.2
	p value	ns	0.015	ns	ns	0.014	ns	ns	ns	ns
2021	T	23.4	121.6 a	145.0 a	28.3 ab	124.2 a	152.5 a	25.3	117.8 a	143.1 a
	P	23.7	96.5 b	120.2 b	22.6 b	107.9 b	130.5 b	21.9	102.7 b	124.6 b
	W	26.7	127.2 a	153.9 a	31.4 a	121.8 a	153.2 a	23.5	128.1 a	151.7 a
	p value	ns	<0.0001	<0.0001	0.017	0.015	0.0002	ns	0.0001	0.0004

Means were separated by ANOVA ($p \leq 0.05$). Different letters indicate differences between treatments within each season. ns = not significant.

In 2019 at site B, cane samples from cordons that were wrapped tightly around the cordon wire had a higher NSC concentration in the intermediate arm section than other treatments, but a lower NSC concentration in the distal arm section (Table 6.4). This was driven by differences in both sugar and starch levels in the intermediate and distal arm sections. No difference was observed in sugar, starch, or NSC levels in the proximal arm section in 2019 or 2020. Cordons which were placed on top of the cordon wire had a lower starch concentration in canes from the intermediate arm section in 2019. Both cordons that were placed on top of the wire and wrapped around the wire had lower intermediate starch concentrations than other treatments in 2020. Minimal differences in starch levels were observed in 2021. In this year, the lowest sugar levels were observed in canes from cordons which were trained in the s-bend, particularly in the distal arm section.

Table 6.4. Average concentrations of sugars, starch, and total non-structural carbohydrates (NSC) in cane samples collected from the different arms sections at site B. T = tightly wrapped, P = placed on top, W = woven, and S = s-bend.

		Proximal			Intermediate			Distal		
	Treatment	Sugars (mg/g)	Starch (mg/g)	NSC (mg/g)	Sugars (mg/g)	Starch (mg/g)	NSC (mg/g)	Sugars (mg/g)	Starch (mg/g)	NSC (mg/g)
2019	T	74.1	125.5	199.6	80.0 a	145.4 a	225.4 a	62.8 b	129.9 c	192.7 b
	P	72.1	120.7	192.8	70.8 ab	116.5 b	187.3 b	78.3 a	132.6 bc	210.8 ab
	W	57.3	126.5	183.9	53.4 c	136.6 a	189.9 b	71.9 ab	150.3 a	222.2 a
	S	62.1	120.4	182.5	66.7 b	139.5 a	206.2 b	78.4 a	145.8 ab	224.2 a
	p value	ns	ns	ns	0.001	0.014	0.002	0.029	0.012	0.024
2020	T	49.7	71.7	121.4	54.1	71.7 b	125.7 ab	45.5	71.9 bc	117.4 c
	P	52.1	74.5	126.6	48.5	70.3 b	118.8 b	53.3	67.9 c	121.2 bc
	W	51.4	75.5	126.9	51.0	77.5 a	128.5 a	51.6	76.8 ab	128.3 ab
	S	51.5	77.9	129.4	50.9	80.5 a	131.5 a	48.3	81.2 a	129.4 a
	p value	ns	ns	ns	ns	0.002	0.053	ns	<0.0001	0.009
2021	T	49.3 b	106.2	155.5	62.9 b	103.8 b	166.7	61.7 bc	108.2	169.9
	P	73.0 a	101.4	174.4	49.6 c	124.2 a	173.8	68.1 ab	103.4	171.5
	W	67.9 a	99.3	167.2	75.3 a	97.7 b	173.1	72.0 a	108.70	180.7
	S	51.0 b	111.2	162.1	53.2 c	113.2 ab	166.5	57.7 c	103.6	161.2
	p value	<0.0001	ns	ns	<0.0001	0.020	ns	0.001	ns	ns

Means were separated by ANOVA ($p \leq 0.05$). Different letters indicate differences between treatments within each season. ns = not significant.

6.3.5. Fertility

No difference was observed in the number of inflorescence primordia (IP) within the compound buds of cane samples collected from the proximal arm section of cordons at either site in any year (Figure 6.7). In 2020, less IP were observed in the buds of cane samples from the distal section of cordons which had been wrapped tightly around the wire than those which had been woven through the plastic clip system at site A. At site B, less IP were observed in the buds of distal canes from cordons which had been trained in the s-bend than other treatments in 2020.

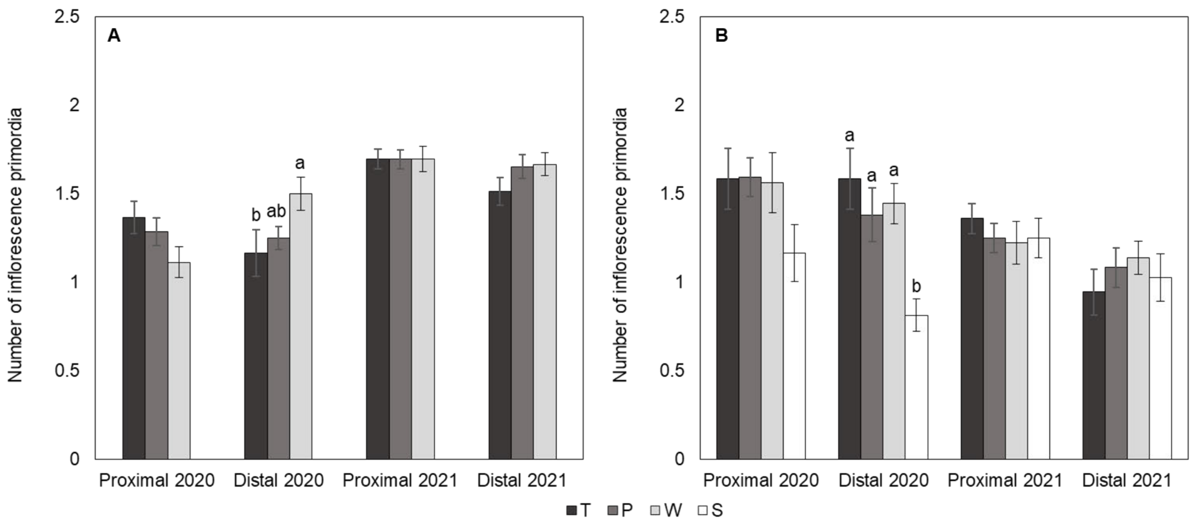

Figure 6.7. Average number of inflorescence primordia (IP) within the compound buds of canes from the proximal and distal arm sections at site A (**A**) and site B (**B**). T = tightly wrapped, P = placed on top, W = woven, and S = s-bend. Means were separated by ANOVA ($p \leq 0.05$), and different letters indicate significant differences between IP number within each season of assessment.

No difference was observed in the amount of buds with primary bud necrosis (PBN) from the canes of any arm section at either site in any year of the study (Figure 6.8).

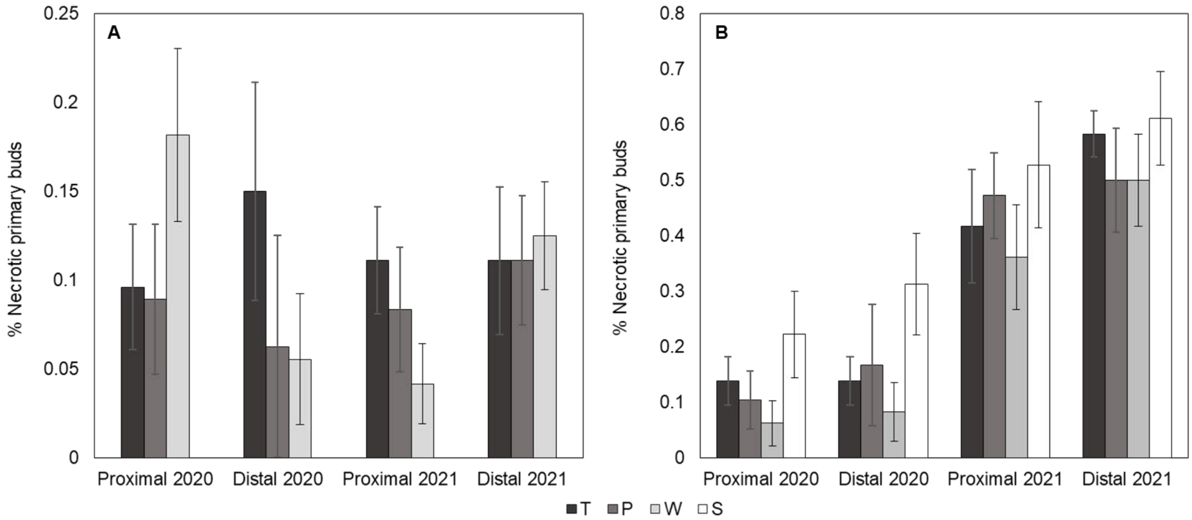

Figure 6.8. Average number of buds of canes from the proximal and distal arm sections with primary bud necrosis at site A (**A**) and site B (**B**). T = tightly wrapped, P = placed on top, W = woven, and S = s-bend. Means were separated by ANOVA ($p \leq 0.05$) and differences were found to be non-significant.

6.3.6. Grape chemistry

At site A, no difference was observed in TSS, pH, or TA between treatments on a whole vine basis in any year (Table 6.5). In 2022, the TSS of grapes from the distal arm section of cordons trained on top of the wire was lower than cordons which were woven through the plastic clip system.

Table 6.5. Harvest parameters of grape samples collected from site A including total soluble solids (TSS), pH, and titratable acidity (TA). T = tightly wrapped, P = placed on top, and W = woven.

		Proximal			Intermediate			Distal			Total		
	Treatment	TSS (Brix)	pH	TA (g/L)	TSS (Brix)	pH	TA (g/L)	TSS (Brix)	pH	TA (g/L)	TSS (Brix)	pH	TA (g/L)
	T	-	-	-	-	-	-	-	-	-	25.4	3.64	4.6
2020	P	-	-	-	-	-	-	-	-	-	26.0	3.75	4.2
	W	-	-	-	-	-	-	-	-	-	25.4	3.71	4.3
	p value	-	-	-	-	-	-	-	-	-	ns	ns	ns
	T	-	-	-	-	-	-	-	-	-	25.8	3.78	5.2
2021	P	-	-	-	-	-	-	-	-	-	25.9	3.80	5.2
	W	-	-	-	-	-	-	-	-	-	26.0	3.80	5.0
	p value	-	-	-	-	-	-	-	-	-	ns	ns	ns
	T	25.6	3.53	6.6	25.6	3.52	6.7	25.2 ab	3.55	6.8	25.5	3.53	6.7
2022	P	25.5	3.59	6.6	25.0	3.56	6.8	24.9 b	3.60	6.9	25.1	3.69	6.8
	W	25.5	3.52	7.0	25.7	3.53	7.1	25.6 a	3.57	7.1	25.6	3.54	7.0
	p value	ns	ns	ns	ns	ns	ns	0.048	ns	ns	ns	ns	ns

Means were separated by ANOVA ($p \leq 0.05$). Different letters indicate differences between treatments within each season. ns = not significant.

At site B in 2020, grapes from the cordons wrapped in the s-bend around parallel wires had a lower concentration of TSS than other treatments (Table 6.6). The same effect was observed again in 2021, where it seemed to be driven mostly by low TSS concentrations in the proximal and intermediate arm sections. Grapes from cordons which were placed on top of the wire had a lower pH than other treatments in 2021, also driven by differences in the proximal and intermediate arm sections. In 2022, grapes from the intermediate section of cordons which were wrapped tightly around the wire and trained in the s-bend had lower pH than those which were placed on top of the wire or woven through the clip system.

Table 6.6. Harvest parameters of grape samples collected from site B including total soluble solids (TSS), pH, and titratable acidity (TA). T = tightly wrapped, P = placed on top, W = woven, and S = s-bend.

		Proximal			Intermediate			Distal			Total		
	Treatment	TSS (Brix)	pH	TA (g/L)	TSS (Brix)	pH	TA (g/L)	TSS (Brix)	pH	TA (g/L)	TSS (Brix)	pH	TA (g/L)
2020	T	-	-	-	-	-	-	-	-	-	30.5 a	3.50	6.4
	P	-	-	-	-	-	-	-	-	-	30.1 a	3.45	6.3
	W	-	-	-	-	-	-	-	-	-	30.2 a	3.41	6.6
	S	-	-	-	-	-	-	-	-	-	29.4 b	3.43	6.7
	p value	-	-	-	-	-	-	-	-	-	0.002	ns	ns
2021	T	25.4 a	3.37 a	7.0	24.9 a	3.34 a	7.1	24.5	3.37	7.1 b	24.9 a	3.36 a	7.1
	P	24.8 a	3.24 b	7.5	25.0 a	3.25 b	7.4	24.2	3.28	7.7 ab	24.6 a	3.26 b	7.6
	W	24.6 ab	3.37 a	7.3	24.8 a	3.33 a	7.7	24.4	3.28	8.2 a	24.6 a	3.33 a	7.7
	S	23.2 b	3.36 a	7.4	23.0 b	3.29 ab	7.6	22.7	3.33	7.8 a	23.0 b	3.33 a	7.6
	p value	0.046	0.007	ns	0.045	0.042	ns	ns	ns	0.012	0.041	0.019	ns
2022	T	27.6	3.65	6.0	27.5	3.67 a	5.9	27.2	3.65	6.0	27.5	3.66	6.0
	P	27.4	3.58	5.9	27.7	3.56 b	6.1	27.5	3.55	6.2	27.5	3.56	6.1
	W	28.1	3.59	6.2	27.9	3.59 b	6.3	27.7	3.62	6.0	27.9	3.60	6.2
	S	27.2	3.61	6.1	27.2	3.60 a	6.3	26.4	3.62	6.2	27.0	3.61	6.2
	p value	ns	ns	ns	ns	0.031	ns	ns	ns	ns	ns	ns	ns

Means were separated by ANOVA ($p \leq 0.05$). Different letters indicate differences between treatments within each season. ns = not significant.

6.3.7. Yield

No differences in yield or yield components were observed at site A in any arm section or on a whole vine basis in any year of the study (Table 6.7).

Table 6.7. Yield and yield component data of site A. T = tightly wrapped, P = placed on top, and W = woven.

	Treatment	Proximal Yield (kg/m)	Proximal Bunch no. (no./m)	Proximal Bunch Weight (g)	Intermediate Yield (kg/m)	Intermediate Bunch no. (no./m)	Intermediate Bunch Weight (g)	Distal Yield (kg/m)	Distal Bunch no. (no./m)	Distal Bunch Weight (g)	Total Yield (kg/m)	Total Bunch no. (no./m)	Total Bunch Weight (g)
2020	T	-	-	-	-	-	-	-	-	-	1.5	21.9	71.5
	P	-	-	-	-	-	-	-	-	-	1.4	23.8	61.4
	W	-	-	-	-	-	-	-	-	-	1.4	21.8	64.2
	p value	-	-	-	-	-	-	-	-	-	ns	ns	ns
2021	T	-	-	-	-	-	-	-	-	-	3.6	38.1	95.0
	P	-	-	-	-	-	-	-	-	-	3.6	39.8	89.4
	W	-	-	-	-	-	-	-	-	-	3.5	39.5	89.4
	p value	-	-	-	-	-	-	-	-	-	ns	ns	ns
2022	T	3.1	45.2	67.5	4.0	51.1	79.6	4.2	48.6	85.8	3.8	48.3	77.6
	P	2.6	34.7	76.5	3.7	48.8	77.5	3.7	42.3	88.0	3.3	41.9	80.6
	W	2.4	38.8	62.7	4.0	50.2	78.4	4.3	49.6	86.4	3.6	46.7	76.4
	p value	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

Means were separated by ANOVA ($p \leq 0.05$). Different letters indicate differences between treatments within each season. ns = not significant.

The yield of the proximal arm section of cordons which were wrapped tightly around the wire was lower in 2021 than cordons which were place on top of the wire or trained in the s-bend at site B (Table 6.8). No other differences in yield or yield components were observed at site B in any arm section or on a whole vine basis in any year of the study.

Table 6.8. Yield and yield component data of site B. T = tightly wrapped, P = placed on top, W = woven, and S = s-bend.

		Proximal			Intermediate			Distal			Total		
	Treatment	Yield (kg/m)	Bunch no. (no./m)	Bunch Weight (g)	Yield (kg/m)	Bunch no. (no./m)	Bunch Weight (g)	Yield (kg/m)	Bunch no. (no./m)	Bunch Weight (g)	Yield (kg/m)	Bunch no. (no./m)	Bunch Weight (g)
2020	T	-	-	-	-	-	-	-	-	-	1.1	20.4	53.1
	P	-	-	-	-	-	-	-	-	-	1.3	22.7	56.4
	W	-	-	-	-	-	-	-	-	-	1.4	24.6	55.5
	S	-	-	-	-	-	-	-	-	-	1.4	23.6	60.1
	p value	-	-	-	-	-	-	-	-	-	ns	ns	ns
2021	T	2.8 b	20.5	134.9	6.1	38.2	156.1	5.8	29.3	192.1	4.9	29.3	163.9
	P	4.3 a	27.8	154.8	5.4	33.7	158.9	4.4	30.3	152.1	4.7	30.6	152.4
	W	3.4 ab	25.1	134.1	5.5	40.1	138.7	4.8	29.8	160.4	4.6	31.7	143.8
	S	4.3 a	29.1	154.1	6.2	36.8	171.4	5.0	30.1	174.3	5.2	32.0	164.1
	p value	0.041	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
2022	T	2.4	31.6	70.2	2.7	32.8	74.7	2.8	28.8	93.4	2.6	31.1	80.3
	P	1.9	26.0	73.4	2.5	33.1	72.1	2.5	29.1	80.3	2.3	29.4	74.7
	W	1.4	24.2	60.1	2.0	36.2	51.7	2.3	29.9	72.1	1.9	30.1	60.7
	S	2.9	37.1	72.4	2.8	39.0	67.1	2.6	32.0	77.8	2.7	36.1	72.2
	p value	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

Means were separated by ANOVA ($p \leq 0.05$). Different letters indicate differences between treatments within each season. ns = not significant.

6.3.8. Mechanical damage

The number of spurs lost to mechanical damage increased each year at both sites (Figure 6.9). At site B in 2021, cordons which were wrapped tightly around the wire lost more spurs to mechanical damage than those which were woven through the plastic clip system or trained in the s-bend.

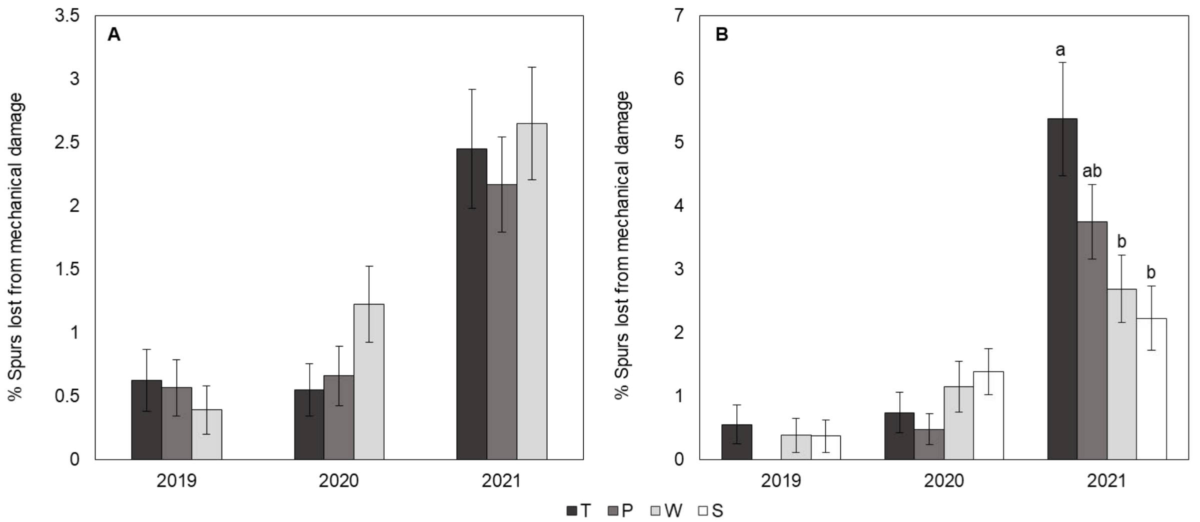

Figure 6.9. Total spur positions lost as a result of breakage from mechanical damage during harvest each year (2019-2021) at site A (**A**) and site B (**B**). T = tightly wrapped, P = placed on top, W = woven, and S = s-bend. Means were separated by ANOVA ($p \leq 0.05$), and different letters indicate significant differences between treatments within each season of assessment. ns = not significant.

6.4 Discussion

Measures of vegetative growth varied considerably between the two sites over the course of the trial. Minimal differences were observed in PAI between the different treatments at site A. At the end of the first growing season, the PAIs of cordons which were placed on top of the cordon wire and secured in place were higher than those which were wrapped tightly around the wire or woven through the plastic clip system centred between parallel wires. While this suggests that this system may have been beneficial in promoting early vegetative growth, this effect was not observed in any of the following years, where no differences in PAI were observed at any point in each season. At site B in contrast, no difference in PAI was observed between treatments in the first growing season, with differences then becoming more pronounced over the following years. Cordons which were trained around two parallel wires in the s-shaped bend had a lower PAI than the other three treatments on many of the imaging dates of growing seasons 2-4, particularly in the last season, where this was the case for all three stages of maturity (pre-flowering, veraison, and harvest) at which vines were imaged. While this training method extended the cordon the furthest spatially into the midrow of all treatments, these low

PAI values were seemingly not due to mechanical breakage. The differences in canopy architecture observed with this training method may have had an impact on bud fertility, as less IP were observed in the buds of distal canes from S cordons than other treatments in 2020. This could have occurred as a result of differences in temperature and light conditions during primordia development, as warmer temperatures favour inflorescence differentiation and lower temperatures favour tendril differentiation (Trought, 2005).

At site B, cordons which were placed on top of the cordon wire had proximal and intermediate arm sections that were of greater circumference than other treatments at the start of the trial. The difference in circumference between treatments at this site decreased over time, with no difference observed in the later years of the trial. No difference was observed in the circumference of distal arm sections in any year of the study at this site, indicating that the treatments did not have a notable impact on the overall size of the cordon arms. At site A, very different results were obtained in that no difference was observed in the circumference of any arm section after the first growing season, with differences then becoming more apparent over the passing seasons. At this site, the cordons which were wrapped tightly around the cordon wire at points displayed cordons which had circumferences that were significantly smaller than other treatments for all three arm sections. This suggests that the tightly wrapped cordons had smaller volumes than the other treatments, which may have had implications on their capacity to overwinter carbohydrates, as the total volume of all perennial structures including the roots, trunk, and cordons contribute towards this storage capacity (Bates et al., 2002). These results agree with Caravia et al. (2015a), who found measurements of arm transversal area (ATA) to be significantly lower in the distal portion of wrapped arms relative to non-wrapped arms in a similar trial after a single growing season.

The smaller circumferences observed in the tightly wrapped cordons at site A correlated with measurements of pruning weight, where T cordons had a lower distal pruning weight than the other two treatments in both 2020 and 2021 as well as a lower distal cane number than other treatments in 2021. This again agrees with Caravia et al. (2015a), who reported pruning mass to be around 20% higher in non-wrapped cordons after a single season, suggesting more favourable conditions for growth. In the final year of the study at site A, the cordons which were woven through the plastic clip system had an overall pruning weight that was much higher than those which were wrapped tightly around the cordon wire or trained on top of the wire. This was driven mostly by differences in the average weight of individual canes in the intermediate (p = 0.013) and distal (p = 0.0004) arm sections, rather than differences in the number of canes, although W cordons did have significantly more distal canes than T cordons. The same result was not reproduced at site B, where there was no difference between pruning weights or their components observed between treatments in any year of the trial. Pruning weight at site B somewhat correlated with observed circumference values, in which differences progressed from minimal to apparently non-existent throughout the course of the trial.

Several studies have suggested that water stress may reduce trunk starch concentration (Dayer et al., 2013; De Bei et al., 2011; Pellegrino et al., 2014). One could make an argument that if lower concentrations of carbohydrates were observed in cane samples collected from the distal sections of cordon arms during winter dormancy, then training method may have been beneficial in allowing the movement of carbohydrates from the distal canes to the perennial structures of the vines such as the roots and trunk for overwintering. In this case, the transiting carbohydrates would have to move through the conduits of the cordon arms, where vascular morphology could be impacted by the constrictive pressure applied from training. When considering the NSC levels of the different cordon arms in this trial however, one common occurrence that was observed was that if the levels of sugars, starch, or total NSC were low in one particular arm section, they were likely to be similarly low in other arm sections as well. Such was the case in 2019, where the concentration of sugars was found to be lower in canes collected from cordons which had been woven through the plastic clip system for all three arm sections at site A. Although these differences were highly significant for all arm sections ($p < 0.0001$), they were not observed again in the following seasons. In the second year a trend began where there was lower starch in the cane samples from cordons which were placed on top of the cordon wire, but only at site A. By the third season this effect was observed in all three arm sections. Overall, the measurements of cane carbohydrate status do not indicate an obvious beneficial or detrimental impact from training method. While the treatments seemingly had some impact on the amount of carbohydrates remaining in cane tissue, the effect was typically not localised to one individual arm section. This suggests that there was likely little to no interruption to the translocation of carbohydrates through the cordons, including those that were tightly wrapped. There may be a more complicated dynamic responsible for the trends observed in differing cane sugar and starch concentrations between treatments. The low levels of starch observed in the canes of P cordons suggests that these cordons may have been more successful in accumulating perennial reserves that could influence growth and productivity in future seasons. Caravia et al. (2015a) also observed a trend (though not significant) of higher trunk starch concentration in vines with non-wrapped cordons. Additional measurements targeting the perennial structures would help to give a more complete picture of the impact of the different training methods on vine carbohydrate status.

Very minimal differences were apparent in the chemistry of grapes harvested from site A over the course of the trial, the only being a lower concentration of TSS in grapes from the distal section of cordons trained on top of the wire than cordons which were woven through the plastic clip system in 2022. No difference was observed between the maturity parameters of the different treatments in any year of the trial at this site on a whole vine basis. At site B a trend was observed where s-bend grapes had a lower TSS than other treatments in the early years of the trial. Interestingly, this effect was not observed in the distal arm section and was driven only by differences in the proximal and intermediate arm sections in 2021. Small differences in acidity (pH and TA) were also observed at this site on an individual arm section and whole vine basis, but did not seem to follow a trend. Yield and yield components varied less than grape chemistry at both sites, indicating that the treatments had very little impact on vine productivity, even in the case of the s-bend vines which suffered from a reduction in PAI in the later seasons. This suggests that the training methods likely had little influence on water and nutrient availability, as the level of these factors at key developmental stages have been found to influence yield components and grape composition (Guilpart et al., 2014; Marciniak et al., 2013; McCarthy, 1997; Naor et al., 1993; Romero et al., 2015). It is possible that differences in yield would be more apparent in a lower vigour setting, as constriction applied via plastic straps on olive trees has been reported to reduce vegetative growth and canopy volume, as well as yield in low vigour cultivars (Tombesi & Farinelli, 2016).

While one of the purported benefits of tightly wrapping arms around the cordon wire is a minimised risk of mechanical damage via increased canopy stability, this trial did not find this to be the case (Figure 6.9). The number of spurs lost to mechanical damage increased each year at both sites as one would expect, given that typical 2-node spur pruning was practiced, and spur positions gradually increased in height each year, increasing their propensity to mechanical breakage (Castaldi, 2011). Surprisingly, at site B in 2021 cordons which were wrapped tightly around the cordon wire lost more spurs to mechanical breakage than those which were woven through the plastic clip system or trained in the s-bend. Mechanical damage is a justified concern for growers as spur positions lost to this avenue of breakage equate to reduced production and a loss of profit. In more extreme cases, spur positions may be lost permanently as the damage to the node may be too deep to support any future growth (Castaldi, 2011). Indeed, this seemed to be the case with some of the breakage observed in this trial, although permanence of damage was not quantified, and damage was assessed only by its new presence on an annual basis. Interestingly, the s-bend treatment lost the fewest spurs in 2021 at site B to mechanical damage. This is surprising given the nature of this training method. While it is not the only treatment that utilised two parallel cordon wires, it is the only treatment in which the cordon was wrapped around the outside of both wires, extending some spur positions further into the midrow. One would expect this treatment to therefore have an increased chance of having the greatest amount of spur positions damaged for this reason, as was the case in 2020. This is especially true as the s-bend cordons had a lower PAI than other treatments in the later stages of the trial, a condition which was apparently not due to mechanical damage. Based on the results of this trial it seems that there is a moderate risk of damage from the use of mechanical harvesters regardless of the method of training employed.

6.5 Conclusion

At this time, it is difficult to conclude if the purported benefits of tightly wrapping developing cordon arms around the cordon wire outweigh the risks. The results of this trial do not demonstrate the effectiveness of the practice in mitigating the risk of cordon rolling, seemingly the primary driver in the decision making of the growers among whom this method is popular. While some mild rolling was observed at site A, the presence of the single foliage wire was enough to prevent the disruption of spur position establishment and no grapes were subject to sunburn damage. In the final season of the trial at Site B, tightly wrapped cordons actually suffered more apparent damage from mechanical harvesting than two of the other treatments. This indicates that alternative training methods may be no more susceptible to this means of damage, and after a few seasons of growth may provide canopy stability that is comparable to tight wrapping. Certain measurements suggest that the vegetative growth of the tightly wrapped cordons may have been negatively impacted by the application of the treatment. These include reduced circumferences of the proximal, intermediate, and distal arm sections of tightly wrapped cordons in the later seasons of the trial at one site. Those same tightly wrapped cordons also had lower intermediate and distal pruning weights than the other treatments in the later seasons. Other measurements however including PAI do not indicate that a reduction in the vegetative growth of tightly wrapped cordons occurred and actually suggest that it was cordons trained in the s-bend suffering from a reduced plant tissue area. It is important to note that the scope of this trial was limited by its timeframe, and that the seemingly negative outcomes developing with the use of certain training methods would likely continue to become progressively worse over time. In the case of the tightly wrapped cordons, this would come as a consequence of the wire applying more pressure to the cordon as it grew, increasing the degree of constriction and embedding the wire within the cordon wood. Further examination of the same cordons over a longer period would provide more insight into the long-term impacts of the different training methods on cordon health and productivity.

6.6 Acknowledgements

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Chapter 7. General Discussion and Future Work

This project provides a better understanding into several areas of research regarding the use of alternative permanent cordon training strategies. The detrimental impacts resulting from the application of certain training methods, while appreciated by some industry members, had beforehand not been quantified in a thorough and meaningful way. As previous research in this area was limited, the results presented in the preceding chapters could provide an avenue to narrow the scope of future investigations. The training methods presented in these studies are all commonplace in certain winegrowing regions. The analysis of the outcomes generated by their use presented in this thesis therefore provide valuable practical insight into their apparent benefits and drawbacks.

One of the focuses of the project was on the relationship if any that exists between cordon strangulation caused by the constriction of tight wrapping, dieback, and the incidence and expression of vascular disease. The results of the survey included in the literature review of this thesis are to the authors' knowledge the first published research regarding this topic. After plating wood shavings collected from cordon samples on potato dextrose agar, the fungal pathogen *Eutypa lata* was positively identified in plates from samples collected from cordons displaying dieback both in the presence and absence of typical Eutypa dieback foliar symptoms as well as from symptomless cordons. Other Diatrypaceous and Botryosphaeriaceous species were identified only in samples from cordons displaying both Eutypa dieback foliar and dieback symptoms. While this small trial did not quantify the impact of tight wrapping on the occurrence of dieback and vascular disease symptom expression, it illustrated the inherent difficulty in relying solely on the expression of foliar symptoms when investigating for the presence of fungal pathogens.

The study presented in Chapter 3 aimed to further investigate the nature of the relationship between cordon strangulation, dieback, and vascular disease symptom expression by assessing their incidence in commercial vineyards displaying varying degrees of cordon decline. The question of whether vines suffering from cordon strangulation are more likely to express symptoms of *E. lata* and other fungal diseases is an interesting one. Previous studies investigating the symptom expression of vascular diseases in vines subjected to other forms of environmental stress including water deficit have had varying results. While numerous studies have suggested that vines may be more likely to express symptoms if their health is already compromised by stress (Edwards et al., 2007a, 2007b; Ferreira et al., 1999; Fischer & Kassemeyer, 2012; Sosnowski et al., 2011a), others have found the opposite to be the case (Sosnowski et al., 2021). The ten vineyard sites included in the two-season study comprised vines aging from 14-24 years old during the first season of assessment, an age point at which the strangulation effect imparted by tight wrapping is readily apparent. Surprisingly, a trend was observed of lower severity of dieback in the cordons displaying the greatest degree of strangulation. This was an unexpected result given that dieback is a condition which is normally associated with advanced cordon age, and in the trial a correlation was observed between degree of strangulation and vine age.

The VitiCanopy app utilised in the trial for the assessment of canopy parameters provided a reliable quantitative measurement of plant area index (PAI), which correlated with visual assessments of dieback and helped to confirm their accuracy. There was a non-significant trend observed for increased foliar symptoms in relation to an increase in the degree of strangulation. It is difficult to assign causality for this trend however, as the occurrence of foliar symptoms may be influenced by a multitude of factors including climatic conditions (Sosnowski et al., 2007). There is therefore not enough evidence to confirm whether the severely strangled cordons were suffering from a reduction in vine defence response exacerbating the onset of trunk disease symptoms. While the results of the survey were not in line with what was expected, the trial was the first to quantify the impact of cordon strangulation on different factors of decline and provides a novel rating scale for the assessment of degree of strangulation that may be useful in future projects.

The investigation presented in Chapter 4 of this thesis examined the question of whether a vigour-based length adjustment during the establishment of permanent cordon arms is a beneficial practice. The problem that this technique is purported to address is that due to the prioritisation tendencies of the vine, it is common for node positions in the centre of new cordon arms to struggle with early vegetative growth. This is especially true in low vigour situations. The results of the study suggest that this treatment was in fact successful in encouraging vegetative growth in this region of cordon arms immediately following establishment. A significantly higher pruning weight, cane number, and cane weight were observed in the intermediate sections of cordon arms after one season of growth. This, in combination with the fact that there was a lower canopy porosity observed in length adjusted vines at the end of the first season, suggests that the treatment achieved its primary goal of ensuring early shoot development in the middle of cordon arms. The problem is that this benefit was only apparent after the first season. Some physiological measurements taken later in the trial including plant area index suggest that the length adjusted vines actually suffered from a reduction in plant tissue area compared to control vines in the succeeding seasons. Measurements from the middle of cordon arms at this stage of the trial including the number and size of canes showed that control vines had no more missing spur positions than length adjusted vines. In this sense, although the treatment did promote the growth of canes in this problem area, there was actually no long-term benefit when compared to the control vines. Based on the results of the trial it could be argued that the length adjustment did provide an early advantage to the cordon arms it was practiced on. It is just that under the conditions of the trial, the control cordon arms were not suffering from low enough vigour that this advantage was pronounced enough to provide the length adjusted vines with any apparent long-term benefit. These results help to narrow the scope of the direction of future research in that it is now clear that any evaluation of the efficacy of this method should be conducted on vines which are more likely to show long-term detrimental effects from a high retained node number. If more of the control vines were missing spur positions in the seasons following establishment, then the lack of missing spur positions promoted by the length adjustment would be of more obvious assistance. The fact that no difference was observed in cordon size (as measured by circumferences of the different arm sections) suggests that the delayed development of the distal arm section inherent to this method did not have a negative impact on perennial wood structure. This is an encouraging result that could make some practitioners more comfortable with adopting the use of the method.

When preparing for the micrological assessment of vascular morphology presented in Chapter 5, it was expected that any differences occurring as a result of the different training methods would be most pronounced in the localised wood of the cordon itself. It was decided to collect cane samples from the distal regions of cordon arms throughout the course of the trial for examination with micro-CT and stereo microscope, as this was a more non-destructive manner of data collection than the direct observation of the cordon. In the final year of the trial, after all other data collection was complete, cordons were then collected for direct examination by micro-CT. It was fortunate that this was possible, as the biggest differences in vascular morphology occurring as a result of training method were indeed the most apparent in the cordons. While some differences between treatments were observed in the morphological properties of the cane samples, they did not follow any apparent pattern. When examining the cordons of one site however, a clear trend was observed in which the cordons that were trained in the most constrictive manner (wrapped around the cordon wire) appeared to be suffering from a range of deleterious effects. These included xylem conduits which were lower volume relative to total cordon volume than other training methods ($p < 0.0001$), as well as thinner individual xylem vessels ($p < 0.0001$), and lower theoretical specific hydraulic conductivity ($p < 0.0001$). The tightly wrapped cordons also had a lower connectivity density than other treatments, indicating that they may be less hydraulically integrated and efficient (Espino & Schenk, 2009; Jacobsen et al., 2012). The least constrictive training method (placing the cordon on top of the cordon wire) had the greatest xylem conduit volume relative to cordon volume (78.7% higher than those which were wrapped tightly around the cordon wire), greater than that of the cordons woven through the plastic clip system centred between parallel cordon wires

(49.8% higher than those which were wrapped tightly around the cordon wire). These results suggest that training method has a very direct and nearly immediate impact on cordon vascular morphology. The fact that these results were produced in cordons which were only four years old at the time of their observation suggests that the resultant condition of reduced xylem functionality is one that is likely to become progressively worse over time. There remain questions however as to how ageing cordons may organise themselves with embedded wires, along with the organisation of new vessels in the medium-to-long term. While the tight wrapping treatment had an obvious visual impact on the structure of the wood of the cordons on which it was applied, they were not yet severely strangled. In fact, using the visual scale for degree of cordon strangulation caused by tight wrapping presented in Chapter 3, no cordon that was observed by micro-CT would have been rated over a 2 out of 4. Older cordons displaying severe (3) or very severe (4) strangulation would likely show even greater reductions in xylem size and connectivity relative to non-strangled cordons. Future research could target older cordons in the 15-20-year range where the harmful impact of tight wrapping is much more visually apparent than those of the age which were imaged in this study. While a direct conclusion regarding vine water status cannot be drawn from the results illustrated in this work, it is safe to say the capacity of the tightly wrapped cordons for normal water transport was negatively affected, as conductivity is determined by vessel structure, size and efficiency (Schultz & Matthews, 1993; Tyree & Ewers, 1991). Xylem vessels and whole xylem conduits of such a reduced size and connectivity could struggle to maintain normal functionality in typical non-stress conditions throughout a vine's lifespan, much less in situations of drought, extreme heat, or in the face of colonisation by vascular pathogens.

Regarding the question of whether tightly wrapping developing cordon arms around the cordon wire is a stressful practice, only one previous study to the authors' knowledge has ever been published. This study, conducted by Caravia et al. (2015a), was limited to a single growing season but reported that the treatment restricted vegetative growth, with tightly wrapped cordons having smaller distal arm transversal areas, and lower pruning weight at the distal arm sections. The study presented in Chapter 6 of this thesis reproduced these results, with the arms of tightly wrapped cordons at one site displaying reduced proximal, intermediate, and distal cordon circumferences as well as lower intermediate and distal pruning weights in the later seasons of the trial. Also reported by Caravia et al. (2015a) was a non-significant trend of higher trunk starch concentration in vines with non-wrapped cordons. While this current project did not investigate trunk starch concentration, it did investigate non-structural carbohydrate concentration including sugar and starch concentration in cane samples collected from the different sections of cordon arms. A similar trend was observed in the later seasons of the trial where cane samples from the proximal, intermediate, and distal arm sections of cordons placed on top of the wire all had lower starch concentrations than other treatments at one site, suggesting they may have promoted the movement of starch to perennial structures including the trunk. Uniformity between the carbohydrate status of the canes of different arm sections was a common occurrence in the trial, suggesting that the treatments had minimal impact on the movement of carbohydrates through the cordon itself. Along with cordons wrapped tightly around the wire and cordons placed on top of the wire, this trial also featured additional training methods that had not been investigated scientifically before. The use of one of these treatments; wrapping the cordon around two parallel wires in a loose, s-shaped bend, seemed to indicate a reduction in seasonal vegetative growth, with lower PAIs observed than other treatments at many points throughout the later seasons of the trial. No reduction was observed in the size of the cordon arms themselves in this case however. Interestingly, the use of this training method, as well as weaving cordons through two parallel cordon wires in a plastic clip system, resulted in less breakage due to mechanical damage at one site than wrapping the cordons tightly around the wire. This is especially notable, as a reduction in the amount of mechanical breakage via improved canopy stability is a reason that many practitioners who tightly wrap cordon arms around the cordon wire utilise the practice.

Overall, the results of this project suggest that cordon training method had a significant impact on numerous indicators of vine well-being. Adjusting the length of new arms in accordance with their apparent vigour had a beneficial impact on early shoot development, especially in the middle of cordon arms. While this benefit was limited to a single season in this trial, further research in a lower vigour setting could better demonstrate its effectiveness in preventing long-term gaps in productive cordon. Constrictive training, predominantly wrapping developing cordon arms tightly around the cordon wire, had a negative impact on vegetative growth at one site including cordons of smaller circumference and lower pruning weights. Particularly strong results were obtained with the investigation into the effects of the different training methods on vascular morphology with the use of micro-CT. The xylem conduits of cordons which had been wrapped tightly around the wire were smaller, and composed of thinner individual vessels than other treatments as well as having less connections per volume between vessels. This suggests that the normal functionality of the cordon in the translocation of water and nutrients could already be compromised by the treatment, a condition that is likely to worsen over time. The major limitation of this project was its timeframe, as severe strangulation observed from the constriction of tight wrapping normally does not become visually apparent until cordons are of a more advanced age than those observed in the greater part of this project. The survey of older, already established vineyards yielded unexpected results, but presents a quantitative scale for the assessment of degree of strangulation imposed by tight wrapping that could prove useful in future research. Further examination of research vines that were trained specifically for the use of this study over an extended timeframe would provide more comprehensive information regarding the total long-term impact of the different investigated training methods. The negative outcomes that were developing with some of the applied treatments, particularly the tightly wrapped cordons, would very likely become more pronounced over time. While vine longevity is a metric that is hard to meaningfully quantify in a short-term study, the results presented in this thesis when taken as a whole suggest that the cordons which were least constricted by their training method performed the best over the course of the trial.

Chapter 8. References

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