Impact of shoot trimming and lateral removal on grape ripening, wine composition and varietal aromas of Riesling

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Amongst management practices in viticulture, the removal of leaves is arguably regarded as one of the most critical steps for final grape and wine quality. Trimming as well as leaf removal in the bunch zone not only alters the leaf area to fruit weight (LA:FW) ratio, but also modifies the canopy microclimate by enhancing air movement and sun exposure of grapes. Viticulturists may take advantage of this to enhance yield components and optimise pest and disease management. However, canopy management may also serve to target certain wine styles by alternating the varietal volatile composition in grapes, or even lower wine alcohol concentrations through lower grape sugar accumulation. Defoliation is commonly used in climate change adaption, and it is one of the most efficient ways of changing berry composition and improving grape and wine quality.

The aim of this research was to assess the changes in grapevine phenology, grape ripening and resulting basic wine chemical parameters, and important varietal volatile compounds by trimming and/or lateral removal at two different time point, bunch closure and veraison.

Defoliation by trimming the canopy to half the original height (FC/HC) and/or lateral removal (L+/L-) was applied at bunch closure and veraison. Key components of berry composition including total soluble solids, pH, Titratable acidity were analysed. Aroma and flavour compounds important for Riesling like β-damscenone, TDN, linalool and geraniol were also quantified.

Results have shown that yield and yield components were not affected by defoliation after fruitset either by trimming or lateral removal. Early or severe treatment both contributed to delayed date of veraison and reduced TSS at harvest. The biggest reduction in TSS was observed when the vines trimmed to half canopy with lateral removal early in the season at bunch closure. The rate of TSS accumulation was thought to be related to the growing degree days (GDD) measured during the ripening period, when the berries progressed from 8 to 18°Brix. Modifying the canopy at bunch

closure decreased juice pH levels more than when the same treatments were applied at veraison. Organic acids concentration increased with early lateral removal. Volatile compounds such as α-terpineol, TDN, geraniol and citronellol also increased in concentrations with leaf removal, especially veraison lateral leaf removal.

This study demonstrated that reducing grape juice sugar concentrations and therefore alcohol in wine, can be achieved by trimming and lateral removing at veraison, without detrimentally influencing compounds that are favorable for Riesling's varietal aroma. This finding suggests that viticulturist may achieve lower berry sugar concentrations without greatly compromising important wine compositional aroma or flavour attributes, which could be desirable for winemakers to produce lower alcohol wines in the context of climate change.

Keywords: canopy management, leaf removal, trimming, grapevine, leaf area to fruit weight ratio, yield, Riesling, soluble solids, veraison, organic acid, volatile compounds, low alcohol wine, climate change, adaptation.

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Table of Contents

List of Tables

List of Figures

Abbreviations

3-SH	3-Sulfanylhexan-1-ol
4-MSP	4-methyl-4-sulfanyl-pentan-2-ol
DOY	Day Of the Year
FW	Fruit Weight
GC-MS	Gas chromatography mass spectrometry
GDD	Growing Degree Days
HPLC	High performance liquid chromatography
IBMP	2-isobutyl-3-methoxypyrazine
LA	Leaf Area
LA:FW	Leaf Area to Fruit Weight
p	Probability
TA	Titratable acidity
TDN	1,1,6-trimethyl-1,2-dihydrinaphtalene
TSS	Total Soluble Solids
VSP	Vertical Shoot Position

Chapter 1

Literature Review

1.1 Introduction

Wine quality is considered to be closely related to berry quality at harvest. Leaf removal via trimming, defoliation or shoot thinning, is regarded as one of the most critical management steps in viticulture that can take place at various stages throughout the growing season. These practices can significantly influence grape physiological development and final quality. Quality grape berries are supported by photosynthetically active leaves, so leaf removal in the bunch zone and trimming alter the leaf area to fruit weight (LA:FW) ratio as well as modify the canopy microclimate by enhancing air movement and sun exposure of grapes. Creating a healthy canopy could also help viticulturists to enhance yield components and optimise pest and disease management. Apart from the basic metrics of berry quality such as total soluble solids concentration (TSS, measured as°Brix), many of components that are responsible for the sensory attributes of a wine are derived from the berry. In addition to berry quality, wine style is also a critical concept of wine quality which can be measured by sensory assessments and/or chemical analyses. Canopy management also serves to target certain wine styles by alternating the varietal volatile composition in grapes. For example, in New Zealand Sauvignon blanc, leaf removal in the cluster zone decreased the contribution of 2-isobutyl-3-methoxypyrazine (IBMP) in the final wine, resulting in less 'green' sensory characteristics (Mosetti et al. 2016).

Previous studies have shown that decreasing the leaf area to fruit weight ratio can impact berry quality by reducing potential grape sugar accumulation and influencing wine style to some extent, and consequently lowering wine alcohol levels and potentially affecting wine aroma and flavour characteristics (Bubola, Rusjan, & Lukić 2020; Parker et al. 2016; Pickering 2000; Pineau et al. 2017). Studies also pointed out the importance of timing of trimming, as the regrowth of laterals may compensate for an early loss in leaf area (Hunter 2017). In light of climate change, these relationships could be exploited in viticulture to counter-steer increasing wine alcohol levels, but this would only be desirable if primary aroma and flavour characteristics are only minimally influenced to meet consumer preferences (Parker et al. 2014; Parker et al. 2015a). A dilemma seems to exist particularly for the Riesling variety, as direct bunch exposure through canopy manipulations is associated with increased concentrations of pre-mature petrol aroma caused by increased 1,1,6-trimethyl-1,2-dihydrinaphtalene (TDN) (Schüttler et al. 2016). The following literature review aims to synthesise current knowledge of the relationship between canopy manipulations to lower grape sugar

concentration and alterations in aroma characteristics, with a focus on Riesling. The identified research gaps then lead to the research questions investigated within this project.

1.2 Grapevine leaves

1.2.1 Roles of leaves and laterals

Grapevines have a very similar structure to many other woody perennials, which has a root system underground to support plant growth by sending water and nutrients to the parts above the soil during the growing season, and where carbohydrates are stored from season to season. The trunk, canes and shoots then support the leaves and shoots growth above the ground. Grapevine leaves have a primary function to harness the light energy and convert carbon dioxide (CO_2) to chemical energy while releasing oxygen (O_2), which defines photosynthesis. The principle of photosynthesis is to use light energy to split water molecules (H_2O) and add hydrogen (H) to CO_2 to form carbohydrates. The carbohydrates produced by photosynthesis are used to support the growth and metabolism of the vine (Creasy & Creasy 2018). All green tissues that contain chlorophyll can conduct photosynthesis and contribute to assimilate production of the vine (Keller 2015a).

Different organs and tissues of the vine act as "sinks" or "sources" depending on whether they are demanding or supplying the photosynthates and assimilates created during photosynthesis and nutrient assimilation. Sinks are organs that cannot provide photosynthate for their own growth demands, and sources are organs that net-export photosynthates and assimilates. Sinks include actively growing vegetative organs like shoot tips, root tips, and expanding leaves, storage organs like shoots or canes, trunks, and roots, and reproductive organs like flowers, developing fruit and seeds. On the other hand, a mature leaf is a typical source organ that is capable of producing more photosynthates than its own needs (Taiz & Zeiger 2010). Woody structures such as trunks and canes act as sources for early season growth and development, as they contain stores of starch amino acids and proteins, which are remobilized in spring to support vine growth before the new leaves start to export energy during the growing season (Holzapfel, Smith, Field, & Hardie 2010).

All plant organs begin their life as sinks, but leaves become sources as they grow. The sink-to-source transition of leaves happens gradually, as leaves need to build up their photosynthetic machinery before they start producing photosynthates. Apical young leaves are mostly sinks, more mature basipetal leaves increase their net carbohydrate contribution, Unfolded leaves have low photosynthesis activity which increases during subsequent leaf expansion (Kriedemann, Kliewer, & Harris 1970). The peak of leaf photosynthesis occurs once a leaf reaches its full size around 30 days after unfolding according to Poni, Intrieri, & Silvestroni (1994), and then gradually declines with advancing leaf age starting around 50-55 days after unfolding. Research has also found that leaves

are net carbohydrate importer until reaching 1/3 of its size, after which they import and export carbohydrates until they become next carbohydrate exporters when they reach about half of their final size (Keller 2015a; Vasconcelos & Castagnoli 2000). Yang & Hori (1980) mentioned that the most basal leaves on the shoot started exporting assimilates when about five or six leaves are present on the shoot. At the time leaves start to export assimilates, the assimilates go to the growing shoot tip and unfolding leaves for expanding the vine structure. Along with shoot growth and the appearance of new leaves, the older leaves find themselves far away from the shoot tip and then change the direction of partitioning toward the shoot base (Wardlaw 1990). Once leaves cease being carbohydrates exporters and sources to other organs, leaves are still photosynthetically active, and their photosynthesis production is enough for their own metabolic demands, only they do not export assimilates (Keller 2015a).

Generally, the closer a source is to a specific sink, the more likely it is the destination of the assimilates. The source to sink relationship between growing shoot tips, laterals, roots, leaves and clusters is dynamic and is influenced by carbohydrates produced as well as the changing sink strength of those organs. Sinks compete with each other, depending on the different developmental stages, there is a hierarchy of relative sink priorities which is sensitive to environmental variables and dependent on the developmental status of the vine (Taiz & Zeiger 2010). For example, inflorescences are weak sinks before fruit set, and the competition of vegetative sinks may affect the ability to set fruit (Winkler, Cook, Kliewer, & Lider 1974). However, once flowers are successfully fertilized, the emerging seeds produce auxin and gibberellin which leads to increased berry sink strength, particularly from veraison onwards (Keller 2015b).

Knowledge about the relationship between leaf age and source activity and size can be useful to manipulate carbohydrate supply throughout grapevine development. During the growth stages of the vine canopy, young leaves are usually present on shoot tips and lateral shoots. Therefore, vines that have not been trimmed and have not had their laterals removed, may have a greater overall canopy photosynthetic output (with the conditions that those leaves are not shaded), which can lead to greater sugar supply to the fruit because there is a larger pool of photo-assimilates available (Candolfi-Vasconcelos & Koblet 1990; Kriedemann, Kliewer, & Harris 1970). Vasconcelos & Castagnoli (2000) also pointed out that "younger" canopies hasten fruit ripening under similar canopy leaf area (i.e. size of source). Therefore, the removal of targeted leaves at different developmental stages, i.e., removing older or younger leaves, may have different implications on the carbohydrate dynamics and grape ripening.

Grapevine lateral shoots that develop during the active shoot growth stage can provide additional photo-assimilating leaf surface area during fruit ripening to support vine growth and berry

maturation. Thus, for moderate vigour varieties, the presence of laterals can act as an important contributor to sugar accumulation in the fruit to improve fruit quality and help accumulate more carbohydrate in the wood (Candolfi-Vasconcelos & Koblet 1990; Parker 2012; Vasconcelos & Castagnoli 2000). Furthermore, research has shown that grape berries on vines with laterals ripen faster and accumulate total soluble solids (TSS) and anthocyanin quicker than those with less or no laterals in the canopy (Hirano, Noda, Hasegawa, & Okamoto 1994; Parker 2012). However, lateral development in vigorous vineyards can become excessive such that air circulation and sun exposure are significantly jeopardized, creating shading and a microclimate which promotes disease development (Gubler, Marois, Bledsoe, & Bettiga 1987; Smart 1985). Smart (1985) concluded that canopy microclimate and source to sink relationships are the two main factors than influence viticultural production and grape quality (see Figure 1.1), and altering source-sink relationships by canopy management can change harvest composition dramatically (Jackson & Lombard 1993).

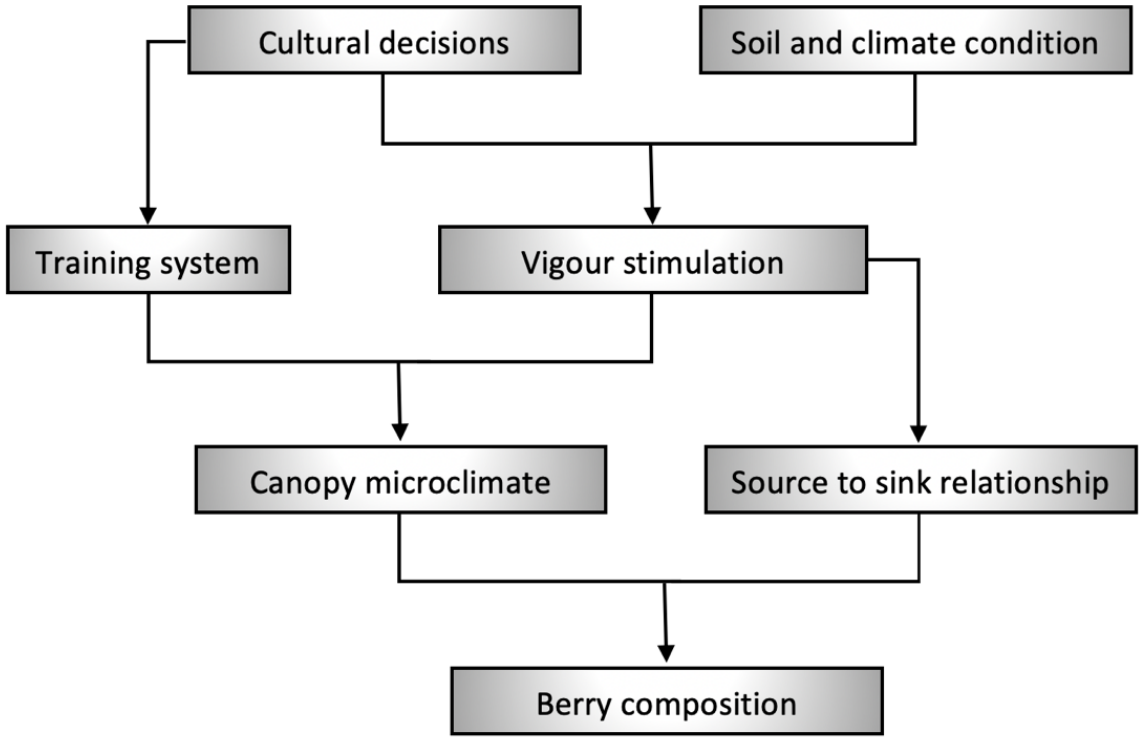


Figure 1.1 Conceptual model to show factors that alter berry composition (Modified from Smart 1985)

Studies have suggested that leaf removal or trimming after flowering may not have detrimental effects on leaf photosynthesis output (Stoll, Lafontaine, & Schultz 2010), but Petrie, Trought, Howell, & Buchan (2003) has shown that vines that have been trimmed with leaf removal have a higher photosynthesis rate on a per-unit leaf area basis, while vines which only have their leaf removed but not trimmed had reduced photosynthesis on a per-unit leaf area basis. This suggested that severe defoliation stimulated leaf photosynthesis rate to compensate leaf area loss, and the results also

indicated that the basal leaves contribute more than the upper leaves to the whole vine photosynthesis.

1.2.2 Altering source-sink relationships

Grapevine canopy management is generally conducted throughout summer and winter. Summer vineyard practices including trimming, leaf removal, and cluster thinning, which can be carried out at different stages through the growing season. Winter practices include winter pruning and trellising. These practices can influence grape berry maturation because changes in LA:FW ratio alter the carbohydrate source-sink balance in the vine.

Leaf removal is a common practice that is applied to modify fruit quality during the growing season. When carried out in the fruiting zone, it allows high-density canopies to have more air circulation and light exposure around the bunches. Defoliation can also change the LA:FW ratio of the grapevine. Grapevine defoliation can affect the yield and composition of grapes at harvest, and the nature of those alterations depends on the timing, position (i.e. leaf age) and degree of the leaf removal/trimming (Bledsoe, Kliewer, & Marois 1988; Poni et al. 2006). It has been found that the leaves unfolding in early spring transit from sink to source more rapidly compared to those unfolding later, which means late formed leaves have progressively shorter periods to contribute to fruit ripening or carbohydrate storage of the vine, and they may even not compensate their growth costs. Thus, viticulture practices that aim at limiting canopy size usually happen in the late growing season to not jeopardize carbohydrates production and consequently berry sugar accumulation or storage build-up.

Due to the apical dominance, the actively growing shoot tips of the grapevine compete with the developing inflorescences for sources as strong sinks (Vasconcelos & Castagnoli 2000). Research has indicated that early trimming increased cluster weight and yield and improved soluble solids concentration if lateral shoot growth is stimulated, and also reduced titratable acidity (TA) due to reduced malic acid by greater cluster exposure to sunlight (Palliotti & Poni 2013). Parker (2012) and Stoll et al. (2013) has also pointed out that trimming shoot tips provokes lateral growth and therefore increases leaf area arising from those lateral shoots. At the early stages of growth, lateral shoots depend on assimilates that are provided by the main shoots, but laterals become net exporters of carbohydrates after two fully expanded leaves present (Hale & Weaver 1962). Apart from providing assimilates to support their own growth, lateral leaves can export carbohydrates to the main shoot and contribute to fruit ripening by balancing out the decreasing photosynthesis capacity of older leaves in the lower position of the shoots (Poni, Intrieri, & Silvestroni 1994) This becomes further evident in the study by (Vasconcelos & Castagnoli 2000) that showed that the most efficient leaves during fruit ripening are the ones located at the top section of the canopy and those

on the lateral shoots. The authors observed that the leaf photosynthesis rate began to decline around 50 days after unfolding. Therefore removing shoot tips by trimming and lateral removal, removes sinks that compete with berry growth but also removes potential sources that will contribute to whole-vine photosynthesis. On the other hand, it has been found that when the vines have their laterals removed periodically with only main shoots retained, sugar accumulation was delayed and occurred at a slower rate, and leaf senescence and abscission were delayed to compensate for the absence of lateral leaves (Parker et al. 2014; Parker et al. 2015a; Parker et al. 2015b; Stoll et al. 2013).

Pre-flowering (before Stage 19) (from the modified E-L Stage by (Coombe 1995)) leaf removal via trimming can increase berry abscission and reduce yield but has little effect on fruitset (Lohitnavy, Bastian, & Collins 2010). According to Poni, Bernizzoni, & Civardi (2008), both trimming and leaf removal in the bunch zone at pre-bloom can cause a huge and abrupt decrease in vine photosynthesis. But this action also triggers the canopy to grow new leaves and increasing their photosynthetic rate due to source: sink ratio decreases (Hunter & Visser 1988). It creates a "younger" canopy for veraison, and more lateral leaves may grow to compensate the early defoliation (Palliotti & Poni 2013). However, eliminating competing growing shoot tips (strong sinks) during inflorescence development (weak sinks) can increase the available carbohydrates for the floral development, which may enhance fruitset (Vasconcelos & Castagnoli 2000). A study carried by Poni et al. (2006) concluded that pre-bloom basal leaf removal results in lower fruitset, cluster weight, berry per cluster, berry size and cluster compactness, but also modify grape quality by improving spray efficacy and light exposure. Palliotti, Gatti, & Poni (2011) also mentioned that basal leave removal at stage 17 (12 leaves separated) delayed the berry sugar loading, lowered the must total soluble solids concentration and resulted in reduced phenolic and anthocyanin concentrations compared to the vines that have not been defoliated.

Trimming alters both source and sink in the canopy, but generally leads to an overall reduction of the source-sink ratio. According to Parker et al. (2014) and Parker et al. (2016), trimming at bunch closure delayed veraison (Stage 35) and slowed total soluble solids accumulation.

Defoliation treatment at fruitset (Stages 27, post-flowering) in Riesling has been studied by Stoll et al. (2013) to see whether changing the LA:FW ratio affected berry maturation and fruit composition. It has been found that the sugar concentrations of Riesling grapes declined at harvest when the LA:FW ratio was significantly reduced, and for the vines that have been trimmed, veraison was delayed by about two weeks, and ripening was delayed by approximately 20 days. It also has been shown in Parker et al. (2015a) and Parker et al. (2015b) that trimming the vines to six leaves at fruitset delayed

veraison, reduced TSS accumulation rate, and lower TSS concentration in the berries at harvest, which could translate into a wine of lower alcohol concentration.

Trimming applied after fruitset (Stage 27) and at bunch closure has shown no or a small difference on yield or yield components (Collins & Dry 2009; Parker et al. 2016). Mosetti et al. (2016) further observed that no yield or berry composition differences occurred with basal defoliation at Stage 29 (berry pepper-corn size), but increasing sunlight exposure in fruiting zone reduced the IBMP and IPMP in Sauvignon blanc grape in early stages of berry development, although no significant difference of berry composition was found at harvest. A recent study by Smith & Centinari (2019) also corroborated that five leaves removed at bloom or fruit set was insufficient to induce a carbohydrate limitation of the berries because early leaf removal vines didn't show any sign of lower fruitset or bud fruitfulness. They also found that there was no compensation via increased in lateral leaf area as a result of their basal leaf removal treatments.

Pre-veraison (Stage 29-34) defoliation (one or two weeks prior to veraison) improves fruit microclimate, and the effect generally depends on the degree of defoliation. Parker et al. (2015a) shown that trimming the vines to six leaves at veraison also slowed TSS accumulation, but the effect is smaller than trimming at fruitset because it did not delay veraison. Petrie, Trought, Howell, & Buchan (2003) has also suggested that leaf removal at veraison reduced soluble solids and sugar per berry at harvest, decreased titratable acidity. Minimal defoliation may lead to a slight increase in sugars and decreased malic acid content in fruit composition via an improvement in canopy microclimate (Hunter, Ruffner, Volschenk, & Le Roux 1995; Palliotti & Poni 2013; Zoecklein et al. 1992).

Defoliation generally affects fruit composition by changing the direction of carbohydrate translocation and reducing the LA:FW ratio. Although decreasing the LA:FW ratio resulted in a delay and slower rate of TSS accumulation, no significant changes have been found in TA and pH of the berries (Parker et al. 2015a). In contrast, Vasconcelos & Castagnoli (2000) found that while tipping at full bloom decreased juice pH; lateral shoot length increased under this situation, which leads to an increased LA:FW ratio compared with the Parker et al. (2015a) study, where lateral regrowth was prevented. Previous research by Bavaresco et al. (2008) also shown that leaf removal in the cluster zone accounted for about 20% of the canopy surface at veraison, decreasing grape acidity in Malvasia di Candia aromatica grapes under cool meteorological conditions but with no obvious impact under warmer and drier meteorological conditions. Likewise, the same leaf removal treatment shown different effect on *Vitis vinifera* L. Croatina, which cool conditions decreased grape soluble solids, but warm conditions did not influence berry composition.

1.3 Berry development and ripening

The beginning of grape ripening phase (veraison) is recognized by berry softening with colour changing, combined with increasing sugars, decreasing acidity, and enlarged volume (Coombe 1992).

Sugars begin to accumulate in mesocarp and exocarp a few days before veraison (Coombe 1987), and Sarry et al. (2004) have suggested that the activation of sugar storage triggers malate produced from the vacuoles, so sugar storage and malate breakdown are activated at veraison simultaneously (Keller 2015b).

Organic acid and protons actively transfer into the fruit vacuoles by H^+ pumps, and the high concentration of H^+ is responsible for the low juice pH. Grape berries accumulate a significant concentration of tartaric and malic acid during berry development and ripening, and these two are the main grape organic acids (Ford 2012). Most organic acids are synthesized and stored inside the berry, starting to accumulate at an early stage of berry development (in the first stage of berry development before the lag phase and veraison). Apart from berry synthesized organic acids, malate can be transported to the berry in phloem (Sweetman et al. 2009). The level of organic acids decreased during fruit ripening (Ford 2012). Malic acid starts breakdown during later stages of berry development (when berries attain veraison) by several enzymes and used for respiration (Webb et al. 1995). Tartaric acid concentration also declines post-veraison in skin and outer pulp fractions due to increased cell size and expansion of the berry, and remain unchanged within other parts of the berry (Possner & Kliewer 1985).

Volatile compounds accumulate during grape ripening, which is a major focus of this study, and these will be reviewed in the next section.

1.4 Volatile compounds in Riesling

Except for sugars, acids and phenolic compounds, volatile compounds are one of the most important groups of compounds in grape berries, responsible for berry and wine sensory attributes. Grape volatile composition is not static but rather changes dynamically throughout grape maturity. The aroma of wine results from a matrix of volatile compounds that varies with grape variety, ripeness, yeast type and activity, pre-fermentative and vinification procedures and ageing (Ebeler 2001; Selli et al. 2006). Aldehydes, alcohols, esters and acids, along with many other compounds present in wines formed a unique aroma profile according to the various concentrations and proportions of these compounds. Complexity is added by various synergetic or antagonistic interactions between individual volatile as well as non volatile wine components that may result in odour intensity amplification or supression (Sáenz-Navajas et al. 2010).

Yield, climate effects and canopy management can all influence volatile aroma compounds. Defoliation, as a canopy management technique, has been studied to improve wine quality by enhancing the composition and concentration of volatile aroma compounds (Diago, Vilanova, & Tardaguila 2010; Verzera et al. 2016). Previous research has found that green aroma components accumulated during early berry growth, and as a berry matures during the third phase of berry development, the contribution of 'green attributes' decreased (Keller 2015b), Pre-veraison has been shown to be a crucial stage for grape aroma development (Kalua & Boss 2010).

As an aromatic variety, the precursors present in Riesling grapes contribute greatly to the final wine style and quality. Most terpenes include norisoprenoids or their glycosylated aroma precursors during the berry ripening even at very advanced stages of maturity. β-Damascenone (megastigma-3,5,8-trien-7-one) with its 'sweet' and 'rose' characteristic is an example. Fang & Qian (2006) found that late maturity wines had much higher concentrations of this compound than the wines made from grapes harvested during early maturity (earlier in stage III of berry development). Prior research has shown that the volatile grape composition, and consequent wine quality of Riesling, may be altered by canopy management such as basal leaf removal just like in the example of like Sauvignon blanc (Wang et al. 2018). Depending on which leaves are removed (i.e. apical/lateral young leaves or basal older leaves) the resulting difference in grape composition could be triggered either by severe changes in sink-source relationships, or by a change in canopy microclimate, or both.

Iland (2011) suggested that vigorous shoot growth may lead to fewer assimilates being directed to the berries, which in turn may limit the synthesis of volatile aroma compounds. In other words, carbon partitioning and allocation may affect the final content of volatile aroma compounds and/or precursors in the berry at maturity. Carotenoids breakdown to produce different bouquets of flavour and aroma compounds in different grape cultivars. Klee (2010) advocated that the resulting profile of aroma volatiles in wines comes from the composition of the carotenoids in grapes. Pre-veraison carotenoid synthesis is induced by light exposure and then regulated by the phytochrome system, so canopy microclimate around the bunch zone may impact the production of carotenoids, whereas low light has been found to be associated with a temporarily decreased carotenoids (Cazzonelli & Pogson 2010).

Analytical capability to separate and identify individual aroma compounds is expanding rapidly nowadays, GC-MS is one of the most common ways to assess volatile compounds quantitatively. Table 1.1 shows some key volatile compounds in Riesling grapes during different development stages. Table 2.2 shows the descriptor, chemical group and odour thresholds of the volatile compounds found in tested wines in this study.

Table 1.1 Key volatile compounds classes discriminating berry development in Riesling (developed from information in Kalua & Boss 2010).

Developmental stage	Volatile compounds
Pre-veraison	Aldehydes
(2–6 weeks post-flowering)	Hexanal
	E-2-Hexenal
	Alcohol
	Hexanol
	Esters
	Z-3-Hexenyl butanoate
	Hexyl Acetate
	Z-3-Hexenyl acetate
	Monoterpene
	Geraniol
	C_{13}-Norisoprenoid
	b-Ionone
	Sesquiterpenes
	a-Caryophyllene
	a-Muurolene
	Calamenene
Veraison	Aldehydes
(7–9 weeks post-flowering)	Hexanal
	E-2-Hexenal
Post-veraison	Aldehydes
(10–15 weeks post-flowering)	Hexanal
	E-2-Hexenal
	Nonanal
	Alcohol
	Hexanol
	Monoterpene
	Geraniol
	Sesquiterpenes
	a-Muurolene

Table 1.2 Volatile aroma compounds identified in tested Riesling wines that are investigated in this research (descriptor, chemical group and odour thresholds).

Analyte	Descriptor	Chemical group	Odour perception threshold (µg/L)	Concertation (µg/L)
Hexanol	Grape juice, resin[1]	Alcohols/C6 compounds	8000[2]	2100-13200[3]
Linalool	Rose, apple-sweet, muscat[4]	Monoterpenes	15[2], 25[5]	1.7-10[3]
α-Terpineol	Floral[6], lily[9]	Monoterpenes	400-500[7]	0.57-16.8[3], 19-145[7]
TDN	Petrol, kerosene[8]	C_{13} norisoprenoids	2[8]	1.3−17.1[8]
Citronellol	Clove, floral[1,6]	Monoterpenes	18[9]	3.70-9.41[10]
Nerol	Sweet rose[6]	Monoterpenes	400-500[7]	2.38-11.23[10]
β-Damascenone	Sweet, rose, baked apple[4,5,11]	C_{13} norisoprenoids	0.05[2,5]	0.29-4.7[3]
Geraniol	Rose, geranium[12]	Monoterpenes	30[2], 130[9]	0.91-44.4[3]

[1] Tao, Li, Wang, & Zhang (2008), [2] Guth (1997), [3] Ferreira, López, & Cacho (2000), [4] Escudero et al. (2007), [5] Bowen & Reynolds (2012), [6] Crespo et al. (2017), [7] Marais (1983), [8] Sacks et al. (2012), [9] Ribéreau‐Gayon, Glories, Maujean, & Dubourdieu (2006), [10] Tomasino (2011) [11] Yuan, Feng, & Qian (2015), [12] Villamor & Ross (2013)

1.4.1 Monoterpenes

Among the volatile compound classes associated with wine aroma, terpenes are major contributors to *V.vinifera* Riesling varietal aroma (Winterhalter, Sefton, & Williams 1990a). In Riesling, they are present in relatively high concentrations and are very obvious in shaping the wine sensory characteristics, and they dominate the varietal aroma of Riesling wines. Nearly 50 terpene compounds have been identified in grapes and wine that have significant effects on wine aroma, and the most prominent terpene compounds occurring in Riesling are linalool, geraniol, nerol and α-terpineol (Malherbe, Moine, & Kramer 2014; Marais 1983).

Terpenes are present in free volatile terpenes and glycosidically bound terpenes (precursors that become volatile with the help of yeasts during the winemaking process) as monoterpenes, sesquiterpenes and C_{13}-norisopernoids which contribute to floral or fruity characteristics of the wine (Black et al. 2015; Skinkis, Bordelon, & Wood 2008). Terpenes can be found in flesh and skins, and are easily extracted with a short skin-contact (Mateo & Jiménez 2000). Generally, the synthesis of terpenes increases with sun exposure, decreases with high temperatures, and the concentration increases with berry maturation, but there are variations between different individual terpenes (Black et al. 2015). However, no studies to date have shown how terpenes change in response to LA:FW ratio manipulation affecting the carbohydrate balance of the vine.

Grape volatile composition changes dynamically over the course of grape ripening. It has been found that terpene production was low at veraison, but some terpenes like geraniol and α-muurolene were produced post-veraison (Kalua & Boss 2010). Environmental conditions should be taken into consideration when deciding about leaf removal treatments to enhance wine qualities. Under cool climate conditions for example, leaf removal decreased grape acidity and trans-resveratrol of aromatic white variety Malvasia di Candia aromatic (Malvasia C.a.); but for the same variety under warmer conditions, leaf removal did not alter acidity and stilbene concentration (Bavaresco et al. 2008).

The pH levels, as well as temperature during fermentation, can also impact the final wine terpene concentration and profile, as those parameters influence the yeast activity and stability of the glycosides. Consequently, the effect of leaf removal on wine terpene concentration may not only arise from altered microclimate or carbohydrate balance directly, but also indirectly by altering the grape titratable acidity and pH (Zoecklein, Hackney, Duncan, & Marcy 1999).

1.4.2 C_{13}-norisoprenoids

Another group of terpenes which are derived from carotenoids degradation existing in grape as tetraterpenoids are the C_{13}-norisoprenoids (Yuan, Feng, & Qian 2015). The most common C_{13}-norisoprenoids in Riesling are β-damascenone and 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN) (Figure 1.2), and they usually have low sensory thresholds (see Table 1.2).

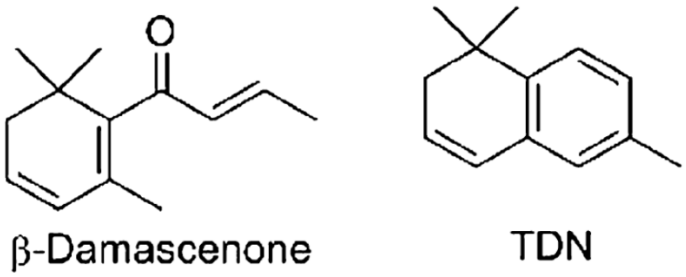


Figure 1.2 Chemical structures of main C_{13}-norisoprenoids in Riesling (Strauss, Wilson, Anderson, & Williams 1987).

β-Damascenone is one of the most ubiquitous plant-derived odorants (Sefton, Skouroumounis, Elsey, & Taylor 2011). Previous research carried by Ferreira et al. (2002) pointed out that when β-damascenone was added to hydroalcoholic solution or model wine, the fruity notes of ethyl cinnamate and caproate were enhanced and the herbaceous aroma of IBMP was masked. Pineau, Barbe, Van Leeuwen, & Dubourdieu (2007) also found that the odor threshold of cinnamate and caproate decreased, and IBMP odor threshold increased after adding β-damascenone into the model wine solution. Both studies indicated that β-damascenone is critical to the wine aroma profile by enhancing fruity perceptions, but the influence of β-damascenone on wine aroma was indirect rather than direct. β-Damascenone has a particularly low odour perception threshold (refer to Table 1.2) and usually quite a high concentration in Riesling wine, which may indicate that instead of monoterpenes, the fruity aromas of this Riesling may be due to norisoprenoids (Skinkis, Bordelon, & Wood 2008). As one of the most important and abounded C_{13}-norisoprenoids, β-damascenone concentration was reported positively related to wine quality score (Ristic et al. 2010).

Another important aroma compound for Riesling grape and wine is 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN), which is usually known as been associated with the sensory characteristics of petrol and kerosene bottle-aged notes when the wine is still young, and it is generally considered undesirable if TDN concentrations are above 4µg/L (Schüttler et al. 2015). The C_{13}-norisoprenoid, TDN, is transformed into glycosidic bound non-volatile precursors by the plant. It is liberated during bottle ageing by acid hydrolysis. The biogenesis of these classes of compounds is affected by grapevine growth conditions. Wines made from Riesling grapes grown in warmer climates have higher concentrations of TDN precursors (Sacks et al. 2012). Moreover, vineyard

management, especially like bunch zone defoliation, affects the development of this compound (Schüttler et al. 2016).

In *Vitis vinifera* Riesling grapes, it was observed that the precursors of TDN were mainly found the juice and not seeds of skins of crushed grapes, and it appeared to develop along with sugar accumulation (Strauss, Wilson, Anderson, & Williams 1987). Early leaf removal directly after fruitset has been found to increase the content of quercetin glycosides in grape skin before veraison (Friedel et al. 2015), and the glycosidic fraction has been identified as the source of precursors for the off-flavour-causing compound TDN (Roscher & Winterhalter 1993). Greater cluster exposure to sunlight and warmer growing conditions have been associated with a higher TDN concentration in grape juice, and total TDN in juice converted to free TDN in finished wine (Marais, van Wyk, & Rapp 1992). According to Kwasniewski, Vanden Heuvel, Pan, & Sacks (2010), the best timing to defoliate in the cluster zone to increase the concentration of both C_{13}-norisoprenoids and carotenoid precursors in the juice is 33 days after berry set. The aroma profile is one of the most important features of Riesling wine, so it is important to find a balance between the precursors that may impact future wine aroma.

It is known that a lot of different aromatic compounds play a role in this sensorial perception. Terpenic compounds, C_{13}-norisoprenoid compounds, sulfur volatile compounds, and varietal thiols are all very important to Riesling. Fruitiness, yellow fruits, ageing notes, citrus fruits, and mineral odors has been identified as the main sensory perception associated with Riesling. Grape skins with a high concentration of lipids are damaged during destemming and crushing process, and C6 compounds with a “fresh green,” “grassy,” or “herbaceous” aromas come from the catabolism of fatty acids. E-2-Hexenal has been found as the most abundant volatile compound in the Riesling grape, and the concentration of it showed a significant increase after veraison (Kalua & Boss 2010). The correlation of measured aroma molecules to Riesling wines’ typicality led to coherent results, showing the importance of the molecules 3-Sulfanylhexan-1-ol (3-SH), linalool, and TDN to the overall perception of typicality of Riesling wines, whereas this approach of linear correlation showed no correlation of varietal thiol 4-methyl-4-sulfanyl-pentan-2-ol (4-MSP) to Riesling wines’ typicality (Schüttler et al. 2014).

1.5 Wine quality and canopy management

Iland (2011) suggested that the quality of wine can be rated according to the type, intensity, complexity, length and balance of its sensory attributes. Naor, Gal, & Bravdo (2002) reported that for Sauvignon blanc, the wine sensory evaluation score decreased with increasing crop load, and that total wine sensory scores decreased with decreasing leaf area to fruit weight below approximately 18 cm^2/g. Another research by Pineau et al. (2017) also provided evidence on the relationship between

leaf removal and wine sensory properties in Pinot Noir grapes. This study has shown that the vines trimmed shortly after fruitset produced fruit with lower soluble solids compared to untrimmed vines, and the subsequent wines made from the trimmed vines were found to have more green and vegetal flavour, and less fruity, spicy, full-bodied characters, and also less overall complexity compared to untrimmed or mildly trimming vines.

A range of compounds contributes to the wine sensory profile. Wine quality is not only decided by the intensity, but also the balance of these compounds. Although most of the 'varietal' compounds are mainly generated, i.e. transferred into their volatile form during winemaking, most of them depend on the pre-fermentation concentration of their respective precursors in grapes, such as IBMP in Sauvignon Blanc. Thus, it is reasonable to say that, to some extent, wine quality is dependent on the quality of berries which are harvested from vines and any physiological change in the vine could make a change in wine.

Climate change is another challenge influencing the date of harvest, alter the speed of ripening and almost certainly create new wine styles compared with regional typicity. Higher alcohol levels and less acidic wines are produced through higher sugar concentrations (Van Leeuwen & Seguin 2006). in harvested grapes and the resulting must. Defoliation may be a way of reducing the speed of berry maturation and tackle the climate changing situation (Parker et al. 2015a; van Leeuwen et al. 2019).

1.6 Conclusion

Manipulating the LA:FW ratio is widely practiced to enhance grape and wine quality, the balance between vegetative growth and reproductive growth, with the aim to produce suitable yield and good quality berries. Lateral shoots, as a part of vegetative tissue, are source for both vegetative growth and reproductive growth. Grape berry quality is determined by season, site, variety, canopy microclimate and sink-source relationships. Leaves, being part of vegetative tissue, provide the photosynthates and assimilates necessary for both vegetative growth and reproductive growth. Berries of acceptable quality are mainly supported by photosynthetically active leaves and influenced by canopy climate around the berries. Also, many of the compounds responsible for the sensory attributes of a wine originate in the berry.

Defoliation is one method to manage source-sink relationships and thus manipulate berry composition. Laterals, as a vital component of grapevine vegetative structure, provide photosynthesis during the later grape growing stages, but also consume energy for their own growth, potentially competing with berries as sinks. Removing laterals completely from the vine and the influence that this has berry composition and later winemaking is an important part of this research.

No study to date has specifically addressed the effect of LA:FW ratio manipulations by removing laterals at different growth stages on aroma or volatiles compounds in Riesling. Managing canopy is one of the most efficient ways of impacting grape berry composition and wine quality. Investigating changes in the LA:FW ratio due to trimming (and understanding the relative contributions of laterals on Riesling berry composition and wine style) will provide useful information on how to use LA:FW ratio manipulations as a technique to delay sugar concentration in the context of climate change.

1.7 Research objective

This study aimed to assess the changes in grapevine phenology, grape ripening and resulting basic wine chemical parameters, and important varietal volatile compounds of the *V.vinifera* cultivar Riesling as a result of reducing the LA:FW ratio via shooting trimming and lateral management. This research will evaluate the consequences of these techniques to produce Riesling grapes of lower sugar concentration. The experimental design focuses particularly on the contribution of laterals in relation to the source-sink balance of the grapevine and the resulting berry and wine quality. In addition, this research aims to consider two different time points of leaf removal at distinct grape developmental stages (bunch closure and veraison) as well as two different canopy types (half canopy with laterals retained, and half canopy with laterals removed). The experimental protocol results in two different carbohydrate balance scenarios at two different time points that can be compared to the non-manipulated control, enabling the following research questions to be addressed:

i) What impact did modify the LA:FW ratio via trimming and lateral removal treatments have on grapevine phenology and grape development?

ii) Have the canopy treatments via trimming and lateral removal efficiently reduced berry sugar at harvest?

iii) How does the canopy treatments via trimming and lateral removal affect the important wine volatile characteristics include C6 compounds, terpenes and C_{13}-norisoprenoids?

In addition, this setup will facilitate discussion around the potential of laterals to replace lost canopy area (i.e. photosynthetically active leaf area).

Knowledge adopted from previous studies confirm that trimming and leaf removal alter the time of the ripening phase of berry maturation and composition, but the contribution of laterals remain unclear. In this study, the focus will be on determining the role of laterals in grape ripening in combination with trimming treatments, investigating how the timing of defoliation influences the

grape/wine quality, and evaluating the relative changes in total soluble solids compared with quality compounds in response to the LA:FW ratio manipulations.

Chapter 2

Material and Method

2.1 Vineyard information

This study was conducted in the 2018/19 growing season in the David Jackson vineyard, Lincoln University, New Zealand. The vineyard is situated at approximately latitude 43° 39’ south, and longitude 172° 28’ east. The soil type is predominantly Paparua and Wakanui series. Two adjacent rows in David Jackson vineyard of *V.vinifera* L. Riesling (clone Gm239-10, rootstock 3309C) was used in this research. The rows were planted in 1998 with a row spacing of 2.5 meters and in-row vine spacing of 1.5 meters and canopy height of 1100m. The grapevine rows were oriented north-south, and the canopies were trained using the Vertical Shoot Positioning (VSP) system on two bilaterally-opposed canes. The first eight vines at each side of the two rows served as buffer, and vines with known trunk disease symptoms or atypical growth were excluded from the experimentation to reduce variability where possible. The growing degree day (GDD) at the research site up from 1 October to 30 April was 923 (10 °C base, calculated using the monthly mean temperature) s, and the long-term average (LTA) was 971. The rainfall received from July 2018 to March 2019 was 274 mm (Lincoln University Season Weather Summaries).

2.2 Experiment design and treatments

The experiment was set up as a randomised block design where seven different treatments were separated in two rows, with treatments replicated four times (one per block), where each replicate comprised of three vines. Leaf area treatments were established by either trimming the canopy to half the height (550mm) compared with the untrimmed full canopy height vines (control) and in combination with retaining (L+) or removing laterals (L-). Treatments were introduced at two different stages of berry development, bunch closure (BC) and veraison (V). All L- treatments had the lateral shoots removed at weekly intervals from the treatment date onward to avoid their influence on the vine carbohydrate balance. Treatments are summarized in Table 2.1.

Table 2.1 Time and degree of trim height and lateral management for experimental treatments.

Treatment	Trim height[1]	Time of trim[2]	Lateral management[3]
Treatment 1	FC	BC/V	L+
Treatment 2	FC	BC	L-
Treatment 3	HC	BC	L+
Treatment 4	HC	BC	L-
Treatment 5	FC	V	L-
Treatment 6	HC	V	L+
Treatment 7	HC	V	L-

[1] FC = Full canopy, HC = half canopy; [2]BC = Bunch closure, V = Veraison; [3] L+ = Lateral retained, L- = Lateral removed.

The schematic of the experiment design in the vineyard can be found in Appendix A.

2.3 Berry sampling, veraison determination and monitoring of berry ripening

The timing of the bunch closure treatment was determined by visually assessing that berries were touching on a bunch (E-L Stage 32). 30 berry samples (samples acrossed the three vines in one replicate) were taken randomly from the bunches from both sides of the row. The first sampling was from four weeks after bunch closure; samples were taken weekly at the initial stage, and bi-weekly samples were taken from pre-veraison until harvest (3rd March 2019). There were 10 times sampling in tatol, which came to 30 x 28 x 10 = 8,400 berries taken from the vines. The average berry weight at harvest is 1.37 g, so the potential yield been removed is 8400 x 1.37 = 11,508 g, or about 11.5 kg. The total yeild of the vines was 142 kg. The weight removed from sampling was 11.5 / 142 = 0.081 corresponding to less than 10% of the final yield. At this low percentage, it should not have impacted ripening dynamics. Veraison progression was monitored for the 30-berry sample by assessing the number of soft berries divided by the total berry number sampled. Berry softness was assessed by lightly pressing on each berry, a berry that and can be crushed easily by finger was considered as a soft berry. When the control vines and vines that haven't been treated before veraison (because these vines haven't delayed veraison) reached 50% berry softness, the veraison treatments were installed (FC V L-, HC V L+ and HC V L-). Veraison day of year was determined by linear regression; values within the range of 25% to 75% were selected for each replicate and used to determine the date at which 50% berry softness via 2-point interpolation (using the two points either side of 50% for each replicate). The speed of total soluble solids accumulation was determined by 2-point interpolation using linear regression between 8 -18 °Brix with day of year.

The 30-berry sample was further weighed using a laboratory scale, then the berries were crushed by hand (pressing on the outside of the sample bag), and the juice was used to assessed TSS (in °Brix) by using a digital refractometer (PR-100, Atago, Japan). pH was measured using a pH meter (Metrohm Ltd., CH-9101 Herisau, Switzerland) throughout grape ripening from pre-veraison until harvest, and

once the percentage berry softness reached approximately 95 – 100, titratable acidity (TA) (g/L) was measured using an Autotitrator 778 Sample Processor and 799 FPT Titrino (Metrohm Ltd., CH-9101 Herisau, Switzerland). The TA was titrated by using a 0.1 N sodium hydroxide solution to titration endpoint of pH 8.2 (Gallander et al. 1987). This berry ripening monitoring was further supported by determining the sugar loading per berry (sugar per berry was calculated using average berry weight, °Brix x 0.59 x 17 x berry weight; sugar loading = (°Brix x 0.59 x 17 x berry weight)/day (Deloire 2011).

2.4 Harvest

Grapes from all treatments were harvested on the 3rd March 2019 by hand into 10kg harvest crates. Any bunches with more than 5% of botrytis (visual assessment) were rejected for experimental winemaking (see section 2) but were included in the yield calculation. Yields were estimated by using average cluster weight of bunches with less than 5% botrytis x total cluster number (all bunches irrespective of botrytis assessment) x vine number for each experimental unit (three vines).

2.5 Leaf area measurement

Leaf area was measured after harvest and before leaf senescence. All leaves from one vine in each experimental unit were plucked and collected (defoliating trunk to trunk). The leaf from main shoots and lateral shoots were collected separately into two bags for all treatments where laterals were present (L+). Leaf samples were weighed then sub-sampled to approximate 100 g per sub-samples. These sub-samples were stored at 4 °C until measurement using a LI-3100 leaf area meter (Li-Cor, Lincoln, NE, USA). The leaf area for the sub-samples was measured, and the original leaf area was calculated by this formula:

$$\frac{Total\ leaf\ area\ (m2)}{Total\ weight\ (kg)} = \frac{Sub{-}sampled\ leaf\ area\ \ (m2)}{Sub{-}sampled\ weight\ \ (kg)}$$

This data was used to extrapolate the leaf area of the original sample size.

2.6 Winemaking

Small scale vinification was conducted for each of the 28 replicates. After the harvest, the grapes were stored in 4°C chiller overnight and subsequently pressed by a Zee press (Stainless Systems Ltd, New Zealand), and the juice was transferred into 1-litre glass demijohns. To avoid oxidation during cold settling, 100 mg/L potassium metabisulphite (PMS) was added using a 10% (w/v) PMS solution into the juice immediately after crushing. The juice was settled at 4°C overnight and racked the next day into clean 1-litre glass demijohns prior to inoculation with 150 mg/L of yeast (LALVIN EC1118™, Australia, re-hydrated according to manufacturers instructions) and with the simultaneous addition of 120 mg/L of nutriferm energy Superferm® (BSG Wine, USA). Fermentations were carried out in a

temperature-controlled room at 12°C. Ferment progression was monitored by weighing the fermentation vessel daily, and once no further weight loss occurred, a Clinitast® tablet (company, country) was used to assess the remaining sugar in the wine. The wines were considered dry when the residual sugar dropped below 4g/L. Nine out of 28 ferments stopped fermenting (reached dryness) gradually by 28 days after the fermentation started. Ferments were considered 'stuck' when the weight stopped dropping for more than two days and the residual sugar was still higher than 4g/L. Stuck ferments were given a DAP addition of 25 mg/L then moved from 12°C ferment room to a 25°C ferment room to support the continuation of fermentation. Once each ferment reached dryness, PMS was added at a rate of 100 mg/L and the wine were stored at 1°C in a temperature-controlled room until all ferments stopped. Racking was carried on 27th May 2019, and bottling was carried on 8th July 2019. Wines were bottled with the addition of 100 mg/L PMS and into 0.375 ml glass bottles, sealed with screwcaps and stored at 4°C before analysis.

2.7 Wine analysis

2.7.1 Chemicals

Analysis of wine reagents and reference compounds used for analyses were purchased from Sigma-Aldrich (New Zealand Ltd) and Merck (New Zealand Ltd). Deuterated standards were purchased from Sigma-Aldrich and EPTES (Switzerland). Ethanol was purchased from Scharlau Chemie SA (Spain), HPLC Grade ACS ISO UV-vis.

2.7.2 Analysis of sugars in wine by High-performance liquid chromatography (HPLC)

Non-deuterated standards were used to produce standard curves: standard concentrations of D-fructose and D-trehalose were established at 0, 50, 100, 150, 200, 300ppm respectively, D-glucose was 0, 5, 10, 50, 100ppm, and Glycerol was 0, 100, 300, 500, 1000ppm. All solutions were stored at 4°C until usage. The method used for sample preparation was described by Phillips et al. (2018). The wine was diluted 1:5 with 12.5% HPLC standard ethanol mixture then filtered through 0.45 µm nylon membrane (Phenex NY, USA) into a vial before samples were injected onto the HPLC.

The chromatography was performed using two columns, a 250 x 4.6mm Prevail™ Carbohydrate ES Columns (Grace Davison Discovery Sciences, USA) with a 7.5 x 4.6mm Prevail Carbohydrate guard column (Grace Davison Discovery Sciences, US). The mobile phase comprised acetonitrile (mobile phase A) and distilled water (mobile phase B) with following phase (% given correspond to distilled water, mobile phase B): runtime 0-5 min, increased from 0-20%; 6-10 min, increased from 20%-50%; 10-11min, decreased from 50%-20%; 11-15min, decreased from 20%-0%. The flow rate was 1.0 ml/min, and the column temperature was set to 20˚C. 10 µl of the prepared sample was injected into

the Shimadzu HPLC system. Glycerol, D- Fructose, D- Glucose and D-Trehalosewere measured by Shimadzu (Shimadzu Corporation, Kyoto. Japan) equipped with an Alltech Model 3300-ELSD detector (Alltch Associates, Inc. United States), where the detector nitrogen flow rate was set t 1.4 L/min at 38˚C. The sugars and organic acids identification were both completed by comparing retention times of standards, and then quantified by the peak area of chromatograms using external calibration standard curves (Phillips et al. 2018). Data processed using LC solution software Lab solution (Shimadzu, Japan). Method used to analyse Sugar was taken from Phillips et al. (2018). The final wine alcohol levels were not measured due to time resources limitation, but treatments resulted in significantly different TSS levels which certainly translate into respective differences in wine alcohol levels given the wines reached dryness.

2.7.3 Analysis of Organic Acids by HPLC

Depending on organic acids concentration range in the sample, the single organic acid standard curve solution was prepared by the organic acid stock standard solution to make the concentration of 0.015, 0.03, 0.15, 0.6 1.5 ppm with 12.5% ethanol. The organic acid concentration varied from 0 to 1500ppm. All solutions were stored at 4°C until usage. The wine was diluted 1:5 with 12.5% HPLC standard ethanol mixture then filtered through 0.45 µm nylon membrane (Phenex NY) into a vial before samples were injected onto the HPLC.

The column used was 250 x 4.6mm, 5µm particle size Prevail™ organic acid column (Grace Davison Discovery Sciences, USA) with a 7.5 x 4.6mm, 5µm particle size guard column (Grace Davison Discovery Sciences, USA). The mobile phase comprised a 25 mM KH_2PO_4 water solution (pH 2.35, adjusted by H_3PO_4), which was filtered through a 0.45 µm cellulose acetate membrane. The flow rate was 0.6 ml/min, and the column temperature was set to 50˚C. The detector wavelength was 210 nm. 20 µl of the prepared sample was injected into the Shimadzu HPLC system (Shimadzu Corporation, Kyoto. Japan). Method used to analyse Sugar was taken from Shi et al. (2011).

2.7.4 Analysis of Volatile Compounds by Gas chromatography-mass spectrometry (GC-MS)

Detailed information regarding all of the eight standards used to generate standard curves for quantitative analysis can be found in Appendix B. A spiked recovery test was used to assess the matrix effect on each analyte. Selected wine samples were spiked with an aliquot of the concentrated composite working standard to the concentration level of standard 3 (6 standards were used in the calibration curve).

Primary standard solutions were prepared in 100 % ethanol (HPLC Grade ACS ISO UV-vis, Scharlau Chemie SA, Spain) for all of the aroma compounds. All the primary standard solutions were stored in amber 40mL vials in a freezer at -20 °C. The working standard was made by diluting primary standards for the individual compounds with a solution of 10 % ethanol in deionised water. This composite standard was then split into small amber vials and stored at -20 °C until it was used.

Standards for GCMS analysis were prepared on the day by diluting from the concentrated composite working standard and made up in reconstituted rotary evaporated de-aromatized wine (14 % ethanol, pH 3.5). Each standard vial was prepared in duplicate and diluted in diluent buffer (5 g L^{-1} tartaric acid in deionized water, adjusted to pH 3.5) standardised to an ethanol concentration of 1.4 % (10-fold dilution of the de-aromatized wine, 14 % ethanol).

Primary standards were also prepared in 100 % ethanol for each of the deuterated analogues used as internal standards. A concentrated composite working internal standard was then made by diluting the primary standards for the individual compounds to a solution of 10 % ethanol in deionised water. This composite internal standard was then split into small amber vials and stored at -20 °C until it was used.

Prior to use the SPME fibre (2 cm long Stableflex DVB/CAR/PDMS combination) was conditioned at 270 ° C in the injection port for 1 hour. Before each sample was run the SPME fibre was conditioned for 10 minutes at 270 ° C in a fibre conditioning station attached to the Combi-Pal auto sampler (Company, country) used with the Shimadzu GCMS instrument. Helium was used in the fibre conditioning station to create an oxygen free atmosphere.

Sample preparation involved pipetting 0.9 mL of wine and 8.06 mL of diluent buffer (5g L-1 tartaric acid in deionised water, adjusted to pH 3.5) into 20 mL SPME sample vials (a 10-fold dilution of the wine), followed by 40 μL of the internal standard solution (5 internal standard mix see Appendix B). 4.5 g of crystalline sodium chloride was then added to the SPME vial just prior to capping. Samples and standards were then placed in a sample tray held at 8 °C awaiting extraction. Samples were incubated initially for 10 minutes at 50 °C during which time the vial was agitated at 500 rpm. After 10 minutes the SPME fibre (2 cm long Stableflex DVB/CAR/PDMS) was exposed to the headspace of the vial for a period of 40 minutes at 50 °C. During this exposure period, the headspace volatiles were adsorbed onto the fibre.

The instrumentation methodology used here was as described in Tomasino et al. (2015). In summary, the GCMS analysis was carried out on a Shimadzu GCMS-QP2010 gas chromatograph-mass spectrometer equipped with a Combi-Pal autosampler ready for automated SPME. GCMS solutions version 4.45 (Shimadzu, Japan) was used as the data acquisition software. The chromatography was

performed using two GC columns in series namely a Rtx-Wax 30.0m x 0.25mm ID x 0.5µm film thickness (Polyethylene Glycol - Restek, Bellefonte, PA, USA) and a Rxi-1MS 15m x 0.25mm ID x 0.5µm (100 % dimethyl polysiloxane - Restek, Bellefonte, PA, USA). Helium was used as the carrier gas with the GCMS set to a constant linear velocity of 33.5 cm sec-1. The injector was operated in splitless mode for 3 minutes then switched to a 20:1 split ratio.

The column oven was held at 35 °C for 3 minutes (during desorption of the SPME fibre), then heated to 105 °C at 3 °C min^{-1} and held at this temperature for 10 minutes before being heated to 140 °C at 2 °C min^{-1} and held for a further 10 minutes, then further ramped to 198 °C at 4 °C min^{-1} before being rapidly heated to 250 °C min^{-1} at 30 °C min^{-1} and held for 9.93 minutes. The total run time was 90 minutes, see table in Appendix B for elution times. The interface and MS source temperatures were set at 250 °C and 200 °C respectively. The MS was operated in electron impact mode (EI) at an ionization energy of 70 eV. All analytes were analysed in single ion monitoring (SIM) mode with selected ions used for the quantification of these analytes.

Dilution of the wine sample was necessary to prevent column overloading when using splitless injection mode. This served to aid both the chromatography and the SPME fibre extraction procedure as it reduced the competition pressure for sites on the SPME fibre, resulting in a more linear response for most compounds. Dilution of the wine samples reduced the matrix effect ensuring ethanol concentrations were therefore diluted to approximately 1.4 %. The spiked wine samples were then diluted 10 times (0.9 mL wine, 8.06mL diluent buffer, 40µL internal standard) in the SPME vial, (refer to Appendix B for details). Spiked samples were analysed in duplicate and compared to their unspiked partners. The concentration difference was then compared with the expected analyte concentration, as determined by standard 3 of the calibration curve, and reported as a percentage.

2.8 Protein stability

Protein stability testing was carried out before bottling. 50ml wine sample from each bottle was collected and filtered by Whatman # 1-grade filter (pore size of 11 µm) (Cytiva, USA) and stored in a 50 ml capacity test tube with screw cap. The test tubes were put in a heat resistant holder with their caps loosely screwed on top, then put into the incubator oven at 80°C for six hours. The tubes were removed from the oven and cool down to room temperature after the heating process. An intense light was used to shine through the test tube and examined the presence of haze. If the wine in the test tube showed a presence of haze by visual assessment, then this wine sample did not pass this test.

2.9 Statistics

The speed of berry ripening (i.e. rate of berry TSS increase) was calculated by the slope of a linear function (y = ax + b) using linear interpolation between 8 and 18 °Brix.

GDDs (base 10°C) were calculated for each plot based on daily GDD from the 8-18 °Brix.

Outliers surpassing the the mean ± 2 x standard deviation have been deleted from the dataset and replaced by auto-generated number by using the MICE package and VIC package in RStudio (RStudio Inc., US) to eliminate interference of the statistics. In a specific case of a replicate belonging to Treatment 1 a yield outlier has been deleted from the dataset due to an unusual small bunch number, which may have been caused by previously not noticed disease or pruning mistakes.

Treatment means were analysed via one-factorial analysis of variance (ANOVA), and separated by unprotected Fisher's Least Significant Differences (LSD) test at $p<0.05$ using the Agricolae package in RStudio (RStudio Inc., US).

All graphs were plotted in RStudio by ggplot2.

Chapter 3

Results and discussion

3.1 Veraison, leaf area and yield

Table 3.1 Veraison, leaf area and harvest data.

	Veraison DOY	Bunch Total	Yield Per Vine (kg)	Leaf area	LA:FW	Berry Weight (g)
T1 (BC V FC L+)	56.9cd ± 1.1	102.0ab ± 22.8	2.3ab ± 0.9	7.2a ± 1.3	3.5a ± 1.1	1.4 ± 0.2
T2 (BC FC L-)	62.0a ± 2.3	105.8ab ± 14.8	2.0b ± 0.5	3.2bc ± 0.4	1.8bc ± 0.8	1.2 ± 0.2
T3 (BC HC L+)	60.9ab ± 2.9	107.8ab ± 16.7	2.1ab ± 0.3	4.3b ± 1.0	2.1b ± 0.7	1.4 ± 0.2
T4 (BC HC L-)	63.3a ± 1.2	99.3ab ± 12.1	1.9b ± 0.3	2.3c ± 0.4	1.2c ± 0.3	1.4 ± 0.2
T5 (V FC L-)	58.9bc ± 1.6	113.3ab ± 14.5	2.7a ± 0.5	3.4bc ± 0.6	1.3bc ± 0.2	1.4 ± 0.2
T6 (V HC L+)	56.3d ± 1.6	118.8a ±16.9	2.4ab ± 0.3	3.4bc ± 0.5	1.4bc ± 0.3	1.4 ± 0.2
T7 (V HC L-)	57.9cd ± 2.5	92.0b ± 14.8	1.8b ± 0.4	2.3c ± 0.4	1.4bc ± 0.6	1.4 ± 0.1

Mean ± one standard error.
DOY = day of year; LA:FW = leaf area to fruit weight ratio.
FC = Full canopy, HC = half canopy; BC = bunch closure, V = Veraison; L+ = lateral retained, L- = lateral removed

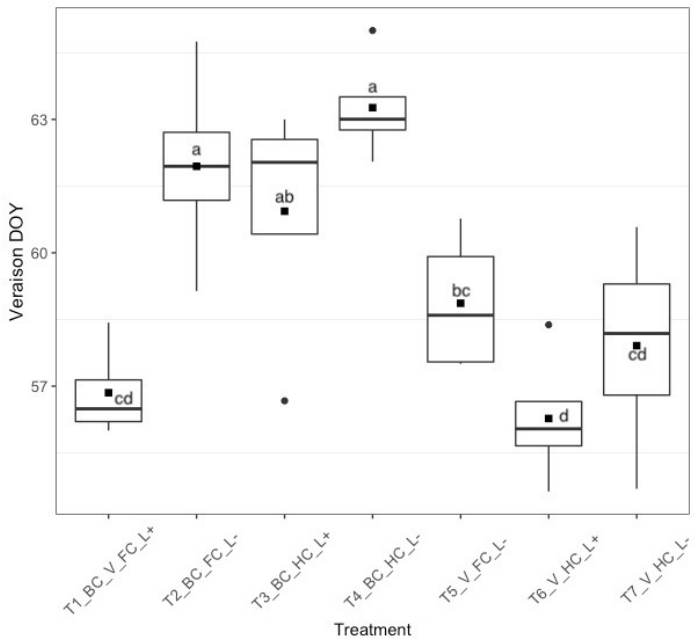


Figure 3.1 Veraison day of year.

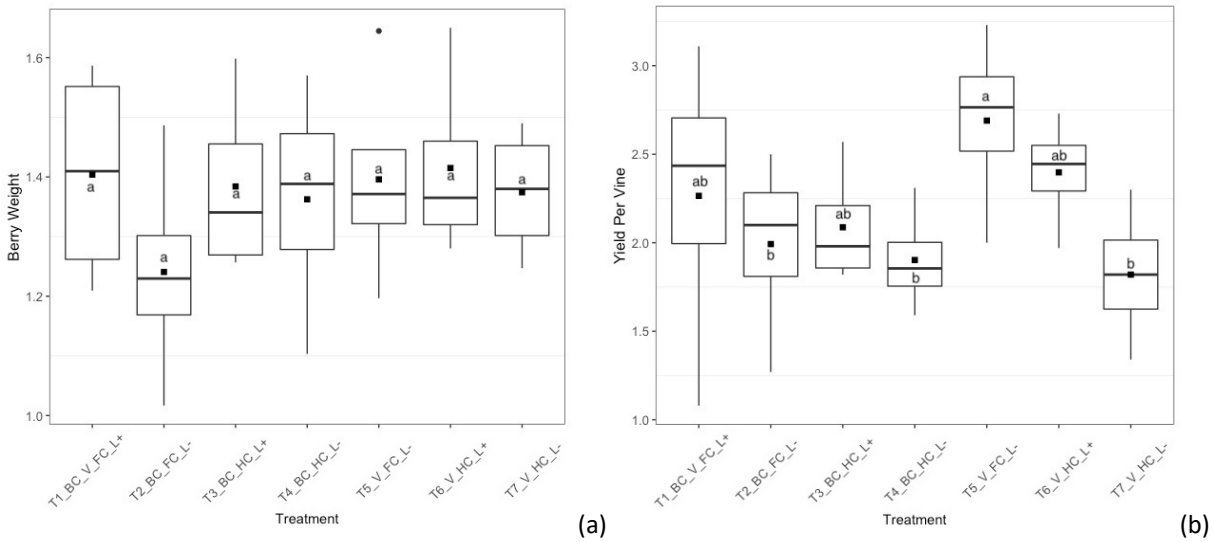
Boxes with different letters were significantly different by Fisher's LSD test ($p < 0.05$); ●: outlier; ■: mean.
DOY= Day of the year; FC = Full canopy, HC = half canopy; BC = bunch closure, V = Veraison; L+ = lateral retained, L- = lateral removed

All defoliation treatments regardless of trimming and/or removing laterals at bunch closure (T2, T3 & T4) reached mid-veraison significantly later compared to the control (T1) (p=9.01e^{-5}) (Table 3.1). By removing the laterals at bunch closure (T2) while maintaining a full canopy (leaves on main shoots), it delayed mid-veraison by five days compared to when no leaves were removed (T1) (Figure 3.1). This

was equivalent in timing to trimming the canopy to half height, irrespective of laterals present (T3 vs T4).

The date of applying veraison treatments was determined by the time of mid-veraison based on berry softness; therefore those veraison treatments (T5, T6 & T7) applied on 27th Feb 2019 (DOY=58) were not significantly different from the control because no canopy management has been applied to those treatments before the time of mid-veraison. This however reinforces the homogeneity of the selected test plot in this study. The significant difference between vines with half canopy at bunch closure (laterals present or absence) compared with the same treatments at veraison (T3 vs T6 and T4 vs T7) was not therefore related to the application of veraison treatments.

There were two possible reasons causing the delay in mid-veraison for vines with reduced canopy due to trimming and lateral removal (T2, T3 & T4): i) the canopy was getting older without lateral shoots' leaves growing actively, and ii) the changing microclimate around bunch zone. The lateral shoots with a greater number of leaves were actively growing, and can provide assimilates as sources for grape berries (sinks) (Vasconcelos & Castagnoli 2000). Other studies have indicated that vines carrying only main shoots compensated for the removed laterals by delaying leaf senescence and abscission (Candolfi-Vasconcelos & Koblet 1990), indicating an upregulation of source activity in response to the removal of leaves. Leaf senescence and abscission were not assessed in this current research project, however, in future studies, these can be included and investigated to see how increasing canopy age and delayed leaf senescence impact the speed of grapevine and berry development.



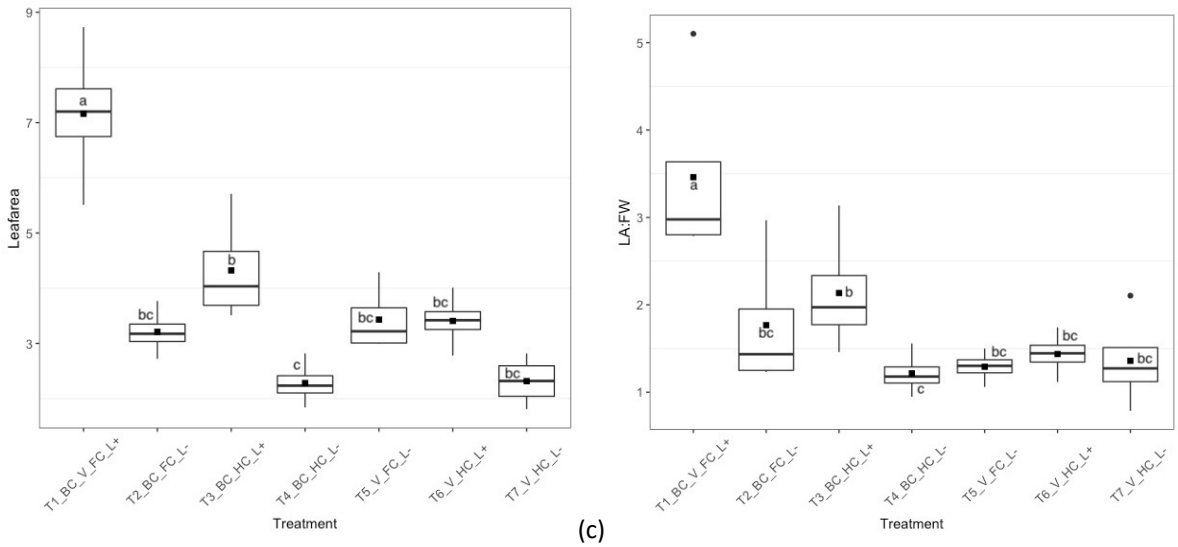


Figure 3.2 Yield and canopy data.

Boxes with different letters were significantly different by Fisher's LSD test ($p < 0.05$); ●: outlier; ■: mean.
FC = Full canopy, HC = half canopy; BC = bunch closure, V = Veraison; L+ = lateral retained, L- = lateral remove
(a): Berry weight at harvest (g)
(b): Yield per vine (kg)
(c): Leaf area per vine (m^2)
(d): Leaf area to fruit weight ratio (m^2/kg)

Vines which had reduced canopy height and laterals removed at veraison (T5) had a significantly higher yield compared to other lateral removed treatments (T2, T4 & T7) (p=0.0005) (Figure 3.2). All other treatments were not significantly different in yield. Vines with laterals removed (T2, T4 and T7) had a reduced source size which was even further reduced if the treatment included canopy trimming (T4 and T7), which may have caused a slight decrease in the overall yield due to lack of carbohydrates under conditions of severe defoliation (Bennett 2002). All treatments were applied after flowering. Therefore the treatments should not have impacted the number of bunches per vine as yield component. Therefore, although there were statistical differences in bunches numbers, there were no trends with regard to types of leaf removal (trimming or laterals) nor the timing of leaf removal.

Apart from the low bunch number for the half canopy lateral removal treatments (T4 &T7) that may have been caused by normal vineyard variation, there were no significant differences in average berry weights. Due to the fact that these yield components were fixed and the timing of the treatments was late in the season, the defoliation had little impact on berry growth. This result has been proved by previous studies; according to Poni et al. (2006) and Palliotti, Gatti, & Poni (2011), early leaf removal (pre-bloom) reduced berry size and fruit set but increased TSS, grape phenolics and anthocyanins due to a slightly decreased LA:FW ratio; however, post fruitset leaf removal had marginal effects on yield and yield components (Hunter, Ruffner, Volschenk, & Le Roux 1995; Palliotti & Poni 2013). Similarly, Parker et al. (2014) and Collins & Dry (2009) found that trimming shortly

after fruitset was not able to alter the yield. However, Wolf, Zoecklein, Cook, & Cottingham (1990) have found a trend that vines trimmed after fruitset have a higher yield than those were not trimmed in two of three years, where many other research found that trimming or leaf removal before berries reached pea size (E-L stage 31) can result in reduced yield (Bennett, Jarvis, Creasy, & Trought 2005; Candolfi-Vasconcelos & Koblet 1990; Poni et al. 2006). Furthermore, previous research mentioned that early leaf removal (pre-bloom) increased TSS (Gatti, Bernizzoni, Civardi, & Poni 2012; Palliotti, Gatti, & Poni 2011; Poni et al. 2006), but this also results in a decrease in yield. Overall though, most studies indicate little yield change from bunch closure onwards, as found in this experiment.

The control (T1) has a significantly higher LA:FW ratios compared with other treatments, which is expected because it had the highest leaf area (Figure 3.2(c)). The treatments with canopies with laterals (T3, T6) generally had higher LA:FW ratios compared to the treatments without laterals. When a full canopy without laterals was established at bunch closure (T2), this treatment had a similar LA:FW ratio to vines with full canopy and laterals established at veraison because the leaf area was limited by removing the laterals in both cases. The leaf area of remaining leaves did not show any increase in size or compensate source size for having laterals removed. In general, results indicated that LA:FW ratio trends predominantly reflected the trend in leaf area, and this is further evidence that the yield is not having much of a confounding effect in the LA:FW ratio calculations.

All treatments were applied after fruit set, which means the leaves on the primary shoot were almost fully expanded at that time, so the half canopy treatment without laterals did not have young or new leaves, and thus the leaf area of these vines was slightly lower than the half canopy vines with laterals. However, a reduced photosynthetic source size by trimming and/or leaf removal could lead to some increases in the rate of photosynthesis per unit leaf area (Hunter & Visser 1988; Petrie, Trought, Howell, & Buchan 2003), although this was not measured in this study. Grapevines have the ability to regulate in response to a relatively low source to sink ratio like restricted leaf area, through higher photosynthetic rates (Hunter & Visser 1988; Kliewer & Antcliff 1970) unless the leaf area is extremely low.

3.2 Berry development and composition

Table 3.2 Grape parameters at harvest and rate of TSS increment.

	Rate of TSS increment(°Brix/day)	TSS(°Brix)	pH	TA(g/L)
T1 (BC V FC L+)	0.33d ± 0.04	20.25a ± 0.25	2.77a ± 0.02	11.50ab ± 0.82
T2 (BC FC L-)	0.41ab ± 0.00	19.30abc ± 0.43	2.69b ± 0.07	11.91ab ± 0.61
T3 (BC HC L+)	0.42ab ± 0.05	19.20bcd ± 0.52	2.68b ± 0.02	12.04ab ± 0.63
T4 (BC HC L-)	0.46a ± 0.02	18.25d ± 0.85	2.69b ± 0.06	12.43a ± 0.82
T5 (V FC L-)	0.39bc ± 0.05	19.58ab ± 0.53	2.70b ± 0.02	11.48ab ± 0.40
T6 (V HC L+)	0.35cd ± 0.04	19.75ab ± 0.17	2.73ab ± 0.04	11.03b ± 0.62
T7 (V HC L-)	0.34cd ± 0.02	18.48cd ± 1.11	2.78 a ± 0.02	11.36b ± 1.29

Mean ± one standard error.
Rate of TSS increment determined as a linear function y=ax+b between 8 and 18°Brix.
TSS = Total soluble solids; TA = titratable acidity
FC = Full canopy, HC = half canopy; BC = bunch closure, V = Veraison; L+ = lateral retained, L- = lateral removed

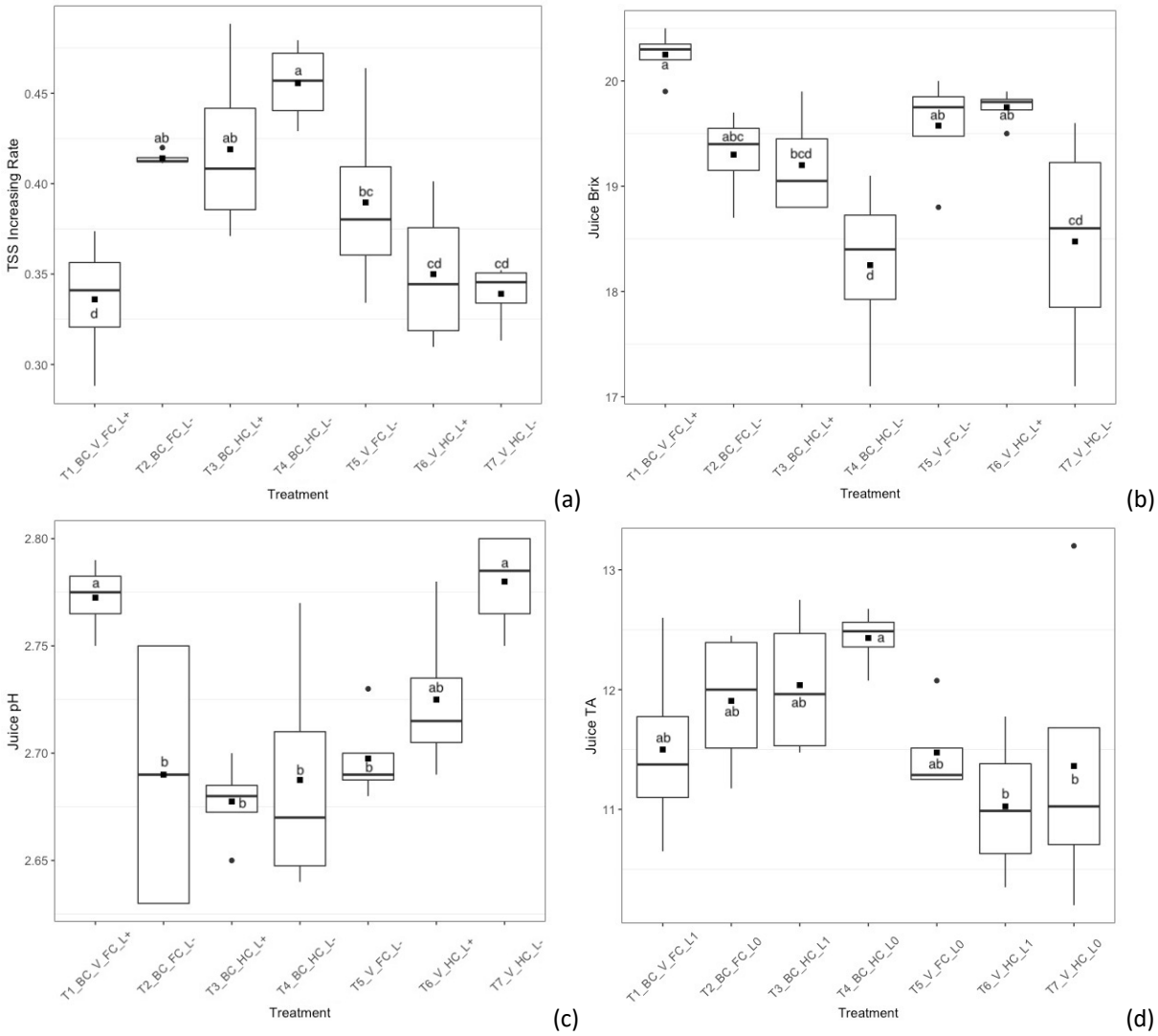


Figure 3.3 Total soluble solids (TSS) accumulation and juice parameters at harvest.

Boxes with different letters were significantly different by Fisher's LSD test ($p < 0.05$); ●: outlier; ■: mean.

TA = Titratable acidity; FC = Full canopy, HC = half canopy; BC = bunch closure, V = Veraison; L+ = lateral retained, L- = lateral removed
(a): Rate of increasing berry TSS concentration (linear interpolation between 7 and 18 °Brix) (°Brix/day)
(b): TSS (°Brix) at harvest
(c): pH at harvest
(d): TA at harvest (g/L)

Vines which had reduced canopy height and laterals removed at bunch closure (T4) had the fastest speed of TSS accumulation but the lowest final TSS (°Brix); vines which had reduced canopy height with laterals (T3) has a similar speed of TSS accumulation rate as T4. Compare to bunch closure (T4), vines which had reduced canopy height and laterals removed at veraison (T7) had similar final TSS concentrations but a much lower rate of TSS accumulation. The TSS increase from bunch closure to harvest has been shown in Figure 3.4, and it can be seen from the figure that treatments with faster TSS accumulating rate (T2, T3, and T4), visually depicting the increased rates determined in Table 3.2 and Figure 3.3). On the contrary, treatments that had slower TSS accumulating rate (T1 and T7) also had more variable accumulation over the time course (for example at DOY63 and 71). Also, the control had a relatively slow rate of TSS accumulation, the same as veraison half canopy treatments (T6 and T7). No significant differences of rates of TSS accumulation or TSS at harvest were found between vines with full canopy and laterals removed (T2) and vines with half canopy and laterals present (T3), this may indicate in this case the leaves on the full canopy may be compensated the lack of laterals in T2 while the laterals compensated the lack of canopy height in T3 and *vice versa*.

Stoll et al. (2013) and Parker (2012) has found that trimming shoot tips provokes lateral growth therefore leaf area arising from those lateral shoots increased and assimilates produced by those leaves were supporting vine growth and berry ripening. This support the finding in this study that vine trimmed to half canopy but with laterals retained (T6) results in higher TSS at harvest compared to the vines that have not been trimmed but removed laterals (T5) at veraison.

Previous research has indicated that greater TSS and rates of TSS accumulation generally occurs in untrimmed vines, so it would be expected that vines with half canopy and laterals removed at bunch closure (T4) should not have had the fastest rate of TSS accumulation. Parker et al. (2014) and Parker et al. (2015a) have reported that rate of TSS accumulation was slowed with shoot trimming at fruitset, but fewer differences if the treatment were carried at veraison. Wolf, Zoecklein, Cook, & Cottingham (1990) found that vines trimmed to 20 leaves had higher soluble solids at harvest compare to the vines trimmed to 10 leaves, but also higher than the control. Vines trimmed to 10 leaves delayed sugar accumulation and this delay persisted until harvest.

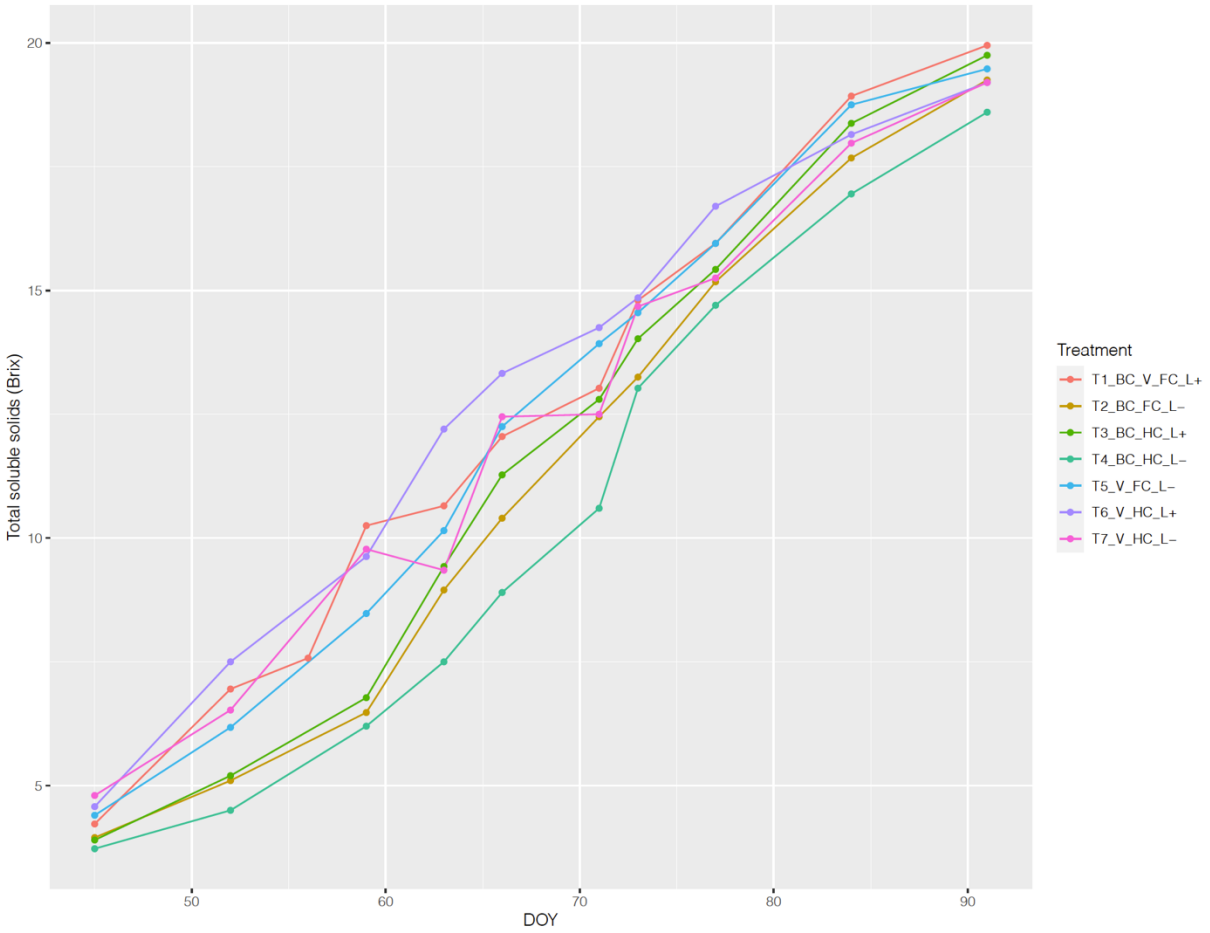


Figure 3.4 Total soluble solids (°Brix) increase during ripening.

DOY = day of year; FC = Full canopy, HC = half canopy; BC = bunch closure, V = Veraison; L+ = lateral retained, L- = lateral removed

Table 3.3 Sugar per berry (mg/g), duration of the period from 8-18 °Brix and Growing degree days (GDD) and daily average GDD (base temperature 10°C for the period from 8-18°Brix).

	Sugar per berry at harvest	Duration of the period from 8-18°Brix	GDD	Average daily GDD
T1 (BC V FC L+)	280.9	30.0a ± 3.4	208.0a ± 31.1	6.9b ± 0.3
T2 (BC FC L-)	239.5	24.2cd ± 0.2	168.9c ± 9.7	7.0b ± 0.5
T3 (BC HC L+)	274.2	24.1cd ± 2.8	170.3c ± 24.4	7.1b ± 0.7
T4 (BC HC L-)	254.2	22.0d ± 1.1	174.2bc ± 11.1	7.9a ± 0.6
T5 (V FC L-)	272.7	26.0bc ± 3.5	169.2c ± 24.9	6.5b ± 0.2
T6 (V HC L+)	272.3	28.9ab ± 3.4	195.6abc ± 29.2	6.8b ± 0.2
T7 (V HC L-)	264.7	29.6ab ± 1.6	205.7ab ± 15.4	7.0b ± 0.3

Mean ± one standard error.
GDD = Growing degree days (base 10); Sugar per berry = °Brix (TSS) x 0.59 x 17 x berry weight;
FC = Full canopy, HC = half canopy; BC = bunch closure, V = Veraison; L+ = lateral retained, L- = lateral removed

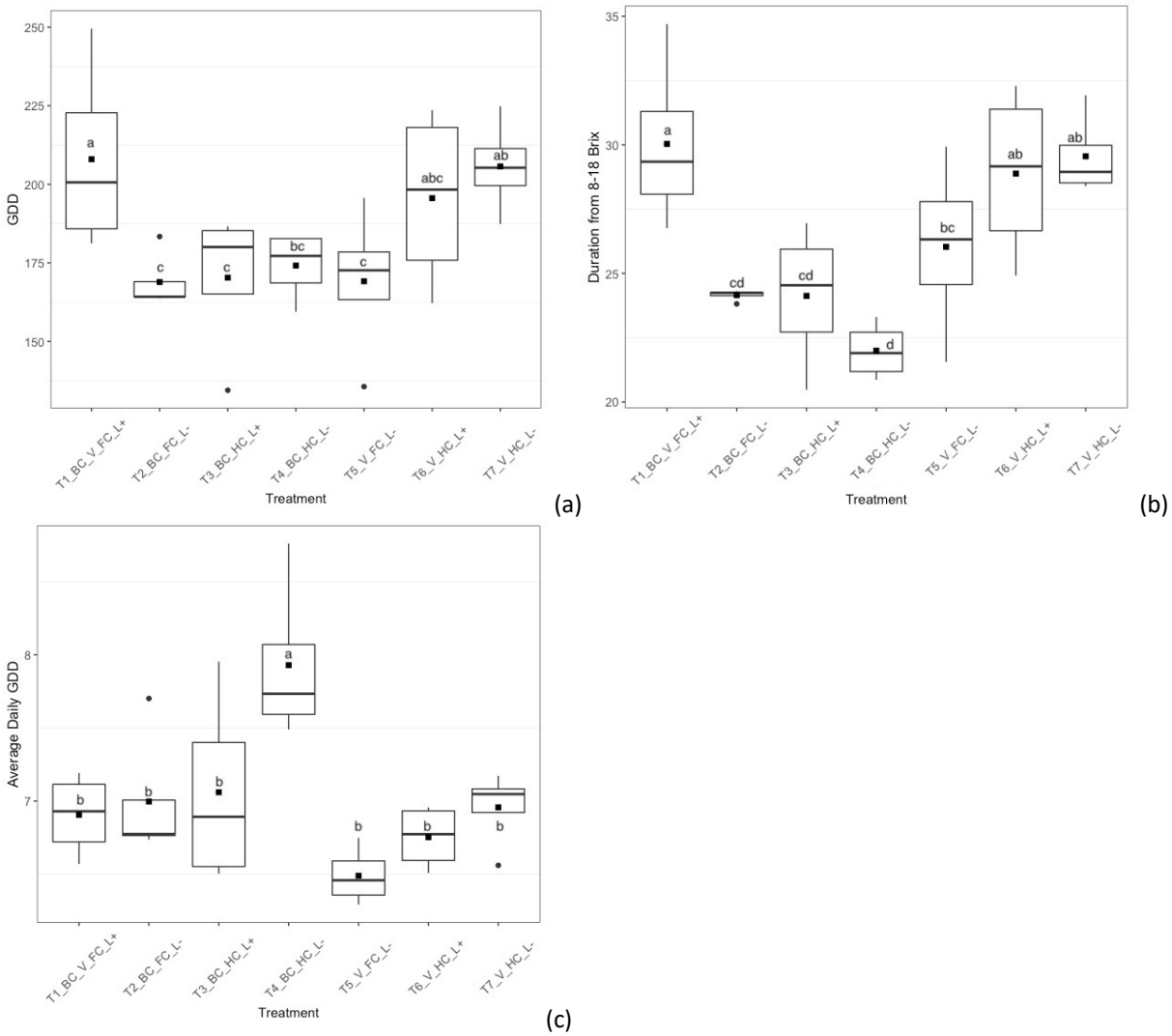


Figure 3.5 Duration of TSS from 8-18 °Brix, GDD and average daily GDD.

Boxes with different letters were significantly different by Fisher's LSD test ($p < 0.05$); ●: outlier; ■: mean.
FC = Full canopy, HC = half canopy; BC = bunch closure, V = Veraison; L+ = lateral retained, L- = lateral removed
(a): Duration of TSS from 8 and 18 °Brix
(b): GDD from 8 and 18 °Brix
(c): Average daily GDD (calculated by GDD/duration)

Climate conditions (GDDs base 10°C) during the TSS accumulation phase (8-18 °Brix) was analysed to see if this was a contributing factor (Table 3.3). Vines which had reduced canopy height and laterals removed at bunch closure (T4) have the highest average daily GDD for the duration from 8-18 °Brix. The GDD analysis indicates warmer conditions for T4 during TSS accumulation which may have contributed to the measured faster rate of TSS accumulation, as temperature is a key element that accelerates plant growth in this case, but due to loss of photosynthesis, However, even though the rate was faster for the vines with reduced canopy height and laterals removed (T4), the final TSSwere lower because the veraison was delayed. Berry weight between treatments was not significantly different, so that the higher rates did not seem to be provoked by for instance enhanced berry transpiration due to increased exposure and therefore altered microclimate. Further research will

need to be done to explore why T4 rates were faster and if the GDD theory is the driver. In summary, trimming at bunch closure with laterals removed delayed veraison and reduced TSS at harvest, despite a faster TSS accumulation rate.

In contrast to the early season canopy treatments, the later conducted canopy trimming (at veraison) did not seem to influence the speed of TSS accumulation to the extent that bunch closure treatments did because it does not trigger as much loss in carbohydrates for berry development as the earlier treatments. However, in both bunch closure and veraison treatments, the extreme source limitation (half canopy and laterals removed) led to lowest final TSS at harvest (T4 and T7). Amongst the veraison half canopy treatments, no significant differences were found compared with the control, with the exception of T5, where TSS accumulation was enhanced.

It is very clear to see from Table 4.2 and Figure 4.3 that the control resulted in the highest TSS at harvest, and that TSS decreased with increasing severity off leaf removal, supporting previously reported trends (Palliotti & Poni 2013; Parker et al. 2015a). Therefore, vines with extremely limited leaf area may struggle to mature fruit. Treatment time is critical for final berry components since bunch closure treatments had lower TSS at harvest compared to veraison treatments, which suggest that early defoliation had more impact on berry composition compared with late season defoliation as shown in many previous studies. Reducing the LA:FW ratio by trimming to half canopy reduced the TSS at both bunch closure and veraison. However, the reduction for the bunch closure treatments was greater than that for the veraison treatments (Palliotti, Gatti, & Poni 2011; Palliotti & Poni 2013; Tardaguila, de Toda, Poni, & Diago 2010; Vasconcelos & Castagnoli 2000).

Modifying the canopy at bunch closure appeared to decrease juice pH levels more than when the same treatments were applied at veraison. The early removal of canopy resulted in lower pH values compared to the control while this was only the case at veraison with full canopy and removed laterals (T5). Trimming to half canopy at veraison, with or without laterals, did not change the pH values compared to the control. The findings presented in Table 3.2 showed that there was no obvious reduction in TSS (°Brix) and higher TA concentrations observed from vines with laterals (more shade than the lateral removed vines), but Percival, Fisher, & Sullivan (1994) demonstrated that shaded canopies produced fruits with lower sugar concentration, increased pH and TA, and a delay in the berry maturation due to lower light intensity on source leaves.

Vines that have been defoliated at bunch closure despite by trimming or leaf removal shown scientifically lower pH level compared to control at harvest. Bledsoe, Kliewer, & Marois (1988) demonstrated that leaf removal at fruitset significantly reduced malic acid, pH and potassium concentration. Research carried by Main & Morris (2004) also shown reduced pH and malic acid in grapes as a result of leaf removal. As a major cation in grape juice, potassium (K) is strongly

correlated with grape juice pH (Walker & Clingeleffer 2016). Bledsoe, Kliewer, & Marois (1988) also proposed that there is a highly significant positive correlation between levels of malate and K in the juice at harvest. It has been found that pH is highly affected by the concentration of organic acids in grape berries, it means that the pH falls as the H^+ concentration rises as defined by the relationship pH = -log10[H^+ (Keller 2015b). Boulton (1980) also advised that the increasing concentration of metal cations like potassium (K+) and sodium (Na+) may influence juice pH by substituting for H^+ and thereby raising the juice pH. Therefore, the decrease in juice pH due to leaf removal was caused by the reduction in both malic acid and K.

3.3 Wine organic acids

It can be observed from Table 3.4 that tartaric acid concentration was higher in early lateral removal treatments (T2 and T4) compared with all other treatments. Keeping the full canopy and removing laterals as the case of T5 also resulted in higher tartaric acid concentrations compared with the control, whereas trimming the canopy to half the full canopy height and removing laterals (T7) significantly lowered tartaric acid concentrations. Lateral removal at bunch closure significantly increased tartaric acid, whereas lateral removal at veraison increased tartaric acid in full canopy treatments but decreased tartaric acid in the half canopy vines. The role of laterals is inconsistent for bunch closure and veraison treatments. All bunch closure treatments ended up with significantly higher malic acid concentrations compared with the control and the veraison treatments, which was very different to previous findings that shown leaf removal decreased malic acid (Main & Morris 2004).

All defoliated treatments result in a lower malic acid concentration, especially lateral removed vines (T2, T4, T5 and T7). Bunch closure treatments (T2, T3 and T4) had higher malic acid compared to veraison treatments (T5, T6, and T7), and the control (T1) had the lowest malic acid concentration. The principle reason leading to the loss of malic acid during fruit maturation is likely to be due to respiration. Malate is the main organic acid stored in vacuoles of grape berry cells, which accumulates from fruit set until veraison. Malic acid is generally considered a possible energy source for berry tissues after veraison (Iland, Dry, Proffitt, & Tyerman 2011). Just before veraison there is a switch from net accumulation to degradation of malate: accumulation of malate from sugar conversion stops, thus imported sugars are accumulated, while stored malate begins to be used for different pathways of the primary and secondary metabolism and malate is consumed. Defoliation in the fruiting zone, improves exposure of fruit to direct light, and cause an increase of fruit temperature, which may lead to malic acid decomposition and improve sugar: acid ratio of the juice (Petrie, Trought, Howell, & Buchan 2003; Ruffner 1982; Ruffner, Possner, Brem, & Rast 1984; Smart & Sinclair 1976).

Table 3.4 Organic acids in Riesling wines (mg/L).

	L-Tartaric acid	L-Malic acid	Malonic acid	Shikimic acid	Lactic acid	Acetic acid	Citric acid	Fumaric acid
T1 (BC V FC L+)	5498.3c ± 198.9	4066.7b ± 119.9	455.9a ± 33.1	35.4a ± 3.1	70.3bc ± 6.1	61.9de ± 19.0	553.7cd ± 23.2	1.4b ± 0.2
T2 (BC FC L-)	5930.7b ± 259.9	4632.0a ± 252.2	385.7b ± 38.3	29.6c ± 1.2	61.5c ± 5.1	89.7bcd ± 24.6	598.5ab ± 44.1	1.4b ± 0.1
T3 (BC HC L+)	5631.3bc ± 422.8	4626.2a ± 92.3	397.8b ± 32.5	33.9ab ± 3.9	78.8ab ± 10.6	70.7cd ± 21.4	524.7d ± 11.0	1.2b ± 0.1
T4 (BC HC L-)	6282.5a ± 131.2	4777.8a ± 216.4	322.7c ± 38.8	23.9d ± 0.4	78.9ab ± 7.0	40.3e ± 6.2	547.8cd ± 37.2	1.2b ± 0.1
T5 (V FC L-)	5702.3bc ± 131.7	4127.6b ± 269.7	403.7b ± 32.9	35.0a ± 4.0	70.1bc ± 3.3	184.0a ± 16.1	609.9a ± 14.6	1.2b ± 0.1
T6 (V HC L+)	5580.3c ± 93.5	4127.0b ± 30.3	423.8ab ± 19.7	32.7abc ± 1.8	72.9ab ± 6.2	112.7a ± 22.5	553.3cd ± 12.2	1.2b ± 0.1
T7 (V HC L-)	5179.4d ± 294.2	4288.1b ± 177.4	381.9b ± 23.3	30.5bc ± 1.3	81.7a ± 6.9	93.4bc ± 13.0	570.0bc ± 6.2	2.2a ± 0.7

Mean ± one standard error.

FC = Full canopy, HC = half canopy; BC = bunch closure, V = Veraison; L+ = lateral retained, L- = lateral removed

Table 3.5 Volatile compounds in trial Riesling wines (µg/L).

	Hexanol	Linalool	α _Terpineol	TDN	Citronellol	Nerol	β _Damascenone	Geraniol
T1 (BC V FC L+)	1193.7bc ± 115.8	19.1bc ± 6.1	11.2cd ± 3.4	0.6b ± 0.02	4.6b ± 0.6	1.5bc ± 0.1	3.5c ± 0.4	5.54a ± 0.31
T2 (BC FC L-)	1300.1b ± 145.7	19.8bc ± 1.3	13.1bc ± 2.6	0.5a ± 0.06	5.2ab ± 0.9	1.5b ± 0.3	3.9bc ± 0.3	4.39cd ± 0.47
T3 (BC HC L+)	1125.4c ± 17.3	12.1d ± 1.5	9.9d ± 2.0	0.4b ± 0.04	4.7b ± 1.2	1.1d ± 0.2	4.2ab ± 0.5	3.66d ± 0.14
T4 (BC HC L-)	1100.0c ± 85.8	17.3c ± 0.6	10.7cd ± 1.3	0.5ab ± 0.08	4.2b ± 0.1	1.3cd ± 0.2	3.4c ± 0.2	4.54bc ± 0.07
T5 (V FC L-)	1289.0b ± 45.7	24.6a ± 4.5	16.4a ± 1.6	0.4ab ± 0.03	5.1ab ± 1.0	1.8a ± 0.1	4.7a ± 0.2	5.59a ± 1.01
T6 (V HC L+)	1125.9c ± 89.6	22.8ab ± 1.5	15.3ab ± 1.8	0.4ab ± 0.02	4.8b ± 0.2	1.7ab ± 0.2	4.6a ± 0.1	5.19ab ± 0.45
T7 (V HC L-)	1424.1a ± 65.1	25.0a ± 1.4	14.8ab ± 0.6	0.5a ± 0.12	5.9a ± 0.4	1.8a ± 0.2	4.7a ± 0.7	5.84a ± 0.49

Mean ± one standard error.

FC = Full canopy, HC = half canopy; BC = bunch closure, V = Veraison; L+ = lateral retained, L- = lateral removed

Control vines and vines with full canopy and lateral removal (T5) had higher shikimic acid concentrations compared with other treatments. Vines that were trimmed to half canopy at bunch closure (T3) with laterals presentand vines trimmed to half canopy at veraison (T6 and T7) had similar concentrations of shikimic acids. Vines which had laterals removal at bunch closure (T2 and T4) have relatively low shikimic acid concentrations in the final wine. Shikimic acid is a product of quinic acid biosynthesis by dehydration (Tessini, Mardones, Rivas, & von Baer 2009), which is a very important precursor for the biosynthesis of aromatic amino acids and flavonoids such as anthocyanins, tannins and flavonols. Shikimic acid content decreases significantly during the ripening of the grapes and therefore its concentration in wine is strongly influenced by the harvest period, and it is predominantly affected by grape variety (Ivanova-Petropulos et al. 2018; Pasquale et al. 2014). Ahn et al. (2008) have found that the nature of the carbon source affected the shikimic acid production, therefore a canopy with larger leaf area that provides more carbohydrate assimilates resulted in higher shikimic acid concentrations in the finished wines. This could explain the lower shikimic acid concentrations observed following lateral removal (T2, T4 and T7), especially for T4 which had the lowest shikimic acid concentration that may have been caused by extreme source limitation in early season.

Acetic acid concentration was well below the detection threshold (600 - 900 mg/L) (Macías et al. 2013), for all treatment, and the differences observed were not systematically related to the different canopy management of the treatments. Malonic acid concentration in wine decreased with reducing canopy size (Table 3.4). Lactic acid concentration has a similar trend difference to malonic acid concentration, which decreased with reducing canopy size, half canopy treatments contain higher concentration compare to full canopy treatments despite application time. In most fruit species, citrate is one of the dominant organic acids, but not with grapes. Citric acid shows a higher concentration for treatments where laterals were removed. However, previous studies haven't found any cause and effect relationship between lactic acid, malonic acid and acetic acid concentrations and defoliation. Given that only tartaric acid, malic acid and shikimic acid changed in response to LA:FW ratios, future research could prioritise focus on these acids that response to the canopy manipulation, and not the others measured in Table 3.4.

3.4 Wine volatile compounds

Given the importance of grape derived volatile compounds such as terpenes and C_{13}-norisopreniods for Riesling wine quality, and their apparent dependence on microclimate and carbohydrate availability upon leaf removal, GC-MS analyses were conducted to evaluate the impact of the canopy treatments on the wine volatile profiles. A total of eight compounds were quantified (Table 3.5), and Table 1.2 summarizes their associated sensory characteristics.

3.4.1 C6 compounds

The absence of laterals resulted in higher wine hexanol concentrations, where vines with half canopy and no laterals (T7) had the highest hexanol concentration, and other treatments with some lateral removal (T2 and T5) also had relatively high hexanol concentration compared with control (Table 3.5). The timing of treatments did not seem to impact the hexanol concentrations, as evident when comparing full canopy lateral removal vines (T2 with T5) or half canopy lateral retained vines (T6 with T3). However, reducing to half canopy at bunch closure irrespective of the presence or absence of laterals results in the lowest hexanol concentrations (T3 and T4). When half canopy was established and laterals were removed at veraison (T7), it resulted in a higher hexanol concentration compare to bunch closure treatment with similar canopy size.

Hexanol derives from lipoxygenase activity, and is the most abundant C6 compound (Table 3.5). Luna et al. (2018) pointed out that the concentration of green odour compound like hexanol was reduced with delayed harvest dates, and similar results have also been observed from a previous study by Bindon et al. (2013) and Schelezki, Šuklje, Boss, & Jeffery (2018). Iglesias et al. (1991) have also reported that C6 aldehyde decreased with increasing grape maturity, and the possible reason is that longer enzyme action time. Apart from grape maturity, Kozina et al. (2008) demonstrated that defoliation right after fruitset (Stage 29) decreased hexanol concentrations compare to control vines, which is supported by our observation, as the bunch closure half canopy treatments showed low concentration in hexanol. Hexanol provides green attributes to wines (Tao, Li, Wang, & Zhang 2008), and is usually present in wines at concentrations above its perception threshold. This green flavour brought by hexanol is not necessarily desired for certain wine style like aromatic Riesling, so extending grape hang time is a solution to avoid this flavour (Schelezki, Šuklje, Boss, & Jeffery 2018). Therefore, low hexanol concentration due to defoliation means less "green" aroma present in the wine, which can be an advantage in Riesling wines.

3.4.2 Monoterpenes

Linalool, α-terpineol and nerol were grouped with treatment time (Table 3.5). Treatments carried out at veraison (T5, T6 and T7) resulted in a higher concentration of these compounds. Despite the strong relationship to treatment time, α-terpineol concentrations appeared to also be influenced by total canopy height, as higher concentrations were found in those treatments that maintained the full canopy (T5 full canopy laterals removed at veraison, T2 full canopy laterals removed at bunch closure and control), in comparison the half canopy treatments (T6 and T7 vines trimmed to half canopy at veraison, and T3 and T4 vines trimmed to half canopy at bunch closure). In fact, T3 and T4 (both trimmed to half canopy at bunch closure) had a significantly lower α-terpineol concentration compare to veraison treatments. The results have shown that the presence of laterals decreases α-

terpineol concentration at a similar canopy height and treatment time. Conversely, lateral removed vines always have higher α-terpineol concentration than the lateral retained vines. Nerol concentration followed a similar trend as α-terpineol concentration, predominantly influenced by the time of treatment but also related to the presence of lateral with little impact with regard to the canopy height observed. Vines with laterals removed at veraison despite canopy height have significantly higher in nerol concentration compare to control, and seems that warmer microclimate might have less to increase nerol despite the loss of carbohydrate. Linalool generally had a similar trend with α-terpineol and nerol, except that the vines that have trimmed to half canopy with laterals removed (T7) had the highest concentration.

It can be seen from Table 3.5 that all treatments established at veraison despite of trimming and leaf removal (T5, T6 and T7) have higher geraniol concentration compare to treatments carried at bunch closure (T2, T3 and T4), but they (T5, T6, and T7) not significantly different from control. Geraniol is an important monoterpene alcohol as it is usually present in greater concentrations and have lower detection thresholds than other major wine monoterpenes (Etiévant 1991). Qin (2015) has reported that geraniol concentration increased with a stronger degree of leaf removal post fruitset. But the concentration of geraniol is significantly below the detection threshold, therefore it can't be entirely concluded whether or not this change impacted the wine sensory perception without a sensory analysis conducted.

Limited source size by defoliation and enhancing microclimate may both be the reason of increased concentration of the volatile compounds. Feng, Skinkis, & Qian (2017) found that basal leaf removal at fruitset had largely impact monoterpenes that contribute to floral and fruity notes to the wine, such as linalool, α-terpineol, geraniol and nerol. The concentration of these compounds were significantly higher in wines from leaf removal treatments than in control, particularly those that were established at veraison, which indicated the importance of microclimate around the bunch zone for their accumulation in grape berries. Joubert, Young, Eyéghé-Bickong, & Vivier (2016) have pointed out that UV-B radiation and other abiotic and biotic stresses triggered monoterpenes synthesis as a mechanism to protect berry tissue. Therefore, light exposure to the berries stimulates the expression of the monoterpene metabolic genes in grape berries (Friedel et al. 2016), and this increases monoterpene glycosidic precursors biosynthesis.

Song et al. (2014) have mentioned in their research that the concentrations of linalool, nerol, and geraniol from ultra-low vigour zones were higher compared to wines from the other vigour zones in a Pinot Noir vineyard. The low vigour vines likely had more exposed bunches, therefore warmer microclimates, possibly similarly as the warmer microclimates that are likely to be present when reducing the leaf area as in treatments with laterals removed (T5 or T7). However, Duchêne,

Butterlin, & Jaegli (2016) observed that linalool synthesis is impaired when grapes are grown at high temperatures, whereas no detrimental effect has been indicated for geraniol. In this study, it has both linalool and geraniol concentration increased with lateral removal canopies, but microclimate was not scientifically measured during the berry ripening. Future studies can additionally research into it.

Citronellol concentration was not affected by neither time nor lateral appearance in general, with the exception of vines that have been trimmed with lateral removal at veraison (T7) which had a significantly higher concentration of citronellol in the wines compared to control. Vines with full canopy and laterals removed, irrespective of treatment time (T2 & T5), and vines that been trimmed to half canopy with laterals removed at veraison (T7), had a higher concentration of citronellol in wines, which could be explained by the enhance microclimate. However, removing laterals in addition to the canopy height reduction at bunch closure (T4) resulted in the lowest citronellol concentration, that could stem from a severely limited carbohydrate supply outweighing the otherwise favorable warmer microclimate (Reynolds, Wardle, & Naylor 1996; Vance, Reeve, & Skinkis 2013). For the situation of T4 (bunch closure half canopy laterals removed), the grapes were very exposed, but the carbohydrates were limited early in the season, and therefore the low citronellol concentrations indicated that the lack of carbohydrate could have outweighed the benefit of warmer microclimate, as seen compared to other lateral removed treatments (T2, T5 and T7).

Carbohydrate supply and microclimate can both contributing to terpene accumulation along with the berry developing stages. Canopy/fruit zone microclimate, especially sunlight climate, could directly affect wine, though other factors, e.g. leaf area to crop load ratio. Fang & Qian (2006) and Song et al. (2014) have also found that the concentration of terpenoids can be enhanced either by better light exposure or increased grape maturity. Microclimate was not measured in this study, therefore no direct conclusion can be drawn from this. But for future studies, microclimate monitoring can be included as a factor that may contribute to volatile compounds concentration changes.

Though these volatile compounds have been investigated in this study, the concentration of most of them did not reach their odour perception threshold (refer to Table1.2). Crespo et al. (2017), Qin (2015), and Skinkis, Bordelon, & Wood (2008) all mentioned that the concentrations of linalool, geraniol, nerol and citronellol increased by leaf removal but that they are usually present at a very low concentration that won't create any organoleptic difference detected in the wine. One critical purpose of this study is to observe that wines made from defoliated vines with berry TSS concentrations and therefore lower wine alcohol levels while still retaining the favorable varietal aroma of Riesling. It has shown that leaf removal had a positive impact on monoterpenes

concentration in wine despite their low concentrations. Though the increasing concentration of these compounds may not be above the sensory thresholds reported.

3.4.3 C_{13}-norisoprenoids

Vines that defoliated later in the season (veraison) can be seen as the key driver for increased β-damascenone concentrations in the wine, while treatments carried out at bunch closure only have very small differences in β-damascenone concentrations (Table 3.5). TSS doesn't seem to be related to β-damascenone concentrations since there were no differences in the control which had the highest TSS and vines trimmed with laterals removed at bunch closure (T4) which has the lowest TSS concentrations.

C_{13}-norisprenoids can be enhanced by sunlight exposure as mentioned earlier (Lee et al. 2007). Previous research found that leaf removal and shoot thinning promoted sunlight exposure, which is associated with both pre-veraison accumulation of carotenoids and the post-veraison degradation to aroma active C_{13}-norisoprenoids (Crupi, Coletta, & Antonacci 2010; Marais, van Wyk, & Rapp 1992). Kemp (2010) has investigated the relationship between aroma and β-damascenone for Pinot Noir wines, and the results showed that wine made from leaf removal vines had more β-damascenone have stronger dark fruit aroma compared to the wines made from non-defoliated vines (Naor et al. 2002). β-Damascenone has a very low odour threshold (0.05 μg/L) (Guth 1997), though the threshold is determined in water/ethanol (90/10, w/w), it is still considered as the threshold β-damascenone concentration in wine usually suggested favorable aroma and better flavour profile. The β-damascenone concentration in this study has been found above the threshold, and defoliation can be an efficient way of increasing β-damascenone concentration and further impact wine aroma profile.

Treatments where laterals were removed resulted in higher TDN concentrations in wine (Table 3.5) which may be a result of the canopy being more open and higher microclimate temperatures (Sacks et al. 2012). TDN precursors in wine grapes have been correlated with warmer growing regions and with greater cluster light exposure, particularly prior to veraison (Ristic et al. 2010). Apart from sun exposure, the impact of laterals remains unknown. TDN is generally absent from the grape berry, but also can be formed by hydrolysis of glycosylated precursors and subsequent rearrangements during fermentation and storage ageing (Winterhalter 1991; Winterhalter, Sefton, & Williams 1990b). Wines made from the vines with lowest leaf area (e.g. half canopy laterals removed vines, irrespective of treatment time, T4 and T7) presented the highest concentrations of TDN compared to control, implicating that it may increase the risk of unfavorable kerosene aroma but this sensory consequence has not been tested in this research.

Apart from the impact of leaf removal, pH is another factor that may affect volatile concentration in wine directly. Low pH and high temperatures increase the hydrolyzation of the non-volatile glycosidic precursors and thus release the volatile aglycones (Maicas & Mateo 2005). An aglycone bound to a glycosidic unit by a β-glycosidic linkage make up a glycosidic precursor, and this binding can be easily broken by acid or enzymatic hydrolysis, liberating other aglycones (such as geraniol, nerol and linalool, and the transformation between them) that can bring floral attributes to the wine even at low odour thresholds (100–400 μg/L) (Crespo et al. 2017). The monoterpenols released from the glycosides by acid hydrolysis are likely to change the wine aroma. The transformation between terpenes has not been discussed in this study due to the limited experiment on grape juice. According to Ribéreau-Gayon (2006), linalool can be further transformed to geraniol during winemaking, and then further changed into citronellol through enzymatic reactions.

In this study, all treatments have been harvested at the same time because one of the main objectives of this study is to investigate the volatile composition in relationship to differing grape sugar concentration (as a result from the treatments) and consequently different alcohol levels. To adress the link between the sugar concentration and volatile composition in accordance with the treatment, future research may aim to harvest the treatments at similar TSS levels, possibly at several times during the ripening phase.

Given time and financial limitations in the project, yeast assimilable nitrogen values were not take of the samples prior to fermentation. YAN is essential for a sound fermtation process and can alter the wine volatile composition. The DAP addition, which was necessary in some of the replicates to ensure fermentation to dryness could have influenced the secondary metabolite composition in the wine. In the current research, the DAP additions to 19 samples out of 28 did not result in obvious differences but by including YAN analysis and a more tailored DAP addition, any ambiguity could be better ruled out.

Most previous studies targeted defoliation by leaf removal around bunch zone with regard to investigating volatile aroma changes, but in this study, the way of creating a more opened canopy with different microclimate was via lateral removal and trimming to half canopy height. Even though these practices were not specifically targeted around bunches, they likely created a more exposed bunch zone potentially creating a microclimate around the bunch zone that differed to the vines where no leaves had been removed at all. Vines which have their laterals removed were considered to have a higher microclimate temperature and stronger sun exposure, but this was not quantified in this study and could be measured in future research. Regardless, the different bunch microclimate environment may have led to higher concentrations of volatile compounds caused by increasing light exposure. Grape berry development is related to multi-environmental and management conditions

and can be altered by, for instance, early source limitation, as has been investigated in this study. Also, future work can focus on the individual differences between experimental plots and use the whole dataset to analyze especially the treatment that have a high amount of variability within plot.

Under the current experiment design of this study, it is hard to conclude if the reason for volatile compounds differences is due to microclimate or carbohydrates because it was not targeted in the first place, but this can be analysed in future experiments. Future studies can further separate bunch zone leaf removal and lateral removal to specify the contribution of leaves and laterals.

Chapter 4

Conclusion

The effect of defoliation on berry maturation and berry composition was investigated in the cool climate growing region of Canterbury, New Zealand, using VSP trained Riesling vines. Vines were treated by trimming (FC/HC) and lateral removal (L+/L-) at bunch closure (BC) and veraison (V).

Results have shown that yield and yield components were not or had a very limited impact by defoliation after fruitset either by trimming or lateral removal. LA:FW ratios generally reflected the leaf area, which were reduced by defoliation. The onset of veraison was delayed by trimming at bunch closure. Early trimming and lateral removal generally resulting in lower TSS concentrations at harvest. Modifying the canopy at bunch closure appeared to decrease juice pH levels more than when the same treatments were applied at veraison. The rate of TSS accumulation was found correlated to heat conditions (GDDs), where vines that trimmed at bunch closure with laterals removed had delayed veraison but the fastest rate of TSS accumulation and shortest duration from 8-18 °Brix due to the high average GDD during their ripening period.

Tartaric acid and malic acid concentration increased with early lateral removal. Vines with larger canopy size resulted in higher shikimic acid concentrations because a larger leaf area provides more carbohydrate assimilates.

Monoterpenes concentration increased with lateral removal, especially at veraison caused by a more open canopy with potentially warmer microclimate and more light exposure to the fruit. β-Damascenone and TDN also increased with veraison trimming and lateral removal. Leave removal and trimming both reduced the canopy size, but laterals is an uncertain increase of canopy density in the system, future research could add a point quadrat analysis to define the canopy density. Increasing β-damascenone may indirectly enhance wine floral and fruity aroma, and increasing TDN may cause higher potential kerosene aroma in wine.

Under the current global warming condition and the pursuit of healthier drinking, reducing grape juice sugar concentrations in order to reduce wine alcohol level is a certain trend of development. Adopting climate change by canopy management such as leaf removal can be achieved at a low cost. This study demonstrated that reducing grape TSS concentrations via defoliation reduced wine alcohol level without greatly jeopardizing the favorable Riesling varietal aroma potential, but potential sensory analysis can be added in future research to conferme that the differences in secondary metabolite concentreation really results in Riesling veriatal aroma or not.

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Appendix A

Trial layout

Table A. 1 Trial layout in the vineyard.

Row 18	Rep	Experiment Unit	Southern End	Row 19	Rep	Experiment Unit
T7 V HC L-	Rep 1	1		T4 BC HC L-	Rep 3	15
T1 BC V FC L+	Rep 1	2		T1 BC V FC L+	Rep 3	16
T3 BC HC L+	Rep 1	3		T6 V HC L+	Rep 3	17
T4 BC HC L-	Rep 1	4		T3 BC HC L+	Rep 3	18
T2 BC FC L-	Rep 1	5		T2 BC FC L-	Rep 3	19
T5 V FC L-	Rep 1	6		T5 V FC L-	Rep 3	20
T6 V HC L+	Rep 1	7		T7 V HC L-	Rep 3	21
T4 BC HC L-	Rep 2	8		T1 BC V FC L+	Rep 4	22
T6 V HC L+	Rep 2	9		T4 BC HC L-	Rep 4	23
T5 V FC L-	Rep 2	10		T2 BC FC L-	Rep 4	24
T1 BC V FC L+	Rep 2	11		T7 V HC L-	Rep 4	25
T2 BC FC L-	Rep 2	12		T6 V HC L+	Rep 4	26
T3 BC HC L+	Rep 2	13		T5 V FC L-	Rep 4	27
T7 V HC L-	Rep 2	14	Northern End	T3 BC HC L+	Rep 4	28

Appendix B

GC-MS curve

B.1 Internal standard information

B.1 Internal standard information.

Internal Standards	ISTD ID No	RT (mins)	Target Ion m/z	Confirming Ions m/z (% to Target Ion)	Purity Stds	CAS No	Supplier
d13-Hexan-1-ol	1	25.78	64	50 (38.28), 46 (37.95)	98 atom %	204244-84-8	***Sigma***
d6-Linalool	2	39.41	124	142 (37.63)	98 atom %	-	***EPTES***
d8-Napthalene	3	52.32	136	108 (9.87), 134 (9.51)	99 atom%	1146-66-2	***Sigma (ISOTEC)***
d4-Beta Damascenone	4	59.56	194	179 (44.39)	95 atom %	217482-71-8	***EPTES***
d6-Geraniol	5	60.71	75	129 (13.80)	97 atom %	66063-44-3	***EPTES***

ISTD = Internal standard; RT = retention time; Target Ion (m/z) = ions of specific mass at specific time

B.2 Quantification parameters for the eight volatile analytes

B.2 Quantification parameters for the eight volatile analytes

Analyte	ISTD Used	RT (mins)	Target Ion m/z	Confirming Ions m/z (% to Target Ion)	Calibration Range µg/L (1/10 dilution)	R^2	Cal Curve Type	Purity Stds	CAS No	Supplier
Hexanol	1	26.43	56	43 (49.03), 55 (47.13)	0 – 499.74	0.9998	Quadratic	98%	111-27-3	Sigma
Linalool	2	39.87	121	136 (23.90) 154 (1.94)	0 - 12.01	0.9999	Quadratic	97%	78-70-6	Aldrich
Alpha terpineol	2	50.66	59	136 (38.07), 121 (47.05)	0 – 15.02	0.9998	Quadratic	98%	10482-56-1	Merck
TDN	3	54.36	172	141 (103.30), 115 (60.83)	0 – 25.01	0.9983	Quadratic	96%	30364-38-6	VeZerf
Citronellol	3	55.35	81	95 (69.69), 109 (28.44) 138 (19.11)	0 – 5.00	0.9999	Quadratic	99%	7540-51-4	Aldrich
Nerol	5	57.49	68	123 (43.03), 139 (20.35)	0 – 1.99	0.9997	Quadratic	97%	106-25-2	Aldrich
Beta-Damascenone	4	59.77	190	175 (52.10)	0 - 1.36	0.9999	Quadratic	98%	23696-85-7	Sigma
Geraniol	5	61.26	69	123 (8.27), 139 (4.33)	0 – 4.51	0.9998	Quadratic	98%	106-24-1	Aldrich

ISTD = Internal standard; RT = retention time; R^2: All fitted standard (calibration) curves were Quadratic functions