Berry composition and wine quality of Pinot noir as affected by leaf area to crop load ratio

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Abstract of a thesis submitted in partial fulfilment of the requirements for the Degree of Master of Horticultural Sciences.

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The effect of vine leaf area (LA) relative to crop load (CL) was investigated in the cool climate growing region of Canterbury, New Zealand, using Pinot noir (clone 10/5) Vertical Shoot Positioning (VSP) trained vines. Three weeks after fruit-set, vine leaf numbers were standardized with two of the four basal leaves retained in all cases. LA treatments were 12 leaves (1L), 6 leaves (0.5L) or 3 leaves (0.25L) per shoot. CL treatments were full crop (1C), half crop (0.5C) or quarter crop (0.25C) by removing equal numbers of primary and secondary clusters, producing five different treatment ratios (TMR), “0.25”, “0.5”, “1”, “2” and “4”. Treatments were applied in a 4-replicate split plot design. Grapes were harvested on April 4, 2014 and replicates 1 & 4 and 2 & 3 were combined to make two wines from each treatment. LA/CL (cm^2/g) was estimated from a subsample of shoot leaf areas divided by total yield per vine after harvest.

Starch dry weight in roots was not affected by crop load, but was decreased with leaf removal, while pruning weight was increased with increasing TMR, indicating that the fruiting capacity for next season could be reduced by limited leaf numbers.

Juice °Brix was negatively, while juice pH was positively, related with increasing levels of leaf removal. Total red pigments, tannin concentration and total phenolics in skin extracts were decreased by greater leaf removal ($p < 0.05$, $p < 0.01$ and $p < 0.05$, respectively). Results suggest shading appeared not to be a limiting factor for the synthesis of phenolic compounds, or to have an impact on fruit composition in general (i.e. potassium, malic or tartaric acids).

The concentration of total red pigments, tannin and total phenolics in wine were not significantly different between treatments, though some visual differences were observed using the CIELab method on native wine samples. HPLC analysis of wine showed that two procyanidins dimers, rutin, caffeic acid and caftaric acid were affected by crop removal only, while quercetin, quercetin-glucuronoide 1 and protocatechuic acid were affected by leaf removal only. Kaempferol-glucoside, *p*-hydroxybenzoic acid and *p*-coumaric acid were affected by both leaf removal and crop removal treatments. GCMS analysis showed the concentrations of fatty acids and some esters were higher

when more leaves and more crop were retained, and that the concentrations of C_6 alcohols were positively related with TMR.

Some wine parameters showed the same patterns as that found for grapes, including pH, colour hue, degree of red pigment colouration, and kaempferol-glucoside concentration, along with the CIELab visual colour pattern. Thus, the fruit characteristics can be transferred into wine.

It appears that the most appropriate LA/CL or TMR to produce quality wines were not the extreme values (e.g. the highest or lowest LA/CL, or TMR ="0.25" or "4") based on the chemical analysis in this study. In fact, lower to medium levels of leaf and crop removal were desired, based on the concentrations of phenolics, volatile aroma compounds, carbohydrate in grapevine roots, along with other parameters measured in this project.

Key words: sunlight exposure, vine balance, Pinot noir, leaf area to crop load ratio, grapevine root, grape skin, phenolic, tannin, anthocyanin, co-pigmentation, wine aroma, organic acid, minerals, SPE, HPLC, GCMS.

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List of abbreviations

TA	Titratable acidity
rpm	Rounds per minute
PAR	Photosynthetically active radiation
nm	nanometer
NaOH	Sodium hydroxide
mm	Millimetre
mL	Millilitre
mg	Milligram
MCP	Methyl cellulose precipitation
LA/CL	Leaf area to crop load ratio
LA	Leaf area
HPLC	High performance liquid chromatography
H_2O	Water
GCMS	Gas chromatography mass spectrometry
g	Gram
CO_2	Carbon dioxide
CL	Crop load
µL	Microliter
VOC	Volatile organic compound
TMR	Treatment ratio
SPE	Solid phase extraction

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Chapter 1 Literature review

1.1. Introduction

Vine balance is defined as the state at which vegetative and reproductive growth can be sustained indefinitely while maintaining healthy canopy growth, adequate fruit production and desired fruit quality, namely sugar levels, acid balance and flavour compounds. Vine balance is complex and dictated by the soil, environment, and overall production capacity of the vineyard. Berries of acceptable quality (commercially often decided by total soluble solids content, e.g. °Brix) are mainly supported by photosynthetically active leaves and influenced by canopy microclimate. Many of the components responsible for the sensory attributes of a wine originate in the berry, though the winemaking process only achieves partial extraction of the berries and modifies these grape-derived compounds by forming new compounds and/or new sensory sensations. Similar to vine balance, the concept of wine quality is complex, but should be related to wine style and can be measured either by sensory assessments or chemical analyses or both.

There is no doubt that wine quality is closely related to two factors, berry quality at harvest and the winemaking process. Thus, it is reasonable to suspect that vine balance can impact berry quality and has an influence on wine quality to some extent. In fact, manipulation of vine balance is widely practiced to enhance grape and wine quality. This review aims to identify the possible influence of vine balance defined by parameters such as LA/CL or yield to pruning weight on canopy characteristic, berry composition and wine quality.

1.2. Grapevine

1.2.1 Photosynthesis

Photosynthetically active radiation (PAR) is often used as measure of the incident light available for photosynthesis. However, some PAR will be reflected or transmitted, depending on leaf age and angle (Smart 1985). In addition, leaf temperature plays an important role in photosynthesis, although the vine can modify its temperature by altering transpiration and changing leaf blade angle (Creasy & Creasy 2009). Asides from this, extremely high or low temperatures can damage the working compounds within the leaf tissue and in turn change the structure of the leaf (Creasy & Creasy 2009). Other factors affecting the rate of photosynthesis include CO_2 availability, water status and cultural practices (Iland et al. 2011). Thus, the photosynthetic rate of an individual leaf can be altered by vine physiological status and modified by canopy microclimate, e.g. light intensity and temperature.

Instead of single leaf photosynthesis, whole vine photosynthesis tends to be more reliable when predicting vine performance and sustainable yield of ripe grapes (Howell 2001). The latter is related to carbon fixation and the vine dry weight, which is easily estimated through pruning weights (Howell 2001). Whole vine photosynthesis can be varied not only by the factors mentioned above, but also can be influenced by meso-climate, season, cloud cover, row orientation, and canopy architecture (Iland et al. 2011).

1.2.2 Canopy architecture and function

The grapevine training system is of central importance in manipulating canopy architecture and canopy microclimate. Kliewer & Dokoozlian (2005) reported that to mature equal amounts of fruit to acceptable quality, divided-canopy vines required less leaf area than single-canopy vines. The reason is that divided-canopy vines have a higher percentage of leaf area at light saturation than single-canopy vines. Besides this, different training systems cooperate with the function of individual leaves. For examples, Cavallo et al. (2001) reported that the median and apical leaves of Bilateral Free Cordon trained vines (where shoots were trained downwards) experienced a more limited light microclimate than the corresponding leaves of the Bilateral Guyot and Bilateral Spur-Pruned Cordon trained vines after veraison, which limited the whole vine photosynthesis of the Bilateral Free Cordon trained vines.

Smart (1985) has reported that about 8 to 10% of PAR striking a leaf passes through it. In a cool climate, the second leaf layer may receive so little PAR that it is below the light compensation point (Howell 2001), meaning that the leaf is a net importer of photosynthates and therefore not contributing to vine growth. Hunter & Visser (1988) further suggested that shaded leaves lack the ability to achieve the rates of photosynthesis of the “sun leaves” even when they are exposed to full sun.

Thus, training system and sun exposed leaf area influences whole vine photosynthesis and in turn, how much carbon can be fixed. Furthermore, clusters need a certain amount and/or intensity of light to initiate and assist secondary biosynthesis, which will be discussed later in this review. A range of indices, such as leaf layer number, exposed canopy surface area, and leaf area to canopy surface area ratio, have been proposed to evaluate canopy performance. Smart & Robinson (1991) developed a scoring system to evaluate canopy performance through assessing what is desired on parameters such as leaf size, leaf colour, shoot length, lateral growth, and the presence of active shoot tips after veraison.

1.2.3 Carbon partitioning and vine balance

Generally, mature leaves are regarded as the main source (net exporter) of assimilates of the whole plant, and berries are regarded as major sinks (net importer) of assimilates and one of the terminal points of solar energy flow. Solar energy flow can be expressed through carbon partitioning, which occurs between sources and sinks. Factors that influence the movement from sources to sinks are identified as their sizes, the capability of sources to generate assimilates and their rates, the rates at which sinks use these assimilates, and finally the distance and the relative position of sink to source (Farrar 1992).

Vine balance is most readily understood when based on an understanding of vine carbon balance. Petrie et al. (2000) suggested that the capacity of a vine to produce dry matter is governed by an interaction between a vine's inherent capacity to fix carbon, and the environment in which it grows. In other words, both vegetative growth and reproductive growth of a vine rely on the fixed amount of carbon primarily produced by its leaves. Berries carried by grapevines are produced at the expense of vegetative growth, by influencing carbon partitioning. When there is an inappropriately high amount of leaves and small amount of crop, the vine will be sink-limited, leading to end-product inhibition of photosynthesis (Farrar 1992; Petrie et al. 2000); on the contrary, if the amount of crop is too high for a certain amount of leaf area (source-limited), the fruit maturation could be delayed though high photosynthetic rates are maintained (Petrie et al. 2000).

Generally, there are two main indices of vine carbon balance that are well-understood: leaf area to crop load ratio (LA/CL) and yield to pruning weight ratio (Ravaz Index). Optimal ranges for these have been proposed with respect to macroclimate (region), cultivar, and vine training system (Howell 2001; Kliewer & Dokoozlian 2005).

In conclusion, leaf area per vine (photosynthate supply) drives carbohydrate production and determines how much crop can be ripened. Provided other factors are not limiting growth and the initiation of fruit primordia, the fruiting capacity of grapevines in a certain climatic region is largely determined by the total photosynthates being produced. The total photosynthates per vine relies on its total leaf area and by the percentage of the total leaf surface area that is at light saturation or above (Kliewer & Dokoozlian 2005). Moreover, distribution of that photosynthate between different organs largely determines the efficiency with which the photosynthate is used for fruit production (Petrie et al. 2000). Certainly, there should be a balance between crop mass (reproductive products) and vine mass (vegetative products) to maintain sustainable production yielding fruit of acceptable quality. However, in a field situation, vine balance also depends on trellis type, row orientation, and canopy development which all influence intra-canopy shading and the proportion of leaves that are

exposed for photosynthesis (Petrie et al. 2000). However, in the wine industry, the sugar concentration, which constitutes a large proportion of fruit' dry matter, is only one aspect of fruit quality. The amounts of other chemical components also relies on vine balance and canopy characteristics, which will be discussed in the next section.

1.3 Fruit

1.3.1 Yield effects

Yield, and the components that make it up, are key parameters of vine balance. As a result of reproductive growth, yield is often considered to be determined by how many nodes are left during winter pruning, management practice and overall growing conditions in past and current seasons.

In terms of vegetative growth as affected by yield, according to Naor et al. (2002), one or two clusters per shoot resulted in no significant sink competition between clusters and vegetative growth at the single-shoot level; however, decreasing the cluster number per vine by shoot thinning resulted in increases in all vegetative parameters (e.g. main shoot length, lateral shoot length and number, shoot diameter, leaf area per shoot and leaf weight), indicating an increase in the relative strength of vegetative growth. Conversely, when the fruiting sinks exceeded vine capacity for dry matter production, the total dry mass of fruit and total dry matter of the current season canopy (including fruit) failed to increased proportionally with increased crop load (determined by levels of shoot thinning) (Miller & Howell 1998).

Cortell et al. (2007) suggested that yield could be low either from high vigour vines having smaller cluster weights, or from low vigour vines having both lower cluster numbers and weights. However, Smart (1985) suggested that the yield could be increased with reduced vine vigour through reductions in shading, thereby improving flower cluster initiation and potential crop load in the following season. Smart (1985) also believed that within a given variety and training system, high yields were associated with more dormant nodes being left during winter pruning and high shoot density per meter row during the growing season, which generally led to dense canopies and more shade in the canopy interior. This phenomenon could be related to the empirical and inverse correlation between yield and quality for winegrapes.

Dami et al. (2006) reported that manipulating sink size by cluster thinning (10, 20 and 30 clusters per vine) at pea-size fruit reduced total yield per vine but increased cluster weight and berry weight, in agreement with a more recent study in New Zealand by King et al. (2012). Thus, attempting to alter yield by reducing cluster number changes the relative sink strength between various organs, in this

case, the total sink strength from fruit was reduced because of fewer clusters. However, other yield components, e.g. berry weight and cluster weight, can compensate for this effect to some degree, mainly because the sink strength of each individual cluster was strengthened due to less competition.

Berry weight is often related to variety, vine vigour and water status (Creasy & Creasy 2009). Removal of about 50% of the foliage a month after anthesis was generally more detrimental to berry weight than to the increase of sugars in the fruit; on the other hand, complete defoliation at veraison reduced berry sugar concentration much more than berry weight (Kliewer & Antcliff 1970). Thus, berry weight is more readily altered pre-veraison through manipulating leaf area, in agreement with Ollat & Gaudillere (1998) who found that leaf removal just after fruit-set strongly reduced berry growth, and that berry size at maturity was proportional to its size before veraison.

To conclude, increasing crop loads caused greater partitioning of dry matter from vine canopy vegetative tissues to fruit. However, if the dry matter increase in the canopy is accompanied with a corresponding increase in crop, it is possible to produce a large quantity of fruit with acceptable quality. In other words, parallel increases in crop level and pruning weight (the latter being a proxy for photosynthetic capacity of the vine) enable production to be increased without affecting balance between sink and source and thereby quality (Smart et al. 1985a). Miller & Howell (1998) suggested that the upper limit to yield in a vineyard is determined by the quantity of exposed leaf area available between veraison and harvest, and that exceeding a vine's capacity for fruit production results in overcropping, which both reduces the value of the present crop and the vine's capacity for cropping the subsequent growing reason. Thus, one of the keys to achieve vine balance is to decide the upper limit of yield which also relies on many factors and has interactions with vine vegetative growth.

1.3.2 Berry composition

Thousands of compounds are found in grape berries. The most important four groups of compounds are sugars, acids, phenolic and volatile compounds, which are most responsible for berry and wine sensory attributes.

1.3.2.1 Sugar

The major sugars in grapes are glucose and fructose. Santesteban & Royo (2006) reported that berry sugar concentration was positively related to leaf area to crop load ratios. But considering the findings from Kliewer & Antcliff (1970), leaf area should be more important during post-veraison in terms of sugar accumulation. At the same time, it should be emphasized that the importance of

maintaining the terminal leaves on shoots throughout the entire growing season, which contribute the most photosynthates after veraison (Kliewer & Antcliff 1970; Hunter & Visser 1988).

One the other hand, King et al. (2012) stated that crop removal rather than leaf removal in the fruiting zone had a positive effect on sugar concentration. However, in that study only apical clusters were removed, and at E-L stage 33 (berries still hard and green). The vines were also unbalanced with excessive shoot growth and dense canopies (the leaf layer number ranged from 2.6 to 3.2 under three levels of leaf removal). Furthermore, only apical clusters were removed, which are known be less advanced in developmentn compared to basal clusters. Thus, although canopy surface leaf area did not change much by treatment, the smaller fruit-sink size may cause less competition between clusters for assimilates and resulted in greater flux into the remaining basal clusters.

Generally, increased vine vigour can cause a decrease in berry sugar concentration, either because of the shading within vigorous canopies or because of the imbalanced source to sink ratio (Song et al. 2014). In a cool climate, an increase in sugar also depends on the intensity of radiation or the duration of exposure to direct sunlight (Smith et al. 1988). In addition, a fraction of sugar in fruit comes from reserves in the trunk, roots and cordons (Kliewer & Antcliff 1970). Thus, large volumes of perennial wood with the concomitant increased in carbohydrate storage volume are often desired (Howell 2001), which could also benefit canopy early development and may buffer the heavy crop load effect for the current season.

1.3.2.2 Titratable acidity (TA) and potassium (K^+)

The total concentration of hydrogen ions in grape juice (and wine), which is determined by titration with a strong base to a particular end point, i.e. pH 8.2, is referred to as titratable acidity (TA). The principal organic acids in grapes are tartaric and malic acids. Kliewer & Antcliff (1970) suggested that leaves have little direct influence on TA of fruit during post-veraison, but during pre-veraison leaves may affect the acidity of fruit either by supplying the metabolites for organic acid synthesis or by supplying the acids themselves. A recent review by Ford (2012) suggests that most of the malic and tartaric acids found in berries is synthesised within the berry. Thus, leaves may not have a directed influence on the synthesis of fruit TA but still may affect the initiation pre-veraison.

TA generally increases with vine vigour, which is believed to be caused by less malic acid degradation due to more canopy shading, as reported by Smart (1985) and Smart et al. (1985b). According to Smith et al. (1988), leaf removal in the fruiting zone pre-veraison, reduced berry TA largely due to a reduction in malic acid, where the longer the fruit was exposed the greater the reduction in TA. No significant response was seen with post-veraison leaf removal, in agreement with the theory of Ford

(2012). The reduction of TA, especially as malic acid, was further shown to be temperature dependent due to an enhancement of malic enzyme activity (reviewed by Reynolds et al. 1986). Aside from the leaf removal, Dami et al. (2006) reported that in a cluster thinning experiment where all the grapes were harvested on the same day, juice TA was not affected by the treatments, indicating that TA may not be affected by crop load.

In addition, potassium (K^+), which is the most abundant mineral in grape berries, plays a role in influencing TA concentration in the berry as well as the juice. Shaded leaves in the upper canopy can accumulate K^+, which can be redistributed to the fruit during ripening (Smart et al. 1985b; Smith et al. 1988). Freeman et al. (1982) also suggested that any conditions that reduce leaf photosynthetic activity could contribute to increased K^+ movement to the berry. It is well understood that excess K^+ in the berry can change TA by displacing H^+ ions. Aside from this, excess K^+ may also affect malic acid metabolism. In theory, K^+ concentration can modify the temperature's effect on cell membrane permeability, which in turn leads to more malic acid being transported to the berry periphery for use in respiration reactions (reviewed by Iland et al. 2011). In other words, upper canopy shading may cause high K^+ in berries, and lower berry TA at maturity.

1.3.2.3 Phenolic compounds

Two groups of phenolics are of high importance in the wine industry: anthocyanins and proanthocyanidins (condensed tannins).

King et al. (2012) reported that anthocyanin concentration in berries was responsive to leaf removal in the fruiting zone but not crop removal. Smith et al. (1988) found that leaf removal in the fruiting zone either five weeks post flowering or at flowering could result in increased anthocyanin content. However, leaf removal at flowering caused considerable vegetative regrowth by veraison, which then reduced cluster exposure, but still increased anthocyanin content in fruit at harvest. Thus, both Smith et al. (1988) and Dokoozlian & Kliewer (1996) believed that light had a vital impact on anthocyanin accumulation especially during the initial stages of growth (pre-veraison).

Cortell et al. (2007) reported that fruit from high vigour vines tended to contain low concentrations of anthocyanin. Their preliminary definition of vigour zones was based on an index calculated using measurements of average shoot length, trunk cross-sectional area, and leaf chlorophyll, which appeared to be closely related to variations in soil depth and water holding capacity according to the descriptions from the authors. However, the variation in anthocyanin concentrations (and composition) among different vigour zones could be mainly due to a combination of light and temperature effects as a result of variations in canopy shading (Cortell et al. 2007).

Proanthocyanidin accumulation reacts in a similar way. The phenomena that the total amount of skin proanthocyanidin, proportion of epigallocatechin extension subunits, average molecular mass of proanthocyanidins, and pigmented polymer content in fruit increased with decreasing vine vigour were reported by Cortell et al. (2005). According to Cortell & Kennedy (2006), when extracted by a model wine system, fruit from shaded clusters had a decreased concentration in and altered composition of proanthocyanidin, especially for skin proanthocyanidins. They also suggested that light exposure could promote pre-veraison skin proanthocyanidin formation and increase post-veraison proanthocyanidin polymerisation.

Generally, both anthocyanin and proanthocyanidin accumulation in fruit are reported to be sensitive to light and temperature. In cooler temperature growing regions where the negative effect of excessive berry temperature on grape composition is not an issue, sunlight exposure tends to be the dominant factor in affecting the accumulation of anthocyanin and proanthocyanidins (King et al. 2012).

1.3.2.4 Volatile aroma compounds and precursors

In wines, aldehydes, alcohols, esters and acids, along with other unidentified compounds present, form a unique aroma profile according to the various concentrations and proportions of these compounds. Some aroma-active compounds present in berries can be transferred into the wine, such as volatile terpenes, sesquiterpenes and methoxyprazines. They persist through vinification and are therefore termed primary aromas. In addition, some precursors in berries can be transformed during winemaking to add volatile characters in wine. In fact, yeast plays an important role in the conversion of precursors to volatile aroma forms (e.g. ethyl esters). These fermentation generated aromas are termed secondary aromas. Other aromas are generated during maturation or aging process post vinification and termed tertiary aromas.

Iland et al. (2011) suggested that vigorous shoot growth may lead to fewer assimilates being directed to the berries, which in turn may limit 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.

On the other hand, volatile aroma compounds in fruit could be altered by canopy microclimate. Descriptive analysis showed that lower shoot density (10 shoots/m row) in Pinot noir resulted in wines with less perceptible vegetative character, more fruit aroma and flavour in comparison to that of higher shoot densities (Reynolds, et al. 1996). According to Ryona et al.(2008), the concentration of methoxypyrazines, which is responsible for some of the herbaceous and vegetative characters in

grapes and wines, could be reduced by sunlight exposure, specifically pre-veraison. On the contrary, the development of norisoprenoids could be enhanced by foliage and cluster exposure or by other factors (Lee et al. 2007). For example, β -damascenone, which is considered to contribute a positive fruity-floral wine aroma, had the highest concentration in fruit when no leaves were removed throughout the season (Lee et al. 2007). Lee et al. (2007) further suggested that leaf layer number may influence norisoprenoid concentrations negatively, independent of sunlight exposure.

Specifically, β-damascenone, β-ionone, linalool and geraniol were believed to be important Pinot noir wine aromas and they are mainly generated during berry development (Fang & Qian 2006). Most authors postulate that β-damascenone and β-ionone could be produced by degradation of carotenoids (Maarse 1991). It has been reported that leaf removal and shoot thinning to promote sunlight exposure, enhanced both pre-veraison accumulation of carotenoids and their post-veraison degradation to aroma active C_{13}-norisoprenoids (Crupi et al. 2010; Marais et al. 1992). In addition, the concentration of linalool and geraniol in wines from ultra-low vigour zones were higher compared to wines from the other vigour zones (Song et al. 2014). Lower vigour vines, however, typically have greater fruit exposure to sunlight (Smart et al. 1985b), which could lead to the increased terpenoid concentration. Thus, canopy/fruit zone microclimate, especially sunlight climate, could have a major effect on primary aromas of Pinot noir wine, though other factors, e.g. leaf area to crop load ratio, remains unclear.

Other secondary aroma compounds that have been reported to be odour active in Pinot noir wine will be discussed in 1.4.2.

1.3.3 Canopy microclimate and source to sink relationship

Through manipulating vine balance, the target is to guarantee fruit quality while pursuing the largest fruiting capacity that maintains sustainable production. Source to sink relationships and canopy microclimate are closely related, which are proxies to show whether the vines are in balance.

On the one hand, either leaf removal (reducing source size) or crop thinning (reducing sink size) modifies canopy microclimate. On the other hand, vine growth and yield are dependent not only on CO_2 fixation capability (whole vine photosynthetic rate), but also on the integrated processes of carbon allocation, accumulation and utilization (Palliotti et al. 2011). Thus, it would be reasonable to conclude that when manipulating the source to sink ratio, the canopy microclimate will be somewhat disturbed, or looking at it from the other direction, canopy microclimate disturbs the source to sink ratio.

There is no doubt that whole vine production of photosynthates depends on the leaf area at or above light saturation point. However, this relies on characteristics of canopy architecture and depends on sink strength and growing conditions, which include vine nutrient status and canopy microclimate. The allocation of photosynthates depends on individual sink size and relative strength, which varies with vine physiological status and growing season (Farrar 1992; Petrie et al. 2000; Kliewer & Dokoozlian 2005). A case in point could be that higher temperature around the fruit, which can be caused by better exposure to the sun, could cause a greater sugar flux into berry, meaning the stronger sink strength was induced by microclimate conditions (Farrar 1992).

Vine vigour has the potential to affect canopy microclimate with respect to sun exposure and leaf surface temperature. The variation in vine vigour, which is associated with grape yield and primary and secondary metabolites of grapes, could result from vineyard attributes and cultural practice. As suggested by Naor et al. (2002), reductions of both vegetative and reproductive sinks allowed a greater allocation of assimilates and reserves to each of the remaining clusters and vegetative growing points. In other words, with proper management, a certain vine vigour coupled with optimum crop load could generate an appropriate source to sink ratio, resulting in good canopy microclimate and yielding quality fruit.

During berry development, various compounds can be produced, accumulated and transformed. The final composition of the berry at harvest is largely dependent on the source to sink relationship and canopy microclimate as discussed before (Figure 1.1). However, vine balance is not only the balance between vegetative growth and reproductive growth, but also the balance between fruit quality and yield quantity, which should take the end use of fruits and economic factors into account. However, the amount of research in this area, especially the effects on quality attributes, is slim.

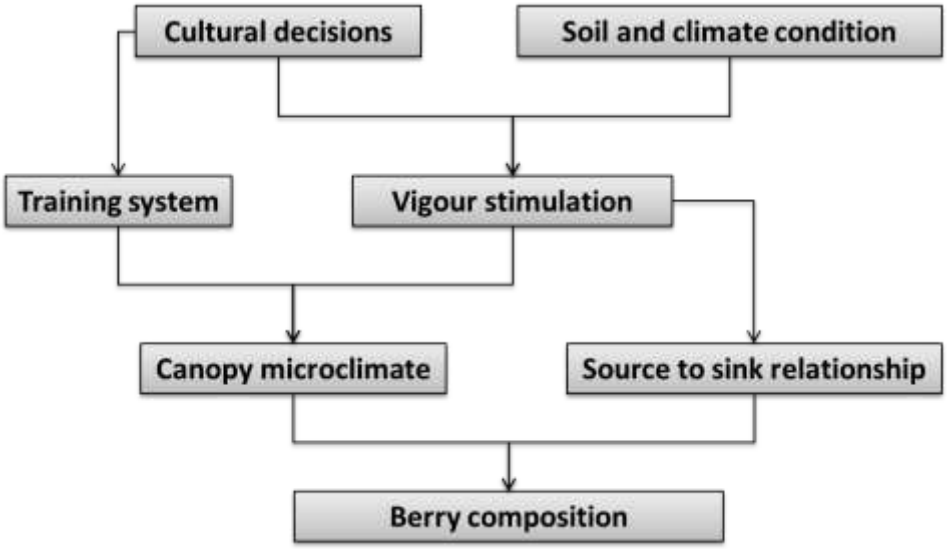

Figure 1.1 Conceptual model to show factors that can alter berry composition (Modified from Smart 1985)

1.4 Wine

1.4.1 Wine sensory elements

Taste and mouthfeel are essential elements of wine quality and major drivers for expert evaluation and consumer liking. Major taste qualities in wine are sweetness, sourness and bitterness, contributed by sugars, organic acids and some phenolic compounds, respectively. Mouthfeel encompasses a number of inter-related tactile sensations, which are mainly caused by the interaction between tannins and salivary proteins. Before actually "tasting" the wine, when one considers the sensory properties of wine, its colour is generally the first attribute evaluated. Anthocyanin is mainly responsible for the colour of red wines. Aside from this, various aroma compounds consist of an aroma profile. One basic idea is that all wines share a common basic aromatic structure formed by ethanol and 27 different aroma compounds, most of them are by-products of fermentation (Ferreira 2010). It is the variation of the concentrations as well as the proportions of these 27 aroma compounds and other volatile compounds that makes the wine unique and exotic.

1.4.2 Evolution of various grape-derived compounds during red winemaking

1.4.2.1 Sugars and acids

Sugars are converted into ethanol during alcoholic fermentation, and how much sugar is left (residual sugar) in wine depends on wine style and winemaking techniques.

As to acids, malic acid can be converted into lactic acids if malolactic fermentation (MLF) is conducted. Lactic acid tends to be softer and more comfortable compared to malic acid in terms of organoleptic sensation, and often gives the wine a slight “buttery” and/or toasty nose and sometimes a certain amount of complexity (Grainger et al. 2005). Excessive K^+ can precipitate tartaric acids and decrease TA in wine. These precipitates can be filtered before bottling, and the pH of resulting wine can be altered (Iland et al. 2011). The pH of the resultant wine has an influence on anthocyanin form, consequently altering the wine colour (Cheynier, et al. 2006).

1.4.2.2 Phenolic compounds

Phenolic changes that are associated with winemaking begin with selective extraction of grape constituents into the must during crushing and maceration. Skins need to be in contact with the juice (and wine if post-fermentation maceration is conducted) for good extraction of colour and tannins.

Once extracted, anthocyanin monomers will undergo chemical reactions mainly in three ways. In the first place, the formation of co-pigments occurs at an early stage, and the equilibrium of co-pigments depends on the concentration of co-factors and the ethanol/water ratio (Somers & Evans 1979). As a result, the wine colour intensity increases and shifts the colour towards purple (Cheynier, et al. 2006). Secondly, anthocyanin can act as the terminal unit of the tannin chain through either an ethyl bridge or C4-C8 (or C4-C6) bond to yield a polymeric pigment. The polymeric anthocyanin could be either pigmented or colourless. Thirdly, with the help from other phenolic acids, an anthocyanin monomer can undergo cycloaddition to form pyranoanthocyanin which seems to be the most stable coloured conformation found in wine and shifts colour from red to orange (Cheynier, et al. 2006). In all, Cheynier et al. (2006) concluded that conversion of anthocyanin into co-pigmented form; or pyranoanthocyanins and ethyl-linked derivatives can shift the colour towards orange or purple tints, enhance colour intensity and stabilise the colour against pH changes or sulphite bleaching. However, free anthocyanin content dropped dramatically especially during the first months after winemaking (Perez-Magarino & Jose 2004).

As for tannins, they can undergo polymerization through ethyl bridge formation and associate with anthocyanin as mentioned above (Cheynier, et al. 2006). Nevertheless, due to the partial extraction, and transformation because of oxidation, polymerisation, degradation and precipitation during the winemaking and aging process, the content of phenolic compounds in wine are less than those predicted from berry composition (Herderich & Smith 2005).

1.4.2.3 Volatile aroma compounds

Wine aroma compounds originate from the berries, and from other volatile products developed during winemaking and wine aging using aroma precursors originally generated in the berry. Oak use and the choice of yeast also influence wine aroma but are not the focus of this review. Production of individual groups of volatile compounds were detailed in work from Clarke & Bakker (2004)and Maarse (1991).

However, no single or group of aroma compounds has been recognized to be responsible for varietal aroma of Pinot noir wine, which is generally considered to be of red fruits evoking particularly the odours of small-stone fruits such as plum and cherry (Moio & Etievant 1995; Fang & Qian 2005; Fang & Qian 2006). 2-Phenylethanol and 3-methylbutanol, which are generated during fermentation, could be very important in contributing to the overall aroma in Oregon Pinot noir wines (Fang & Qian 2005), but, only four odour active peaks were common among six wines (two vintages × three grape maturities produced within the same vineyard): linalool, 2-phenylethyl acetate, 2-phenylethanol and an unknown methionol impurity (Mirandalopez et al. 1992). In addition, *p*-hydroxybenzaldehyde and 2-phenethanol were the major components in distinguishing Californian and Pacific Northwest Pinot noir wines (Brander et al. 1980). As the original producer of Pinot noir, Burgundy Pinot noir wines were characterized by ethyl anthranilate, ethyl cinnamate, 2,3-dihydrocinnamate and methyl anthranilate, but these were still regarded as minor constituents in terms of concentration (Moio & Etievant 1995). Later papers reported that the concentration of these four compounds were below the sensory thresholds, but that they may act synergistically with each other or other compounds to contribute to perceived aroma (Aubry et al. 1997; Fang & Qian 2006). In New Zealand, Kilmartin & Nicolau (2007, as reviewed by Kemp 2010) suggested that β-damascenone and β-ionone as well as C_6 alcohols, higher fermentation alcohols, carboxylic acids and esters were important aroma compounds in Pinot noir wines. A prior study by Tomasino (2011) at Lincoln University, showed that some fatty acids could contribute to different Pinot noir wine styles. In addition, canonical correlation analysis of chemical and sensory data combined with further addition/omission tests showed that four compounds, namely benzaldehyde, ethyl decanoate, ethyl octanoate and 2-phenylethanol could be important aroma compounds in New Zealand Pinot noir wines (Tomasino et al. 2015). Rutan et al. (2014) reported that β-damascenone, ethyl octanoate, ethyl hexanoate, ethyl isovalerate, isovaleric acid and 3-mercapto-hexanol were the most important odorants in Central Otago, New Zealand Pinot noir wines based on odour activity values.

The secondary aromas that have a contribution to a typical Pinot noir wine aroma profile are introduced below.

By enzymes, C_6 alcohol and aldehydes, which are generally considered to be responsible for “green” characters in wine, are generated from aerobic oxidation of linoleic and linolenic acids (C18:2 and C18:3) (Tomasino 2011). This process mainly occurs at different points between harvesting and alcoholic fermentation, such as transporting, crushing, pressing, must heating and grape maceration, though it happens in grape itself as well (Clarke & Bakker 2004; Oliveira et al. 2006). Acetaldehyde can be consumed by either reduction or oxidation into ethanol and acetic acid, respectively, with the lowest concentration being found at the end of fermentation (Ugliano & Henschke 2009).

At high concentrations, fatty acids are associated with rancid, cheesy and vinegar-like aromas (Song et al. 2014). Most fatty acids present in wine are biosynthesized during fermentation by yeasts and bacteria (Maarse 1991). Octanoic acid and decanoic acid are abundant in wines, which result from yeast metabolism of lipid during fermentation (Maarse 1991; Song et al. 2014). However, the presence of propanoic, butanoic and 3-methylbutanoic acids is due to the micro-organisms, in other words, they are by-product of the yeast metabolism of proteins (Clarke & Bakker 2004).

The generation of straight chain higher alcohols or fusel oils are also largely due to fermentation. However, certain higher alcohols may originated from grape-derived aldehydes and by the reductive denitrification of amino acids or via synthesis from sugars (Perestrelo et al. 2006). In addition, fusel alcohols can be derived from amino acid catabolism via a pathway that was first proposed a century ago by Ehrlich (1907). Thus, with the addition of specific amino acids, the production of certain branched-chain fusel oils may be promoted, as suggested by Clarke & Bakker (2004).

Esters, which are responsible for “fruity” and “floral” aromas, are generally considered to be generated during fermentation through acyl-SCoA by yeasts (Fang & Qian 2006; Ugliano & Henschke 2009). Their formation can be affected by many factors, such as yeast strain, fermentation temperature, oxygen availability, grape nutrient composition, nitrogen level, pH, SO_2 levels and overall matrix conditions (Clarke & Bakker 2004; Ebeler & Thorngate 2009; Perestrelo et al. 2006; Vianna & Ebeler 2001).

1.4.3 Wine quality and vine balance

Iland et al. (2011) suggested that the quality of wine can be rated according to the type, intensity, complexity, length and balance of its sensory attributes. Naor et al. (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.

Kassara & Kennedy (2011) reported that there was a positive correlation between wine quality rating (assessed by winemakers) and skin-derived tannins. At the same time, skin tannin concentration can be altered by canopy microclimate (Cortell & Kennedy 2006) and by vine vigour (Cortell et al. 2005) as mentioned above. Thus, wine quality has some connections to vine vigour and the canopy climate with respect to tannin concentration.

In addition, wine colour density, total anthocyanins and nonbleachable pigments all had a high correlation with wine grade judged by winemakers (Kassara & Kennedy 2011). Cortell et al. (2008) reported that wines made from low vigour vines had a much greater formation of pigmented polymers. Castro et al. (2005) reported that vines of low shoot density make good wine by improving colour intensity. However, Iland et al. (2011) mentioned an unpublished study with Pinot noir in a cool climate in which a positive linear relationship was found between wine quality rating (judged by winemakers) and berry colour when the anthocyanin concentration was in the range of 0.3-0.8 mg/g berry weight; values above 1.0 mg/g were rated downwards possibly because of the resultant wine was out of style for a Pinot noir wine.

Volatile aroma compounds play an important role in the sensory properties of wines. However, limited research was reported on relating wine scores with individual aroma compound in Pinot noir wine, or on a larger scale, in red wine or grape fruit. Ristic et al. (2010) reported that a positive relationship between wine quality score and hydrolytically-released β-damascenone concentration for both berries and wines has been found in Shiraz. Song et al. (2014) reported that the concentration of esters, some terpenoids (linalool, nerol and geraniol), and C_{13}-norisoprenoids vitispirane and β-ionone were higher in wines made from low vigour Pinot noir vines. Among them, esters (being mainly produced during fermentation and aging) concentration depends on nutrient density in the grapes, i.e. nitrogen availability (Dennis et al. 2012; Song et al. 2014). The concentration of terpenoids can be enhanced either by better light exposure, or by increased grape maturity primarily caused by low vine vigour and in turn, better light exposure (Skinkis et al. 2010; Fang & Qian 2006; Song et al. 2014). C_{13}-norisprenoids can be enhanced by sunlight exposure as mentioned earlier (Lee et al. 2007). In addition, leaf removal produced Pinot noir wines that had more dark fruit aroma than non-defoliated wines (Kemp 2010); herbaceous aroma scores increased with increasing pruning weight in Sauvignon blanc wines (Naor et al. 2002).

In all, a range of compounds contribute to the wine sensory profile. Wine quality is not only decided by the intensity, but also the balance of these compounds. Although some compounds are mainly generated during winemaking, most of them depend on the pre-fermentation concentration of their respective precursors in grapes. 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.

1.5 Conclusion

Manipulation of vine balance is widely practiced to enhance grape and wine quality. A certain amount of vegetative growth is appreciated to support reproductive growth, yielding good quality berries. Leaves, being part of vegetative tissue, are the energy source 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. Dense canopies, which are often formed by too much vegetative growth, are not beneficial for berry development. In general, an "open canopy" with well exposed leaves and fruit has scored highest in wine quality by taste panels (reviewed by Kliewer & Dokoozlian 2005).

Although the winemaking process only achieves partial extraction of the fruit and modifies these grape-derived compounds by forming new compounds and/or new sensory components, many of the compounds responsible for the sensory attributes of a wine originate in the berry. The concept of wine quality is complex, but should be related to the wine style and can be measured either by sensory assessments or chemical analyses or both. Thus, it is reasonable to conclude that wine quality is largely dependent on the quality of fruit which are carried by grapevines and modified by grapevine state—vine balance.

1.6 Objective of this project

Pinot noir vines were treated with three levels of leaf removal and three levels of crop removal to disturb the vine balance. Previous study by Pasch (2014) mainly focused on the phenological stages and physiological status of the vine as affected by the treatments, i.e. photosynthesis, sugar accumulation, coloration process along the growing season. This study is a further analysis and builds on work done by Pasch (2014). The main focus of this study is to discover whether some potential sensory attributes of grapes and wine can be affected by the various leaf and crop removal treatments. Most sensory attributes of grapes and wine are generally considered to be generated from or affected by the compounds or the matrix of compounds in grapes. Thus, one key part of this research is to identify which compound(s) can be affected by the treatments, and what would this mean in terms of the sensory properties of the wine, at the same time as identifying whether there would be any carry-over effect existing between grapes and wine.

Chapter 2 Materials and methods

The experimental design, treatment application, calculation of leaf area to crop load ratios, berry and root sample collection, and winemaking were reported by the previous research student Pasch (2014). All the other methods reported in this Chapter were done within this study.

2.1 Site and materials

This study was conducted on 2013/ 2014 growing season in the David Jackson vineyard, Lincoln University, New Zealand. The vineyard is situated at approximately latitude 43° 39' degrees south, and longitude 172° 28' east. The soil is predominantly Paparua and Wakanui series. The growing degree day (GDD) accumulation at the research site up through April was 949 (10 ℃ base) on a monthly basis and 965 on a daily basis, where the long term average (LTA) through April is 924 and 971, respectively. The precipitation received from July, 2013 to March, 2014 was 427.5 mm with the pattern shown in Figure 2.1.

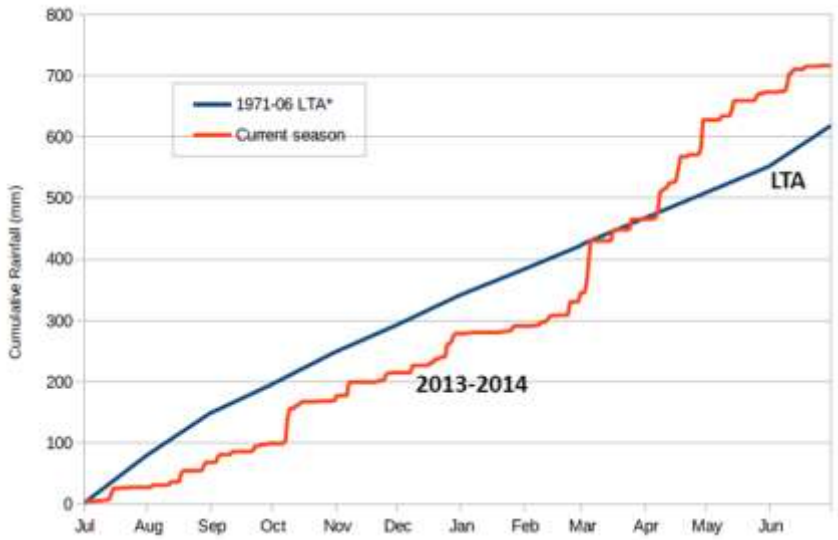

Figure 2.1 Cumulative rainfall (mm) for 2013-2014 vintage. Note: LTA values were calculated from the 1930 through the 2010 vintages

Two adjacent rows of mature vines of Pinot noir clone 10/5 (planted in 1996) on Riparia gloire at 1.6 m in-row spacing in north-south oriented rows were the subject of study. All the vines were trained to a vertical shoot positioned system and pruned to two bilaterally-opposed canes.

The experimental design was three leaf removal and three crop removal treatments in four replicated blocks (two blocks in each row, see Figure 2.2). It was a split plot design with three vines

per plot. The reason to use a split plot design was to avoid shading or other effects caused by adjacent vines with a different treatment.

Leaf	Crop	Rep	Leaf	Crop	Rep
--	--	Buffer vine	--	--	Buffer vine
0.25L	1C	3	1L	0.25C	2
0.25L	0.5C	3	1L	0.5C	2
0.25L	0.25C	3	1L	1C	2
--	--	Buffer vine	--	--	Buffer vine
1L	0.25C	3	0.25L	1C	2
1L	0.5C	3	0.25L	0.25C	2
1L	1C	3	0.25L	0.5C	2
--	--	Buffer vine	--	--	Buffer vine
0.5L	1C	3	0.5L	0.5C	2
0.5L	0.5C	3	0.5L	0.25C	2
0.5L	0.25C	3	0.5L	1C	2
--	--	Buffer vine	--	--	Buffer vine
0.25L	1C	4	0.25L	1C	1
0.25L	0.5C	4	0.25L	0.5C	1
0.25L	0.25C	4	0.25L	0.25C	1
--	--	Buffer vine	--	--	Buffer vine
0.5L	1C	4	0.5L	0.25C	1
0.5L	0.5C	4	0.5L	1C	1
0.5L	0.25C	4	0.5L	0.5C	1
--	--	Buffer vine	--	--	Buffer vine
1L	1C	4	1L	0.5C	1
1L	0.5C	4	1L	1C	1
1L	0.25C	4	1L	0.25C	1
--	--	Buffer vine	--	--	Buffer vine

Figure 2.2 Experimental design. L, the levels of leaf numbers; C, the levels of crop loads.

2.2 Treatments

2.2.1 Leaf removal treatments

All shoots were cut to retain 14 leaves as some nodes did not have leaves when the treatments were applied. Thus, some shoots were greater than 14 nodes but still had 14 leaves. Two of the four basal leaves were retained for all the treatments. Three levels of leaf removal treatments were applied post fruit-set. Full leaf number (1L) was considered as retaining all leaves above 4th node, and 12 leaves were retained in total. Half leaf number (0.5L) was considered as retaining 4 leaves above 4th node following the pattern of removing every other leaf, and 6 leaves (4 leaves above 4th node plus 2 basal leaves) were retained in total. Quarter leaf number (0.25L) was considered as retaining 1 leaf above 4th node, and 3 leaves (1 leaf at the top of the shoot and 2 basal leaves) in total. Leaf removal treatments were on per shoot basis, the apical leaf was retained under all treatments.

2.2.2 Crop removal treatments

Three crop removal treatments were applied post-fruit set on a per vine basis. Full crop obtained (1C) was considered as retaining all clusters. Half crop obtained (0.5C) was considered as removing every other cluster. Quarter crop obtained (0.25C) was considered as retaining every fourth cluster. The last two crop removal treatments were by random removal, so as not to preferentially leave basal clusters.

2.2.3 Treatment ratio (TMR)

Treatment ratios were calculated by the formula: leaf levels divided by crop levels and listed below.

Table 2.1 Treatment ratio (TMR) calculation method and its relationship with treatments

Treatments	**0.25L**	**0.5L**	**1L**
0.25C	1	2	4
0.5C	0.5	1	2
1C	0.25	0.5	1

2.3 Winemaking process

Winemaking was done by Ludwig Pasch, a research student. An excerpt from his thesis (Pasch, 2014) regarding the winemaking is reproduced here:

> Grapes were processed in the experimental winery of Lincoln University, located about 500 m distance to the experimental plot. Immediately after harvest, grapes were destemmed manually and 2 to 3 kg of grapes, depending on the available quantity, grapes were put for fermentation in plastic buckets of 10 L volume. Grapes of replicates 1 & 4 and 2 & 3 were processed together, which means there were two wines per treatment made. It was not possible to vinify each replicate separately due to low quantities of grapes, especially for the "25C" crop level treatment. After destemming, 60 ppm SO_2 in the form of potassium metabisulfite was added to the grapes in order to suppress wild yeast activity. A cold soaking was performed for 15 h at 4 °C. Grape juice samples were taken from the free run juice after cold soaking. These samples were stored in a freezer at - 20 °C until further analysis were made. After cold soaking, the sugar content of the must of each experimental wine was determined using a digital refractometer (PAL1 Pocket Refractometer, Atago Co. LTD, Tokyo, Japan). All musts were

adjusted to the same sugar content by adding sucrose. The must with the highest sugar content was set as a reference.

After the cold soaking, grape must was heated up to 20 °C and inoculated with a selected yeast strain (BGY Enoferm Burgundy, Lallemand Inc., Montreal, Canada). The dry yeast was rehydrated in warm water at 35 °C for 30 minutes. Then grape must was added. After another 30 minutes yeast activity could be observed visually and the yeast slurry was allowed to cool down to the temperature of the must. The must was inoculated with 60 g hL^{-1} yeast. An addition of 30 g hL^{-1} of diammonium phosphate was applied to ensure a sufficient nitrogen supply for the yeast, since some of the grapes were infected with Botrytis.

Alcoholic fermentation took place in a temperature-controlled room at 26 to 27 °C. The cap of the fermenting grapes was pushed down three times a day (8 am, 2 pm and 8 pm). When initial sugar concentration was decreased by 30 % another 20 g hL^{-1} of diammonium phosphate was added to the ferments. The process of fermentation was monitored every 24 h by measuring temperature and sugar concentration. Sugar concentration was measured using a hydrometer. When sugar concentrations reached -1 °Brix fermentation was considered as finished, followed by a post-fermentation maceration of 24 h. After fermentation, grapes were pressed manually and the wine was filled in plastic bottles of 1 to 3 L volume. After 24 h of sedimentation, the wine was racked off the lees and filled again in plastic bottles. Freeze-dried lactic bacteria of the strain *Oenococcus oeni* (Viniflora® CH16, CHR Hansen, Hørsholm, Denmark) were added (0.6 g hL-1) to initiate malolactic fermentation. Malolactic fermentation took place in a temperature controlled room at 20 °C. The conversion of malic acid into lactic acid was monitored by paper chromatography. After malolactic fermentation, the wine was stored for four weeks at 15 °C and then bottled. The wine was carefully racked off the lees and filled into 0.375 L glass bottles and sealed with screw caps. At bottling, the concentration of free SO_2 was adjusted to 25 mg/ L free SO_2 with potassium metabisulfite solution.

2.4 Research methods on vine and fruit

The objectives of the present study was to evaluate the effects of leaf and crop removal on fruit ripening, vine performance, fruit and wine composition. The first part of this study, namely the

effects of LA/CL on fruit quality and vine performance have been assessed previously by Pasch (2014) and these results are referred to as appropriate.

2.4.1 Leaf area to crop load ratio (LA/CL)

All the grapes were hand harvested on 4th April, 2014.

LA/CL (cm^2/g) was calculated from leaf area per vine divided by total yield per vine. In detail, total yield per vine (g), a calculated and potential value, was the total cluster number per vine multiplied by the average undamaged cluster weight per vine. Leaf area per vine (cm^2) was estimated firstly by measuring the area of every single leaf off two shoots per vine by a leaf area meter (LI-3100 Area Meter, Li-Cor, Lincoln, NE, USA) to get an average leaf area per shoot, and then multiplying that by the number of shoots on that vine. Leaf samples were taken 14 days post-harvest but prior to leaf fall.

2.4.2 Pruning weight

Pruning was conducted in fine weather condition on days mainly in August, 2014. Two cane pruning (Double Guyot) was practiced, and pruning weight (one-year old growth) was recorded vine by vine. A formula of 25 nodes per kilogram of pruning weight was then applied to conduct balanced pruning.

2.4.3 Carbohydrates determination

Sections of vine root near the base of the trunk were taken from each vine four weeks post-harvest, each about 10-15 mm in diameter and 10 cm long. The three root sections from each plot were pooled, washed with water, blotted dry and the fresh weight recorded before being put in a -20 ℃ freezer for three weeks. The roots were then cut into 1 cm pieces freeze-dried for 48 hours, ground (Retsch ZM 200, 18000 rpm) and passed through a 1 mm mesh. The prepared samples were stored in air-tight bags at -20 ℃.

Two standard colorimetric tests were used to measure the concentration of soluble sugar and starch in grapevine roots. These included the Anthrone test for soluble sugars and o-toluidene test for glucose from enzyme digested starch (Allen et al. 1974; Rose et al. 1991).

2.4.3.1 Soluble sugar extraction

Freeze dried and finely ground 100 mg root samples were extracted in 10 mL of 80% ethanol in a 85 °C water bath for 10 minutes (mixing occasionally), then centrifuged at 1680 *g* for 10 minutes to allow for better solid/liquid separation. After decanting the supernatant to a labelled container, the pellet was re-suspended in 5 mL of 80% ethanol and re-extracted until the supernatant was clear

(generally once more). The supernatants were pooled together and duplicate 1 mL aliquots used for analysis. Root tissue extracts, along with five glucose standards in the range of 0 to 0.6 mg/mL, were mixed thoroughly with 10 ml of Anthrone reagent (1.5 g anthrone, 1.0 g thiourea and 1000 mL 70% concentrated sulfuric acid) by vortexing, and then heated at 85 °C for 15 minutes for the colour reaction to proceed. Ten minutes of cooling down with tap water was conducted before measuring the absorbance at 625 nm using a Helios Alpha spectrophotometer (Unicam UV-Vis spectrometry). Standard curve for glucose was derived using linear regression equation. The soluble sugar (SS) concentrations (% dry weight) were calculated using the following equation:

SS (% dry weight)(mg/mg) = C (mg glucose/mL)×extract volume (mL) ÷ sample dry weight (mg) × 100

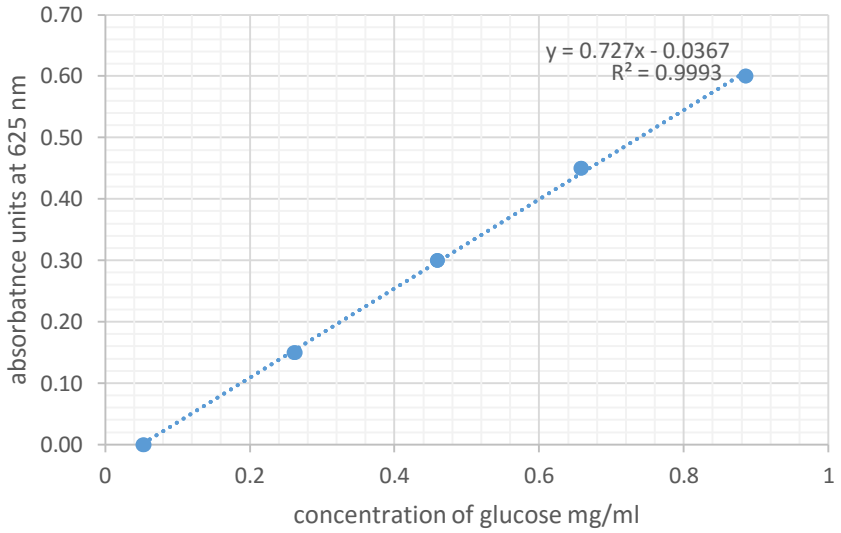

Figure 2.3 Glucose equivalent calibration curve at 625 nm

2.4.3.2 Starch digestion

Following soluble sugar extraction, the remaining solid tissue (pellet) was dried in an oven at 65 °C for 2-3 hours to remove ethanol and water. Once dried, the samples were re-suspended in 5 mL distilled deionised water, capped, and heated in a water bath at 85 °C for 1 hour to allow for starch gelatinisation. They were then quickly cooled in a 10 °C water bath for 15 minutes. One mL of starch digestion solution (398 enzyme units/ml α-amylase (Sigma A-6255), 1.96 enzyme units/ml amyloglucodase (Sigma A-3514), adjusted to pH 5.1 using sodium acetate buffer) was added to the sample and incubated at 50 °C for 48 hours, mixing occasionally. After incubation, the samples were centrifuged at 2500 rpm for 10 minutes and the supernatants were collected for colorimetric analysis. Supernatant aliquots of 0.1 mL in duplicate along with six glucose standards in the range of

0 to 3.5 mg/mL were mixed with 5 mL of o-toluidene reagent (1.0 g thiourea, 940 mL glacial acetic acid and 60 mL o-toluidene), capped, vortexed, and heated for 20 minutes in a water bath at 85 °C to allow for colour reaction. Ten minutes of cooling down with tap water was conducted before measuring the absorbency at 635 nm using a Helios alpha spectrophotometer (Unicam UV-Vis spectrometry).

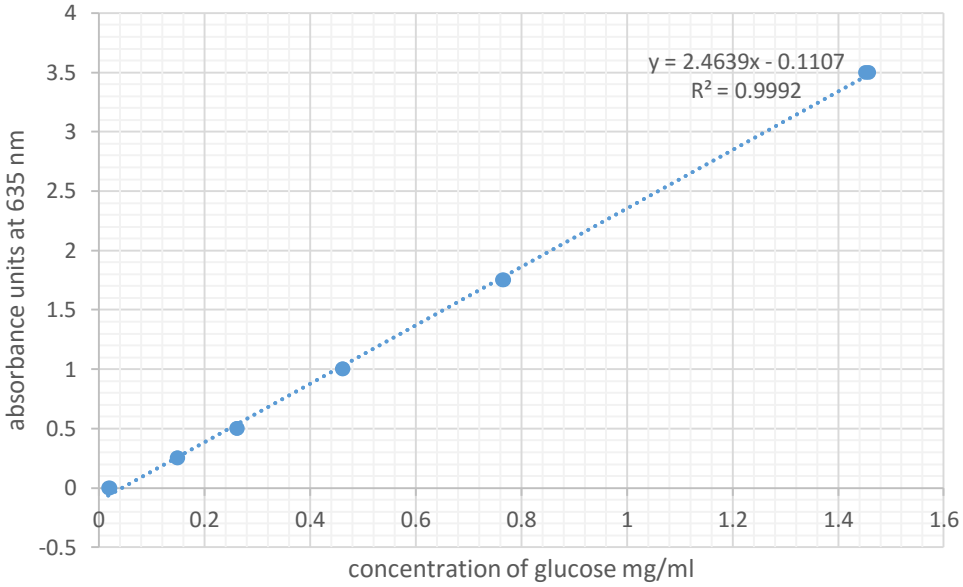

Figure 2.4 glucose equivalent calibration curve at 635 nm

The standard curves from the glucose standards were derived using linear regression equation (Figure 2.4). The weight (mg) of starch in the sample was calculated using the equation below:

% dry weight (mg/mg) = Yg × v × hf/dw × 100

Yg = glucose concentration (mg/mL)

v = original volume of starch extract (5 mL distilled deionised water + 1 mL starch digestion solution)

hf = starch hydrolysis factor (0.9)

dw = original sample dry weight (mg)

Recovery rates were obtained by adding 0.2 mL H_2O or 0.2 mL of a known concentration of glucose to testify the efficiency of Anthrone reagent. The results were 79.4% and 71.0% for 0.15 mg/mL and 0.30 mg/ml glucose spike, respectively. The same protocol was used to testify the efficiency of o-toluidene by adding 0.02 mL H_2O or a known concentration of glucose, the recovery rates were

105.7% and 113.0% for 0.50 mg/mL and 1.00 mg/mL glucose spike, respectively. These recovery rates were deemed acceptable.

2.4.4 Sample preparation

The material preparation method was previous used at Lincoln University by Kemp (2010) and further modified by Patricio Mejias (pers. comm.), and adopted here. Fifteen berries from each grape samples were randomly selected and their fresh weight were recorded. Samples were firstly defrosted at room temperature before the berry skins and seeds could be manually separated from the pulp. Fresh weight and seed number were recorded for seed samples before being frozen with liquid nitrogen to prevent further oxidation and were freeze-dried at -20 ℃ for approximately 48 hours. The dry weight was recorded and the seeds were ground into powder with a Polytron PT 3100 homogeniser for 5 minutes at 22000 *g*. Sixty milligrams of powdered seeds was extracted with 10 ml 50% ethanol combined with 1 mL water (11 mL solution in total), and then mixed on a Shafter Orbital shaker for 60 minutes at 30 rpm followed by centrifugation at 1960 *g* for 5 minutes.

At room temperature, the fresh weight of skins were recorded and then the material homogenised after adding 8 mL of water. Approximately 1 g homogenates was extracted with 10 mL 50% ethanol by mixing on a Shafter Orbital shaker for 60 minutes at 30 rpm followed by centrifugation at 1960 *g* for 5 minutes.

2.4.5 °Brix, titratable acidity and pH

For each berry sample, fifteen berries were randomly selected for measurements. Frozen berries were left to stand in fifteen individual test tubes at room temperature for one hour before measurements. Then, the berry was gently crushed with a plastic rod. A small volume of juice from each berry was used to measure °Brix using a digital refractometer (PR-101, ATAGO CO., LTD), recorded as individual °Brix and the rest of the juice was pooled into a centrifuge tube for juice from the other berries. The remaining solids in test tubes were also collected in another centrifuge tube. To extract more tartaric acid out into the juice, both the collected juice and remaining solids were held in a ~45℃ water bath for 20 minutes. The remaining solids were centrifuged for 10 minutes at 3000 rpm. The supernatant was combined with collected juice and further centrifuged for 10 minutes at 3000 rpm. Afterwards, a fraction of this juice sample (1.5 mL) was stored at -20 ℃ for minerals and organic acids analyses. Another °Brix reading was obtained from the total volume of juice and recorded as juice °Brix.

pH was measured by pH meter (SP-701, Suntex Instruments Co., Ltd., Taipei, Taiwan) and Titratable acidity was measured by titration to pH 8.2 by standardised NaOH solution following the method from Iland et al. (2000).

2.4.6 Minerals and organic acids analyses

2.4.6.1 Organic acid analysis by HPLC

One mL of saved sample mentioned above was taken out and 4 mL of DI water was added before being filtered through 0.2µm nylon filter membrane. Three hundred microliters of this was used for organic acids analysis by Shimadzu HPLC with UV-Vis detector (Shimadzu Corp. Kyoto Japan) (Shi et al. 2011). Sample injection volume was 20 µl. The identification of these organic acids was obtained by comparing retention time of standards. Sample quantification was determined by the peak height of chromatograms using the external calibration standard curve; all data were processed using LC solution software.

The column used to separate and analyse organic acids was an Alltech Prevail ^{TM} organic acid column (250 x4.6mm, 5µm particle size; Grace Davison Discovery Sciences) with a guard column (7.5 x4.6mm) with the same packing. The mobile phase was 25 mM KH_2PO_4 (pH 2.5, adjusted by H_3PO_4) filtered through a 0.45 µm membrane. The flow rate was 0.6 mL/min, and the column temperature was 50˚C. The detective wavelength was 210 nm. Sample injection volume was 20 µL.

A mixed standard stock solution was prepared by dissolving L-tartaric acid, L-malic acid, and citric acid in DI water. The concentration of L-tartaric acid and L-malic acid were 4000 ppm; and the concentration of citric acid was 2000 ppm. These standard stock solutions were kept in 4˚C fridge.

Working standard solutions were made by the above mixture standard stock solutions with DI water, concentration varied from 0, 2, 4, 10, 20, 40, 100, 200, 400, 1000, and 1600 ppm. The prepared organic acid standard solutions were kept in 4˚C fridge.

2.4.6.2 Mineral analysis by ICP-OES

The rest of samples were used for mineral analysis by ICP-OES (Inductively Coupled Plasma Optical Emission Spectrophotometer) (Nölte 2003).

Calibration standards and internal standards were serially diluted from Merck ICP standard solutions. The plasma gas flow was set to 15.0 L/min with 1.20 kW power. Aux 1.5 L/min, nebulizer 0.9 L/min, seaspray nebulizer and cyclonic spraychamber were used.

2.4.7 Total phenolics, total tannins and anthocyanins (colour measurements)

Total phenolics of seed extracts and skin extracts and total red pigments of skin extracts were measured by spectrophotometer (UV-1800, Shimadzu Corp., Kyoto, Japan) at 280 nm and 520 nm, respectively. For skin extracts, the method was slightly modified from Iland et al. (2000), by using 1.1 M HCl instead of 1 M HCl and adding 0.1 mL extracts into 1 mL HCl. Thus, 11 times dilution was gained instead of 101 times dilution but the final pH remained pH 1. Other colour parameters, i.e. colour density, colour hue, estimate of the concentration of SO_2 resistant pigments and degree of red pigment colouration, were conducted as described by Iland et al. (2000).

Colour density (a.u.) = $A_{520} + A_{420}$

Colour hue = A_{520}/A_{420}

Total red pigments = A^{HCl}_{520}

Total phenolics = $A^{HCl}_{280} - 4$

Estimate of the concentration of SO_2 resistant pigment = A^{SO2}_{520}

Degree of red pigment colouration (%) = A^{520}/A^{HCl}_{520}

Total tannins was measured using the methyl cellulose precipitable (MCP) tannin assay using the 1mL assay (Sarneckis et al. 2006) in 1.5 mL disposable UV-Cuvettes.

As the 1 mL assay was used, the volumes of samples and reagents of the MCP Tannin Assay for wine and grape extract samples were slightly different (Table 2.2).

Table 2.2 Volumes of sample and reagents for MCP Tannin Assay for wine and grape extract

	Treatment				**Control**			
Sample type	Sample volume	Polymer	Salt	Water	Sample volume	Polymer	Salt	Water
Wine	25 µl	300 µl	200 µl	475 µl	25 µl	0 µl	200 µl	775 µl
Grape extract	100 µl	300 µl	200 µl	400 µl	25 µl	0 µl	200 µl	700 µl

The dilution factor for skin extract was 10.

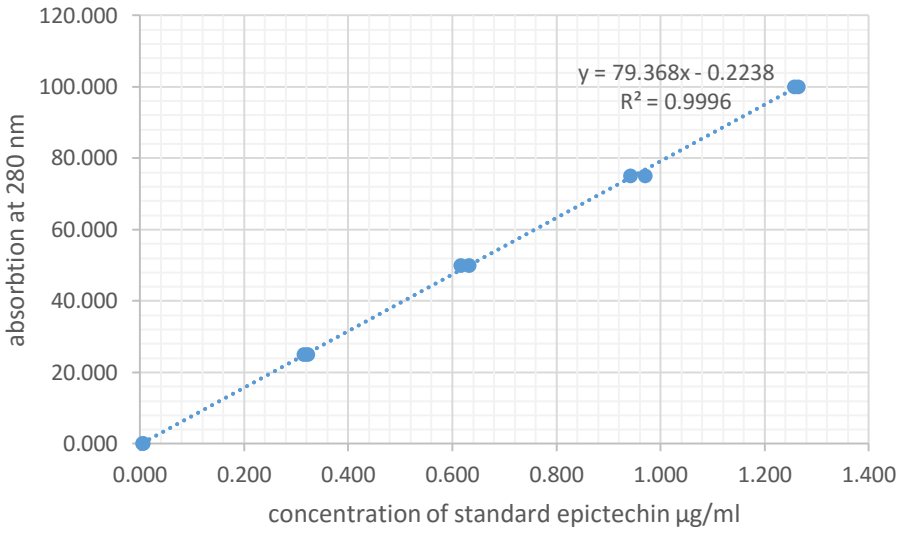

Figure 2.5 Epicatechin equivalent calibration curve

Tannin concentration in skin extracts (mg/g) was not calculated because skin extracts were considered as the "ingredients" of wine which is always in liquid form.

2.4.8 CIELab

The CIELab space is a uniform three dimensional space defined by the colorimetric coordinates L*, a* and b* (C. I. E. 1986). The measurements were made with a UV-Vis spectrophotometer using 0.2 cm pathlength cells. The measurements were taken at four wavelengths: 450, 520, 570 and 630 nm. The further calculation and colour simulation were gained by a software developed by the Research Colour Group at the University of La Rioja. The software can be downloaded from this link *http://www.unirioja.es/color/descargas.shtml*.

2.4.9 Phenolic analysis by high-performance liquid chromatography (HPLC)

HPLC methods will be presented in detail in 2.5.5.

2.5 Research methods on wine

2.5.1 Basic analyses

Alcohol, residual sugar, pH, titratable acidity (TA), free SO_2, and total SO_2 were determined using the methods described by Iland et al. (2000).

Alcohol content was determined by ebulliometer. The principle of this method is the depression of the boiling point of water/alcohol mixture with atmospheric pressure as the reference. For the measurement of residual sugar, Clinitest kits were used to decide whether wine samples need to be

diluted. Then the wine samples were de-colourised by carbon. After filtration, the Rebelein method was applied to determine residual sugar.

pH was measured by using calibrated pH electrode (SP-701, Suntex Instruments Co., Ltd., Taipei, Taiwan). Approximately 30 mL of wine sample was placed in thick-wall 250 ml conical flask and applied vacuum for 2-3 minutes until no bubbles come up. Then the titratable acidity (TA) was measured by titration to pH 8.2 by standardised NaOH solution following the method from Iland et al. (2000).

The aspiration method was used for the determination of SO_2 by firstly converting the free forms of SO_2 to molecular SO_2 by lowering the pH of the sample to pH 1. Free SO_2 was determined by passing a stream of air through the sample for 12 minutes at room temperature. Molecular SO_2 removed in the gas was passed through a hydrogen peroxide solution that can oxidise it to sulphate (SO_4^{2-}). The H^+ ions generated were then determined by titration with standardised NaOH solution. Bound SO_2 was then determined by boiling the same sample for 10 minutes while the stream of air was again passed through.

$$\mathbf{SO_2\ (mg/L) = C\ [NaOH] \times V\ [NaOH] \times [1/2] \times M\ [SO_2] / V_{wine}}$$

C [NaOH] desirably to be 0.01 M and was standardised by known concentration of HCl.
V [NaOH] was the amount of NaOH used for the titration.
[1/2] was the mole ratio of SO_2 to NaOH.
M [SO_2] was the molecular weight of SO_2 which is 64 g/mol.
V_{wine} was the volume of the sample.
When C [NaOH] was 0.01 M and V_{wine} was 20 mL, the equation can be simplified as

$$\mathbf{SO_2\ mg/L = V\ [NaOH]\ (mL) \times 16}$$

Total SO_2 was calculated from the sum of free SO_2 and the bound SO_2.

2.5.2 Fractionation of phenolic compounds by solid phase extraction (SPE)

Solid phase extraction (SPE) was applied to all the wine samples to produce three fractions following the method from Jeffery et al. (2008) with minor modifications. An Oasis HLB cartridge (3 mL, 60 mg, 30 µm) (Waters, Rydalmere, NSW, Australia) was utilized as follows. The cartridge was conditioned with 2 mL of ethanol followed by 2 mL of water, with the water being left level with the top frit of the cartridge. The wine sample was firstly centrifuged and 1 mL was applied to the cartridge under gravity. When the wine volume was completely adsorbed, the cartridge was dried with a gentle stream of nitrogen gas (approximately 1L/minute) for 15 to 20 minutes. The cartridge was washed

with 40 mL of 95% acetonitrile/5% 0.01 M hydrochloric acid (F1) and eluted with 5 mL of methanol containing 0.1% formic acid (F2), followed by 300 µL of neat formic acid prior to 2.7 mL of 95% methanol (F3). The solvent was removed in vacuum on a rotary evaporator at 40 mbar with a 35 °C water bath, and the fraction containing formic acid (F3) was dried further with a gentle stream of nitrogen gas (normally requiring 10 minutes). The samples were dissolved in 1 mL of 10% ethanol/0.1% formic acid for HPLC analysis, with the exception of F3. Once dried, F3 had 10 µL of formic acid added, followed by 10 µL of 10% ethanol/0.1% formic acid, and finally the remainder of the 10% ethanol/0.1% formic acid was added to give a final volume of 1 mL. Fractions were stored in 1.7 mL Eppendorf tubes at – 20 ℃ until further analyses.

2.5.3 Total phenolics, total tannins and anthocyanin (colour measurements)

Total phenolics and colour measurements on native wine samples were measured following the methods from Iland et al. (2000). Total tannins was measured following the MCP tannin assay (Sarneckis et al. 2006) introduced in 2.4.7. The dilution factor for wine was 40.

Total phenolics and total red pigments of the three wine fractions (F1, F2 and F3) were also measured by five-fold dilution to give a proper reading by spectrophotometer at 280 nm and 520 nm. The solution used for the dilution were 10% ethanol/0.1% formic acid for F1 and F2, 10% ethanol/1.1% formic acid for F3. The sum values of the total phenolics and total red pigments from the three fractions were recorded as Sum Phenolics and Sum Red Pigments. After being divided by total phenolics and total red pigments of native wine, two recovery rates were gained which can be used as the total phenolics recovery rate and total red pigments recovery rate for the solid phase extraction, respectively.

2.5.4 CIELab

This methods were introduced in 2.4.8 and applied on native centrifuged wine samples.

2.5.5 Phenolic analysis by high-performance liquid chromatography (HPLC)

HPLC was carried out on both skin extracts and wine F1 (Kemp et al. 2011; Cheynier & Rigaud 1986; La Torre et al. 2006). A Shimadzu LCMS 2010 equipped with system controller CBM-20A was used, with two pumps: Pump A (LC-20AD) and Pump B (LC-20AD). Auto sampler was SIL-20AC with temperature set at 4°C, and the injection volume was 10.00 µL. PDA detector was SPD-M20A. The column used was Grace Davison (Alltima C18, 250 mm × 2.1 mm, 5 µm), temperature controlled at 25°C. Two solvents were used to separate phenolic compounds, solvent A was deionized water contains 2.0% acetic acid, and solvent B was methanol contains 2.0% acetic acid. Flow rate was set

to 0.4mL/min. The HPLC solvent gradient was shown below. The PDA Detector was D2&W lamp, scan wavelength from 200nm to 600nm.

Table 2.3 HPLC solvent gradient for phenolic analysis

Time	Solvent A%	Solvent B%
0	98	2
8	85	15
24	80	20
38	69	31
57	30	70
63	0	100
68	0	100
69	98	2
80	98	2
80	stop	stop

2.5.6 Wine aroma compounds analysis by gas chromatography mass spectrometry (GCMS)

Head space solid phase micro extraction gas chromatography mass spectrometry (HS-SPME-GCMS) was used to quantify selected aroma compounds in wine samples following the method from Tomasino et al. (2015). Three different HS-SPME-GC-MS methods were required to achieve the desired separation and sensitivity needed for accurate quantification.

- Fatty acids run: acetic acid, butanoic acid, 3-methylbutanoic acid, 2-methylbutanoic acid, hexanoic acid and octanoic acid (Table 2.4);
- Traces run: linalool, β-damascenone, geraniol, ethyl hydrocinnamate, β-ionone and ethyl cinnamate (Table 2.5);
- Aldehyde, esters and alcohols: benzaldhyde, trans-3-hexenol, cis-3-hexenol, hexanol, 1-heptanol, 3-methylbutanol, 2-phenylethanol, ethyl acetate, ethyl butanoate, ethyl hexanoate, ethyl octanoate, ethyl decanoate, ethyl-3-methylbutanoate, ethyl isobutyrate, ethyl pentanoate, ethyl lactate, ethyl heptanoate, hexyl acetate, and isoamyl acetate (Table 2.6).

Table 2.4 Quantification parameters for the 6 volatile organic acid analytes.

Analyte	ISTD[a]	RT (mins)	Target Ion m/z	Confirming Ions m/z (% to Target Ion)	Calibration Range µg/L (1/10 dilution)	R^2 [e]	Purity of Standards (%)	CAS No	Supplier
d_4-Acetic acid	(1)	12.13	46	63 (72)	-	-	99.5 A%	1186-52-3	Sigma-Aldrich
d_7-Butyric acid	(2)	14.46	63	46 (27), 58 (7)	-	-	99.5 A%	73607-83-7	CDN isotopes
d_{11}-Hexanoic acid	(3)	17.03	63	77 (43), 93 (12)	-	-	98 A%	95348-44-0	Sigma-Aldrich
d_2-Octanoic acid	(4)	19.55	62	74 (33) 102 (12)	-	-	98 A%	64118-36-1	CDN isotopes
Acetic acid	1	12.19	43	60 (82), 45 (84)	0 – 136,773[b]	0.9999	99.7	64-19-7	Sigma-Aldrich
Butanoic acid	2	14.58	60	43 (22), 55 (9)	0 – 520[c]	0.9998	99	107-92-6	Sigma-Aldrich
Isovaleric acid	2	15.13	60	87 (18)	0 – 200[b]	0.9999	99	503-74-2	Sigma-Aldrich
2-Methyl-butanoic acid	2	15.15	74	57 (66)	0 – 99[b]	0.9998	98	116-53-0	Sigma-Aldrich
Hexanoic acid	3	17.19	60	73 (41), 87 (12)	0 – 532[d]	0.9997	99.5	142-62-1	Sigma-Aldrich
Octanoic acid	4	19.54	60	73 (56), 101 (20)	0 – 499[d]	0.9999	99	124-07-2	Sigma-Aldrich

[a] Internal Standards used are in brackets. [b] Six standards were used to create the calibration range, however, fewer standards were used where appropriate; [c] Five standards; [d] Four standards. [e] All fitted standard (calibration) curves were Quadratic functions

Table 2.5 Quantification parameters for the 6 compounds in the Trace profile.

Analyte	ISTD[a]	RT (mins)	Target Ion m/z	Confirming Ions m/z (% to Target Ion)	Calibration Range (1/10 dilution)	R^2 [e]	Purity of Standards (%)	CAS No	Supplier
d_3-Linalool	(1)	39.96	74	124 (19)	-	-	99 A%	1216673-02-7	CDN isotopes
d_3-β-Ionone	(2)	69.41	180	46 (88), 138 (11), 181 (13)	-	-	100 A%	-	Lincoln
d_5-Ethyl trans-cinnamate	(3)	77.15	136	108 (64), 181 (25)	-	-	99.4 A%	856765-68-9	CDN isotopes
Linalool	1	40.09	121	136 (30)	0 – 11.7[b]	0.9998	97	78-70-6	Sigma-Aldrich
β-Damascenone	2	60.21	190	91 (61), 105 (43)	0 – 0.98[c]	0.9998	1.3% wt[f]	107-92-6	Sigma-Aldrich
Geraniol	1	61.45	123	93 (157)	0 – 0.98[c]	0.9988	98	106-24-1	Sigma-Aldrich
Ethyl hydrocinnamate	3	64.71	104	107 (40), 178 (19)	0 – 0.65[b]	0.9998	99	2021-28-5	Sigma-Aldrich
β-Ionone	2	69.41	177	135 (16), 178 (9)	0 – 0.65[b]	0.9999	96	14901-07-6	Sigma-Aldrich
Ethyl cinnamate	3	77.19	131	103 (60), 77 (41), 176 (21)	0 – 0.98[d]	0.9996	99	103-36-6	Sigma-Aldrich

[a] Internal Standards used are in brackets. [b] Seven standards were used to create the calibration range however less standards were used where appropriate; [c] Six standards; [d] five standards. [e] All fitted standard (calibration) curves were Quadratic functions. [f] A dilute solution in 190 proof ethanol.

Table 2.6 Quantification parameters for the 19 compounds in the Alcohol and Esters profile.

Analyte	ISTD[a]	RT (mins)	Target Ion m/z	Confirming Ions m/z (% to Target Ion)	Calibration Range µg/L (1/10 dilution)[b]	R^2 [c]	Purity of Standards (%)	CAS No	Supplier
Methyl acetate	(1)	5.31	43	74 (19), 42 (10)	-	-	99.8%	79-20-9	Sigma-Aldrich
d_5-Ethyl butanoate	(2)	11.65	93	34 (95), 106 (15)	-	-	100 A%	-	Lincoln
d_3-Isoamyl acetate	(3)	14.81	46	90 (13), 76 (8)	-	-	100 A%	1219804-75-7	Lincoln
d_5-Ethyl hexanoate	(4)	19.28	93	74 (35), 34 (30)	-	-	100 A%	-	Lincoln
d_3-Hexyl acetate	(5)	20.53	46	64 (29)	-	-	99 A%	1219805-39-6	Lincoln
d_{13}-Hexan-1-ol	(6)	22.07	64	50 (42), 78 (31)	-	-	98 A%	204244-84-8	Sigma-Aldrich
d_5-Ethyl octanoate	(7)	26.66	106	74 (107), 134 (31)	-	-	100 A%	-	Lincoln
d_6-Benzaldehyde	(8)	28.25	82	112 (98), 110 (91)	-	-	98 A%	17901-93-8	Sigma-Aldrich
d_5-Ethyl decanoate	(9)	33.34	93	106 (37), 120 (7)	-	-	97 A%	-	Lincoln
d_5-1-Phenyl ethanol	(10)	36.49	112	84 (89), 127 (28)	-	-	98 A%	90162-45-1	Isotech
Ethyl acetate	1	6.73	61	70 (95), 73 (33), 88 (33)	0 – 17,912	0.9991	99.5%	141-78-6	Fisher
Ethyl Isobutyrate	2	9.54	71	88 (36), 116 (28)	0 – 89.3	0.9999	99%	97-62-1	Sigma-Aldrich
Ethyl butanoate	2	11.77	88	101 (16), 60 (34)	0 – 64.8	0.9999	99%	105-54-4	Sigma-Aldrich
Ethyl isovalerate	2	13.12	88	57 (76), 85 (71)	0 – 9.91	0.9999	98%	108-64-5	Sigma-Aldrich
Isoamyl acetate	3	14.94	43	87 (23), 73 (18)	0 – 132	0.9996	99%	123-92-2	Sigma-Aldrich
Ethyl pentanoate	4	15.48	88	85 (90), 101 (26)	0 – 1.23	0.9997	99%	539-82-2	Sigma-Aldrich
Isoamyl alcohol	6	17.46	42	70 (89), 41 (82)	0 – 58,763	0.9999	99%	123-51-3	Sigma-Aldrich
Ethyl hexanoate	4	19.45	88	60 (33), 101 (25)	0 – 170	0.9997	99%	123-66-0	Sigma-Aldrich
Hexyl acetate	5	20.64	43	61 (25), 84 (19)	0 – 4.78	0.9999	99%	142-92-7	Sigma-Aldrich
Ethyl lactate	6	21.98	45	75 (7), 47 (2)	0 – 18,188	0.9999	98%	687-47-8	Sigma-Aldrich
Hexan-1-ol	6	22.56	56	55 (48), 84 (5), 41 (36)	0 – 905	0.9999	99%	111-27-3	Sigma-Aldrich
trans-3-Hexen-1-ol	6	22.81	67	82 (64), 100 (5)	0 – 28.5	0.9999	98%	928-97-2	Sigma-Aldrich
Ethyl heptanoate	4	23.14	88	60 (33), 113 (33)	0 – 1.75	0.9999	99%	106-30-9	Sigma-Aldrich
cis-3-Hexen-1-ol	6	23.49	41	67 (90), 82 (43)	0 – 57.1	0.9998	98%	928-96-1	Sigma-Aldrich
1-Heptanol	6	26.08	70	56 (88), 41 (78)	0 – 19	0.9999	99%	111-70-6	Sigma-Aldrich
Ethyl octanoate	7	26.95	101	70 (79), 129 (29)	0 – 250	0.9996	99%	106-32-1	Sigma-Aldrich
Benzaldehyde	8	28.14	77	106 (97), 51(44)	0 – 191	0.9999	99%	100-52-7	Sigma-Aldrich
Ethyl decanoate	9	33.63	88	101 (37), 115 (8)	0 – 277	0.9985	99%	110-38-3	Sigma-Aldrich
2-Phenyl ethanol	10	39.29	91	92 (62), 122 (31)	0 – 19,211	0.9992	99%	60-12-8	Sigma-Aldrich

[a] Internal Standards used are in brackets. [b] Six standards were used to create the calibration range. [c] All fitted standard (calibration) curves were Quadratic functions

2.6 statistical analysis

All the statistical analyses, including but may not limited to Duncan test, Tukey test, two-way ANOVA, PCA and CVA, were done with Genstat 18th edition. When analyzing the data according to treatment ratios (TMR), where the data was unbalanced in each categories, a Duncan means separation test was used instead of Tukey Test to give more liberal and avoid overprotecting a probably false null hypothesis against type I errors (incorrect rejection of a true null hypothesis). The interactions between leaf area and crop load treatments were considered when conducting two-way ANOVA. The methods, especially the parameter selection methods, of CVA and PCA are detailed in Chapter 3.

Chapter 3 Vine characteristics and fruit composition

3.1 Introduction

Pruning is a common practice in vineyards, mainly aiming to facilitate vineyard management, produce grapes of a desired quality, produce fruitful shoots, regulate the vegetative growth of the vine, and thus, regulate vine balance. Pruning weight can be regarded as representative of vegetative yield, which gives some indications of vine size and vine fruiting capacity (Howell 2001).

Determination of soluble sugar and starch content is important in many aspects of grapevine biology. Such knowledge is particularly relevant to studies of grapevine source to sink relations, and in understanding areas such as the utilisation of carbohydrate reserves.

Hundreds, possibly thousands, of compounds are found in grape berries. The major compounds in berries that could affect wine quality include sugars, acids, minerals, phenolics, some volatile compounds and the overall matrix condition, e.g. pH and the proportion/balance between each compound. To release these compounds during the winemaking process also relies on the physical characteristic of the berry.

The aim of this chapter is to investigate how vines, and fruit carried by the vines, would respond to leaf and crop removal treatments. In other words, the main idea is to discover whether vine and fruit characteristics can be affected by the treatments, and to discover which factor(s), namely source to sink ratio and/or vine canopy microclimate, is the fundamental reason leading to the variables in vine and fruit characteristics. Thus, the first part of this chapter mainly concerns vine characteristics with an emphasis on LA/CL, pruning weight and carbohydrate status in roots, followed by berry physical characteristics, and finally, the concentration/content of important compounds in berries/juice before vinification.

3.2 Results and discussion

3.2.1 Vine characteristics

Based on the calculations presented in Figure 3.1, a high correlation (R^2=0.86) between LA/CL (the actual ratio calculated from leaf area divided by fruit weight, in cm^2/g, refer to Section 2.4.1) and TMR (a virtual number with no unit, refer to Section 2.2.3) was found. Generally, for the same TMR, the LA/CL obtained from different treatments were slightly different (Figure 3.1). For example, there is a trend that for TMR “2”, 0.5L/0.25C treated vines had higher LA/CL than 1L/0.5C treated vines; on

the contrary, for TMR “0.5”, there was a tendency that 0.25L/0.5C treated vines had lower LA/CL than 0.5L/1C treated vines.

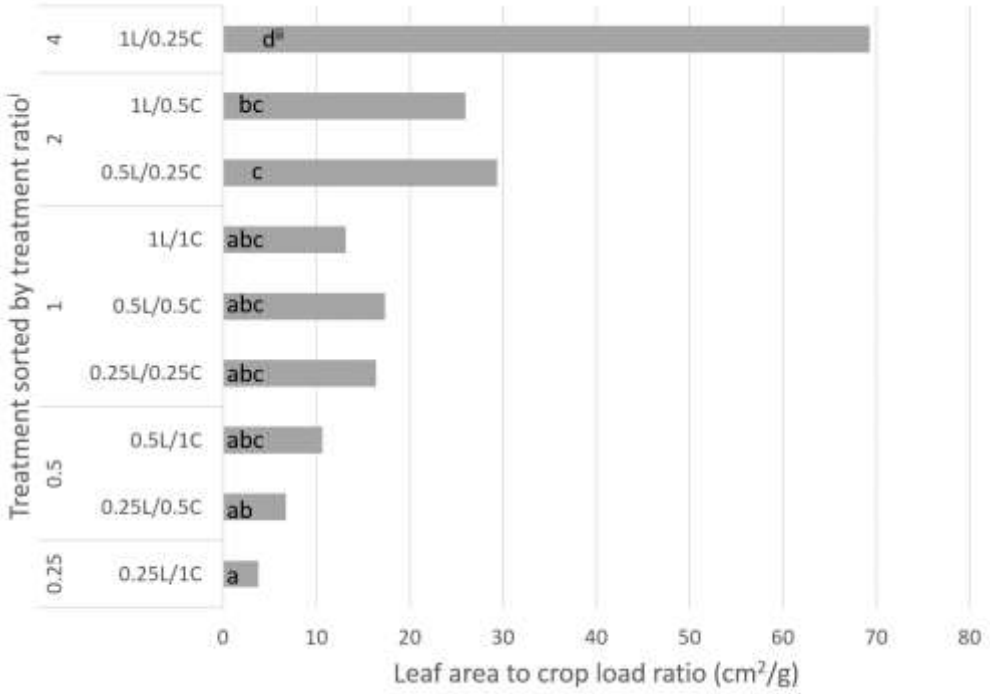

Figure 3.1 Leaf area to crop load ratios (LA/CL) (obtained from the raw data of Pasch 2014)

[i] TMR = level of leaf area (e.g. 0.25, 0.5, 1) / level of crop load (e.g. 0.25, 0.5, 1)

[ii] Bars with different letters were significantly different by Tukey test ($p < 0.05$)

It is suspected that when subjected to TMR of “0.5”, 0.25L is too severe compared to 0.5L, and leaves of 0.25L treated vines have to compensate their growth to support berry development. This was further confirmed with the fact that individual leaves were substantially larger for the 0.5L treatment compared to the 1L treatment, but individual leaf size was smallest for leaves from 0.25L treatment (Pasch 2014). Though all the treatments were applied soon after fruit-set and leaves at that time were almost fully expanded, the sudden defoliation may have triggered leaves that had not yet finished development (for example, from the 0.5L treatment), causing them to further expand. However, in the 0.25L treatment (2 basal leaves and 1 apical leaf) there were only three leaves per shoot, and even though the apical leaf was not fully expanded at this stage, there may not have enough carbohydrate available to support expansion. It is interesting that Pasch (2014) noted that the single leaf photosynthetic rate was lower for 0.25L/0.5C than 0.5L/1C, while the latter had the highest photosynthetic rate among nine treatments. Thus, grapevines have the ability to compensate for a relatively low source to sink ratio, i.e. restricted leaf area, through higher photosynthetic rates, as revealed before (Kliewer & Antcliff 1970; Hunter & Visser 1988; Petrie et al.

2000), however, grapevines with extremely low leaf area (0.25L) could not compensate for this as their photosynthetic rate remained relatively low.

There was no significant difference in pruning weight between different treatments. TMR did affect pruning weights (Figure 3.2), however, no linear relationship can be found between pruning weight and TMR (R^2=0.12). In terms of the two extreme TMRs, "0.25" did result in lightest pruning weights, indicating that vines with extremely high crop loads coupled relative to leaf area strive for limited assimilates and as a result, the vine compensated through a reduction in vegetative growth. On the contrary, another extreme TMR "4" did not result in higher pruning weight, suggesting that either the dry matter produced was not located into shoots, or the total dry matter production did not increase in parallel with TMR, possibly due to end-product inhibition of photosynthesis (Farrar 1992; Petrie et al. 2000).

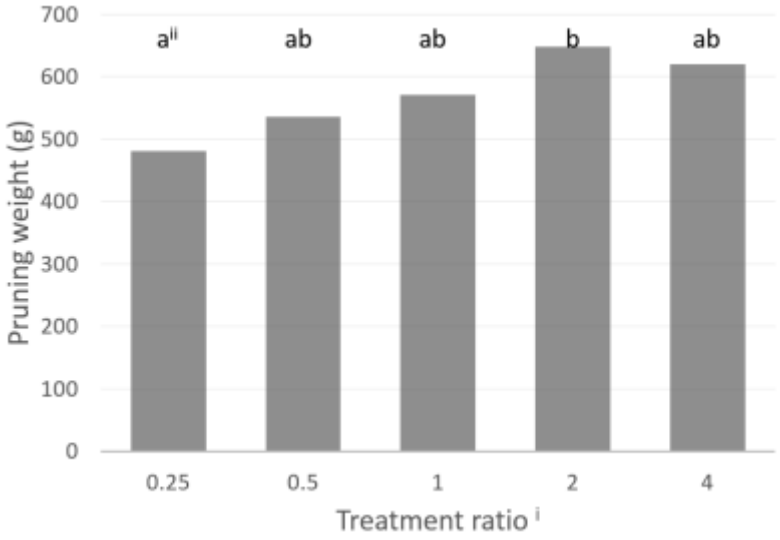

Figure 3.2 Pruning weight

[i] TMR = level of leaf area (e.g. 0.25, 0.5, 1) / level of crop load (e.g. 0.25, 0.5, 1)

[ii] Columns with different letter were significantly different by Duncan test ($p < 0.05$)

LA/CL is not the only indicator of vine balance; the Ravaz index (yield/pruning weight) is also used and this measure emphasises that the ratio of fruit to wood is the key to achieving both fruit quality and consistent production (Howell 2001). Previous studies revealed that these two indices had a negatively linear relationship. When LA/CL falls in the range of 7 to 14 cm^2/g, the Ravaz index normally would be in the range of 5 to 10, which is the indication of vines growing in balance (Kliewer & Dokoozlian 2005).

However, in this study, the linear correlation between LA/CL and Ravaz index was weak (R^2=0.30, Figure 3.3), possibly because under the extreme treatments, the vines were quite unbalanced. The range of LA/CL in this study was from 3.11 to 85.07 cm^2/g. However, the range that Kliewer & Dokoozlian (2005) collected in their research was from 5 to 16.5 cm^2/g. Thus, linear correlation between CA/CL and Ravaz index might only exist within a limited range, but this is not the case for this study (data not shown). In this study, when LA/CL fell in the range of 10 to 20 cm^2/g, the yield/pruning weight ratio was always below 4. The reason could be that in a cool climate region, with limited light intensity, limited heat summation during the growing season, shorter postharvest period before dormancy and other limiting factors, more leaf area is needed to mature fruit as well as having a desirable vegetative growth (Kliewer & Dokoozlian 2005; Howell 2001).

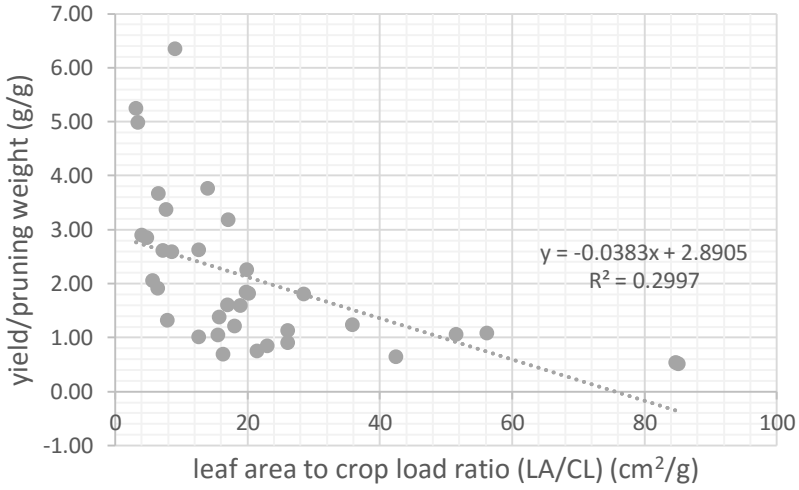

Figure 3.3 Relationship between LA/CL and Ravaz index

Soluble carbohydrates in roots remained unaffected by treatment (Table 3.1). However, starch dry weight and total non-structural CHO (due to the changes in starch) were affected by leaf removal treatments. Generally, 0.25L led to less starch and less total non-structural CHO in roots. Furthermore, there was a trend (except for 1L/0.25C) that higher TMR would lead to higher starch content in roots, in agreement with Zufferey et al. (2012), who reported that both starch content and total non-structural carbohydrate at harvest were strongly affected by the ratio of sun exposed leaf area to fruit weight. The exception to this was 1L/0.25C which did not retain the highest starch content in root, and which may result from the same reason as why it did not achieve the highest pruning weight (Pasch, 2014), which is end-product inhibition of photosynthesis. Hunter et al. (1995) reported that by 33% leaf removal either at “pea size” or veraison, the remaining leaves of partially defoliated vines were able to sustain normal metabolic functions in the roots. However, in this study, 75% defoliation (0.25L) was too severe for Pinot noir in a cool climate. Similar results were gained

using mature Chardonnay grapevines growing in the Lincoln University vineyard by removal all but the four basal leaves (Bennett et al. 2005). These authors suggested that it is also possible that the reduction in starch concentrations in roots at harvest by defoliation 4 weeks post-bloom could result from the remobilization of CHO reserves to ripening fruits during the growing season.

Table 3.1 Carbohydrate status in grapevine roots

Treatment	Soluble solids (% dry weight mg/mg)	Starch dry weight (% dry weight mg/mg)	Total non-structural CHO (% dry weight mg/mg)
Leaf area 0.25L			
0.25C	4.29	5.66 ab[i]	9.95
0.5C	4.26	5.48 ab	9.74
1C	4.59	5.11 a	9.70
Leaf area 0.5L			
0.25C	4.35	7.13 b	11.48
0.5C	4.14	7.08 ab	11.23
1C	4.16	6.74 ab	10.91
Leaf area 1L			
0.25C	4.19	6.87 ab	11.05
0.5C	4.29	7.21 b	11.51
1C	4.43	7.43 b	11.86
Leaf removal effect[ii]	n.s.	$p < 0.001$	$p < 0.01$
Crop removal effect	n.s.	n.s.	n.s.

[i] Means followed by a different letter were significantly different by Tukey test ($p < 0.05$)

[ii] Treatment effects were listed according to two-way ANOVA test, no interactions found.

3.2.2 Berry physical characteristics

There was no significant difference in terms of seed number, seed fresh weight, and water content in seeds by the leaf and crop removal treatments (Table 3.2). Seed dry weight responded to leaf removal treatment, with 0.5L resulting in the highest seed dry weight. Skin fresh weight increased with leaf number retained, which could result from bigger berries. In fact, berry size seemed to be significantly affected by extreme leaf removal (0.25L) (Pasch 2014). Pasch (2014) showed that berry weights were 1.39 g, 1.46 g, and 1.49 g for 0.25L, 0.5L and 1L treatments, respectively; in terms of berry diameter, the figures were 1.24 cm, 1.28 cm and 1.28 cm, respectively. However, there was no correlation between skin fresh weight per berry and berry weight (R^2=0.0006). Mathematically, berry weight and skin fresh weight may have some correlation providing that the berries of different treatments are of similar density and that the thicknesses of the berry skin are also similar. Since skin fresh weight was not correlated with berry weight, the leaf removal treatments may be having some influence on skin fresh weight.

Table 3.2 Physical characters of berry parts

Treatment	seed number (15 berries)	seed fresh weight per seed (mg)	seed dry weight per seed (mg)	water content in seeds	skin fresh weight per berry (mg)
Leaf area 0.25L					
0.25C	26.0	34.35	18.65	0.46	372.3
0.5C	28.3	33.38	17.74	0.47	380.2
1C	29.5	33.95	18.08	0.47	382.0
Leaf area 0.5L					
0.25C	24.8	36.22	19.61	0.46	410.1
0.5C	25.8	35.82	19.42	0.46	396.5
1C	25.0	37.58	19.92	0.47	393.7
Leaf area 1L					
0.25C	28.5	34.41	18.57	0.46	434.6
0.5C	25.5	34.64	19.07	0.45	410.2
1C	28.3	34.53	18.50	0.46	404.3
Leaf removal effect[i]	n.s.	n.s.	$p < 0.05$	n.s.	$p < 0.05$
Crop removal effect	n.s.	n.s.	n.s.	n.s.	n.s.

[i] Treatment effects were listed according to two-way ANOVA test, no interactions found.

3.2.3 Fruit/juice composition and skin colour properties

°Brix, titratable acidity (TA) and pH were measured using frozen berries sampled at harvest. TA remained unaffected by treatments, but both juice Brix and juice pH were significantly affected, especially by leaf removal treatments (Table 3.3).

King et al. (2012) reported that leaf removal treatments had no significant effect on °Brix, TA and pH at harvest, but crop removal enhanced ripening by increasing °Brix and decreasing TA, and sometimes increasing pH. However, that phenomenon was not pronounced in this study, possibly because of the differences in climate, treatment and cultivar. Working in Hawkes Bay, King et al. (2012) treated Merlot vines pre-veraison with three levels of leaf removal in the fruiting zone and three levels of crop removal targeting apical clusters, the latter of which is a departure from how crop loads were adjusted in the present study.

Table 3.3 Juice °Brix, juice titratable acidity (TA) and juice pH (from frozen berries)

Treatment	Juice °Brix	Juice TA	Juice pH
Leaf area 0.25L			
0.25C	18.60 ab[i]	5.90	3.64 c
0.5C	18.45 ab	6.32	3.62 c
1C	18.20 a	6.28	3.59 bc
Leaf area 0.5L			
0.25C	19.88 b	6.39	3.57 bc
0.5C	19.55 ab	6.42	3.48 ab
1C	18.75 ab	7.01	3.47 ab
Leaf area 1L			
0.25C	19.43 ab	6.87	3.52 abc
0.5C	19.88 b	6.37	3.52 abc
1C	19.28 ab	7.08	3.42 a
leaf removal effect[ii]	$p < 0.001$	n.s.	$p < 0.001$
crop removal effect	n.s.	n.s.	$p < 0.01$

[i] Means followed by a different letter were significantly different by Tukey test ($p < 0.05$)

[ii] Treatment effects were listed according to two-way ANOVA test, no interactions found.

It was also reported that leaf elimination led to a higher titratable acidity in fruit at early stages of ripening and the differences in acidity diminished along with fruit maturation (Kliewer & Antcliff 1970), in agreement with Ollat & Gaudillere (1998). In fact, during berry maturation, °Brix normally increases, TA drops and pH increases (Iland et al. 2011). In this study, it is interesting to note that higher °Brix accompanied with lower pH was achieved for higher leaf levels (Figure 3.4), indicating that maturity, which is a common factor caused by moderate higher leaf levels (sufficient source) and result in higher °Brix and higher pH, was not the reason leading to this phenomenon. On the contrary, sunlight exposure could be the reason. Pasch (2014) reported that there was a positive linear correlation between leaf number removed and PAR inside canopy. More leaves could lead to more photosynthesis and larger sugar flux into the fruit, but shading could also lead to less degradation of acids and accumulation of potassium. However, later analysis showed that there was no significant difference between leaf removal treatments in terms of the concentration of malic acid, tartaric acid and potassium. Based on this, sun exposure may not be an influencing factor for the higher °Brix and lower pH values found at higher leaf levels, which links in with Pasch (2014) finding that there were no shading effects between the treatments.

°Brix and pH results from Pasch (2014) also presented the same pattern (although with lower pH) as in second time measurement. Based on a comparison of juice TA results from Pasch (2014) and juice TA from frozen materials, the second time measurement had higher values possibly due to some

plant tissue break down that released more acids. However, juice TA generally decreases after freezing and thawing (reviewed by Garcia et al. 2011). Thus, the heating procedure could be the reason for more acids being released (Threlfall et al. 2006). Garcia et al. (2011) reported that there were high correlations between fresh materials and frozen materials on values of pH and TA, and further suggested that the main reason for an increasing pH after freezing storage was acid salification, which is due to the formation of potassium hydrogen tartrate, a process enhanced during the process of freezing and thawing.

It is known that TA and pH are not linearly correlated (Iland et al. 2011). TA in this study was not affected either by leaf removal or by crop removal. Thus, differences in pH between leaf levels may not result from difference in acid concentration. Walker & Blackmore (2012) reported that the H^+ ions concentration in grape juice and wine decreased as K^+ concentration increased, resulting in increased pH, but the relationship was dependent on variety.

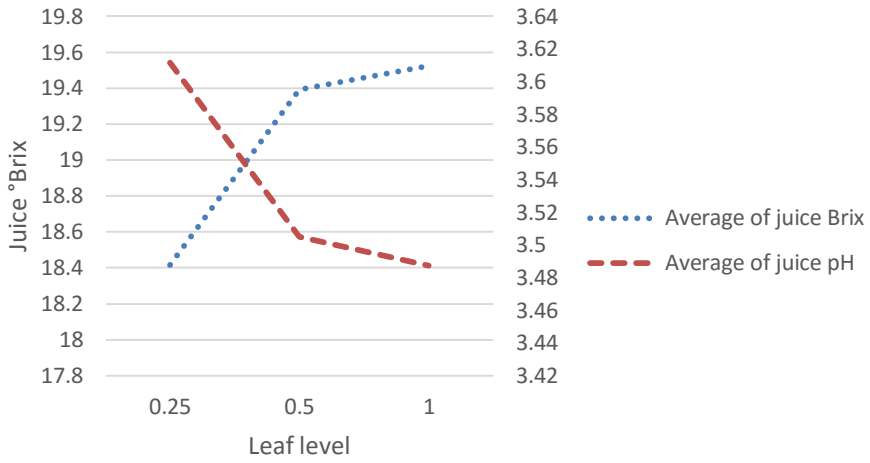

Figure 3.4 Juice °Brix and pH by leaf levels

For °Brix variation within a group of 15 individual berries, no statistical difference could be found in terms of standard deviation (Table 3.4). However, under the treatment of "0.25L", extremely low °Brix can be found, in contrast to extremely high °Brix under the treatment of "0.5L" and "1L". Thus, vines with extremely limited leaf area may struggle to mature fruit especially when crop load was inappropriately high. Crop removal treatments alone did not make a big contribution to the extremes (data not shown).

Table 3.4 °Brix and standard deviation of 15 individual berries from the same treatment

Treatment	Mean	Standard deviation[i]
0.25L		
0.25C	18.65 ab[ii]	1.192
0.5C	18.52 ab	0.999
1C	18.12 a	1.593
0.5L		
0.25C	19.86 d	1.365
0.5C	19.70 cd	1.627
1C	18.96 bc	1.655
1L		
0.25C	19.49 cd	1.423
0.5C	20.02 d	1.700
1C	19.27 bcd	1.384
Leaf removal effect[iii]	$p < 0.001$	n.s.
Crop removal effect	$p < 0.001$	n.s.

[i] Standard deviation was calculated from each treatment of each replicate

[ii] Means followed by a different letter were significantly different by Tukey test ($p < 0.05$)

[iii] Treatment effects were listed according to two-way ANOVA test, no interactions found.

Three organic acids were detected in juice (Table 3.5). No significant difference can be seen between the nine treatments. Only citric acid was shown to be affected by crop removal treatments. However, both the highest value and the lowest value of citric acid concentration were achieved under full leaf area (1L). Thus, the pattern of citric acid concentration suggested that the effect of crop levels on citric acid concentration could be more pronounced under full leaf area, although statistically no interaction was found.

Malic acid is generally considered a possible energy source for berry tissues after veraison (Iland et al. 2011; Ollat & Gaudillere 1998). The principle reactions causing the loss of malic acid during fruit maturation are likely to be of a respiratory nature, which is temperature related. Kliewer & Bledsoe (1987) reported that TA, malic acid, and pH in fruit were all significantly reduced by leaf removal. In this study, two of the four basal leaves were removed for all the vines, which gave vines in all treatments a somewhat similarly exposed fruiting-zone microclimate: the intent was to keep solar radiation conditions, even under different treatments, similar. However, Pasch (2014) reported that severe leaf removal did result in higher PAR around fruiting zone, but even with full leaf retained in this study, visible gaps were evident in the fruiting zone.

Table 3.5 Organic acid content of juice from 15 crushed berries per plot.

Treatment	malic acid (μg/ml)	tartaric acid (μg/ml)	citric acid (μg/ml)
Leaf area 0.25L			
0.25C	4017	4553	350.8
0.5C	4353	4730	356.5
1C	4225	4967	340.5
Leaf area 0.5L			
0.25C	4138	4759	357.4
0.5C	4132	4636	347.7
1C	4469	4832	340.0
Leaf area 1L			
0.25C	4585	4855	365.1
0.5C	4096	4758	363.2
1C	4253	5251	331.9
Leaf removal effect[i]	n.s.	n.s.	n.s.
Crop removal effect	n.s.	n.s.	$p < 0.05$

[i] Treatment effects were listed according to two-way ANOVA test, no interactions found.

Potassium concentration remained unaffected by various treatments (Table 3.6). Kliewer & Bledsoe (1987) found that potassium concentration was significantly affected by leaf removal treatments, and there was a significant direct correlation between potassium and malic acid concentration in fruit. However, in this study, neither titratable acidity nor malic acid was significantly affected by leaf removal treatments. Thus, that potassium content remained unaffected seemed reasonable.

On the other hand, phosphorus (P) concentration was significantly affected mainly by leaf removal treatments, while higher crop loads generally led to lower P concentration. Most of the previous studies on P were concentrated on nutrition uptake and deficiency (WenJuan et al. 2009; Kuranaka et al. 1975), and reports on P and its influence on source to sink ratio or vine microclimate were rare. A study on apples showed that P metabolism was a limiting factor in spontaneous ester production within the fruit (Brown 1968). Moreover, P widely exists as an component of amino acids, which could be further synthesised into volatile fatty acids and esters during fermentation (Clarke & Bakker 2004; Reynolds 2010). Thus, the concentration of P may have some indication of the potential production ability for volatile fatty acids and/or esters; this will be further discussed in Chapter 4.

Table 3.6 Mineral content of juice from 15 crushed berries from 36 samples.

Treatment	K (µg/ml)	P (µg/ml)
Leaf area 0.25L		
0.25C	1712	240.8 b[i]
0.5C	1735	234.5 b
1C	1721	220.7 ab
Leaf area 0.5L		
0.25C	1605	225.1 ab
0.5C	1479	217.4 ab
1C	1493	203.8 ab
Leaf area 1L		
0.25C	1620	208.1 ab
0.5C	1547	214.7 ab
1C	1551	190.6 a
Leaf removal effect[ii]	n.s.	$p < 0.01$
Crop removal effect	n.s.	$p < 0.05$

[i] Means followed by a different letter were significantly different by Tukey test ($p < 0.05$)

[ii] Treatment effects were listed according to two-way ANOVA test, no interactions found.

There was no interactions between the two types of treatments, leaf removal treatments and crop removal treatments that were identified in seed and skin phenolic profile or colour parameters. For seeds, total seed phenolics on a per berry basis and total tannin in seed powder (w/w) remained unaffected by all the treatments, in agreement with Ristic et al. (2010). Cortell et al. (2005) reported that total tannin per seed was not significantly affected by vine vigour. They also suggested that the total amount of tannin in seed was independent of vine vigour and/or environmental conditions. In this study, no significant difference in tannin between leaf treatments was observed when considering "seed tannin per berry".

Skin total phenolics and total tannin on a per berry basis (for calculation formula see note in Figure 3.5) were reduced by leaf removal treatments ($p < 0.05$ and $p < 0.01$, respectively. Figure 3.5). A high correlation was found between tannin concentration and phenolic concentration in skin extracts on a per berry basis (R^2=0.95). Previous studies showed that total tannin in skin decreased with increased vine vigour (Cortell et al. 2005), although Downey et al. (2006) pointed out that it was uncertain whether that experimental fact resulted from the difference in vine vigour or from an indirect effect of changes in canopy architecture resulting in differential fruit exposure. Ristic et al. (2010) reported that tannin concentration in skin was significantly reduced in fruit from shaded vines (where the canopy was wrapped in bird nets) compared to no canopy manipulation and well-trained-highly-exposed treated vines. In this study, Pasch (2014) reported that leaf removal

significantly increased fruit exposure to sunlight by measuring PAR, but even non-defoliated vines had a fairly high PAR interception (e.g. the ratio of inside canopy PAR to above canopy PAR was between 0.18 to 0.30 for 1L treated vines compared to 0.54 to 0.72 for 0.25L treated vines). However, both tannin and phenolic concentration on per berry basis increased when more leaf was obtained. Thus, source to sink ratios could be the reason leading to this change in phenolics and this will be discussed further below (see Figure 3.6 and 3.8).

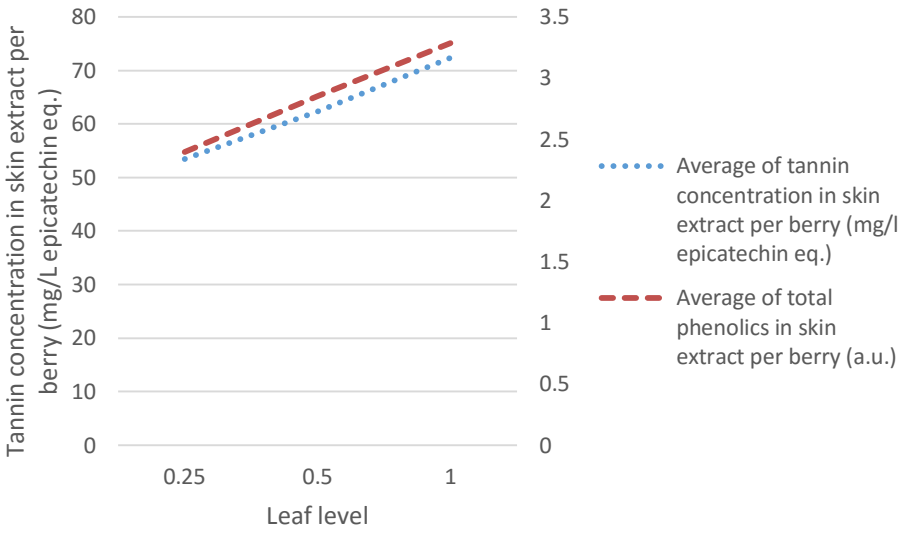

Figure 3.5 Skin total phenolics and total tannin sorted by leaf levels. Note: tannin/phenolic in skin extract per berry = (tannin or phenolic concentration in skin extract solution/grams of skin mass used for extraction) × single berry skin fresh mass

It appeared that for skin extracts, all colour properties but the estimate of SO_2 resistant pigment per berry were affected by leaf levels. Generally, higher leaf levels led to higher colour density, lower colour hue, lower degree of red pigment colouration and higher total red pigment per berry (Table 3.7).

Since wines are solutions, changing those parameter listed in Table 3.6 and Table 3.7 into x per litre could be more meaningful in terms of relating the results to what could happen during and after vinification. Here we regard all the berries as the same density, and divide the original data by grams of berry (Figure 3.6). It is clear that for skin extract samples, total tannin, total phenolics, total red pigments, colour density and colour hue per gram of berry weight all followed the same pattern as revealed in Table 3.7 and Figure 3.5.

Table 3.7 Colour properties of skin extracts on a per berry basis[i]

Treatment	Colour density per berry (a.u.)	Colour hue per berry (a.u.)	Degree of red pigment colouration	Estimate of SO2 resistant pigment per berry (a.u.)	Total red pigments per berry (a.u.)
Leaf area 0.25L					
0.25L/0.25C	0.402	1.182 abc[ii]	0.116 cd	0.051	1.469
0.25L/0.5C	0.416	1.210 bc	0.118 d	0.050	1.508
0.25L/1C	0.367	1.231 c	0.111 bcd	0.050	1.441
Leaf area 0.5L					
0.5L/0.25C	0.525	1.126 abc	0.107 abcd	0.062	2.255
0.5L/0.5C	0.487	1.116 abc	0.101 abc	0.051	2.175
0.5L/1C	0.398	1.162 abc	0.113 cd	0.046	1.557
Leaf area 1L					
1L/0.25C	0.569	1.079 ab	0.096 a	0.061	2.724
1L/0.5C	0.564	1.087 ab	0.102 abc	0.064	2.572
1L/1C	0.475	1.067 a	0.098 ab	0.052	2.248
Leaf removal effect[iii]	$p < 0.05$	$p < 0.001$	$p < 0.001$	n.s.	$p < 0.001$
Crop removal effect	n.s.	n.s.	n.s.	n.s.	n.s.
Leaf removal ×crop removal	n.s.	n.s.	$p < 0.01$	n.s.	n.s.

[i] parameters on per berry basis = (the original results of that parameter/the actual skin mass used for extraction) × single berry skin fresh mass

[ii] Means followed by a different letter were significantly different by Tukey test ($p < 0.05$)

[iii] Treatment effects were listed according to two-way ANOVA test.

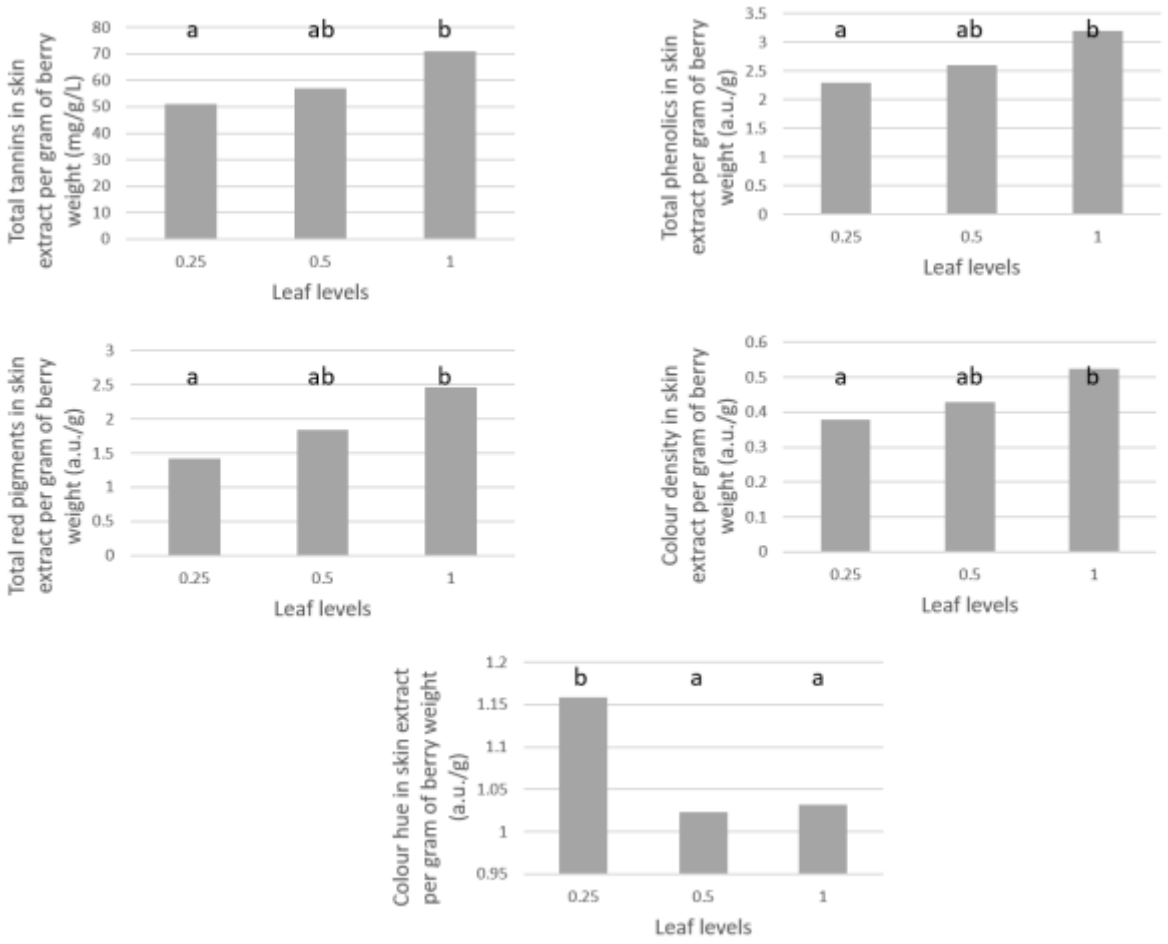

Figure 3.6 Phenolic/colour properties of skin extracts (on a per gram of berry weight basis) by leaf removal treatments. Columns with different letters were significantly different by Tukey test ($p < 0.05$)

Thought a certain amount of variation can be seen, the results were interesting because most of the previous studies suggested that flavonoid synthesis was favoured with greater sunlight exposure and moderately higher temperature in fruiting zone (Ristic et al. 2013; Ristic et al. 2010; Downey et al. 2006; Price 1994; Cortell & Kennedy 2006). However, greater sun exposure (measured by PAR) caused by leaf removal failed to increase total phenolics, total tannins and most of the colour parameters for skin extracts. In fact, even the canopy of non-defoliated vines can be considered open and there was no significant difference in shoot number per metre of row between treatments (Pasch 2014). Thus, any shading effect is not pronounced in this study. It is then reasonable to consider that changes to whole vine photosynthates produced from leaves, which is the origin for materials used for phenolic (flavonoids) synthesis, could be the reason for this phenomenon.

By using CIELab, a visual view of what these skin extracts looked like is presented in Figure 3.7. It is obvious that even though vines with lower leaf area had higher degree of red pigment colouration,

they failed to show more redness visually (as tested by a*(redness/greenness), data not show), the pattern of which is most readily to be identify in Rep.3 (data not shown). Visually, skin extract from 1L treated vines had the most redness under all crop levels, although crop level could have another effect, with 1C having the lightest colour at all leaf levels.

Figure 3.7 Approximate skin extracts colour of Rep 3 generated from CIElab method. Note: skin extracts colour were a lot lighter than a red wine solution because of approximate 11 times dilution (refer to 2.4.4 for detail).

HPLC was conducted on skin extract samples (SK). The parameters selected were based on a recent study on New Zealand Pinot noir wine by Liu (2014). Gallic acid, hydroxybenzoric acid, catechin, vanillic acid, epicatechin, *p*-coumaric acid, and procyanidin 2 were not detected in any of the 36 skin extract samples. Other compounds, protocatechuic acid, caftaric acid, epigallocatechin, caffeic acid, syringic acid, ferulic acid, epicatechin gallate, quercetin, gallocatechin, procyanidin 1 were only detected in some samples, but not all (Table 3.8). The two procyanidins are both dimers (as confirmed by mass spectrometry), where procyanidin 1 was eluted at 12.98 min and procyanidin 2 was eluted at 21.2 min.

Table 3.8 Phenolic compounds that have been detected in certain skin extract samples

Name of the compounds	Numbers of samples that have been detected	Mean value[i](mg/L)
Protocatechuic acid	4	0.223
Caftaric acid	1	0.262
Epigallocatechin	2	1.912
Caffeic acid	2	0.066
Syringic acid	17	0.129
Ferulic acid	1	0.099
Epicatechin gallate	1	0.005
Quercetin	1	0.482
Gallocatechin	26	1.278
Procyanidin 1	24	0.868

[i] Mean value was calculated from the samples have been detected.

Quercetin and caftaric acid are responsive to cluster exposure (Price 1994; Spayd et al. 2002), but in this study, one figure from one sample is not very informative. The possible reasons why those compounds listed in Table 3.8 were not detected in all the samples are: the skin extract samples was too diluted (approximately 11 times dilution compared to a common wine solution); those compounds were not as soluble into 50% ethanol as in a wine system; or one hour of extraction may not have been long enough.

Mavidin-3-glucoside, rutin, kaempferol-glucoside, quercetin-glucuronide 1 and quercetin-glucuronide 2 were detected in all 36 samples. Both quercetin-glucuronides have the same ionization mass in negative field: 477(-), but have different retention times: 49.2 min and 52.0 min, respectively. The results are listed below (Table 3.9).

Table 3.9 Phenolic compounds that have been detected in all the skin extract samples (unit: mg/L)

Treatment	Malvidin-3-glucoside	Rutin	Kaempferol-glucoside (quercetin equiv.)	Quercetin-glucuronide 1 (quercetin equiv.)	Quercetin-glucuronide 2 (quercetin equiv.)
0.25L					
0.25C	19.32	4.50	1.75	3.63	1.67
0.5C	19.91	4.85	1.78	4.03	1.49
1C	19.05	3.59	1.91	3.61	1.33
0.5L					
0.25C	28.73	5.69	1.59	4.47	1.90
0.5C	27.69	5.22	1.62	4.09	1.83
1C	20.61	4.51	1.70	3.92	1.57
1L					
0.25C	34.58	5.21	1.44	4.06	1.95
0.5C	33.35	5.01	1.36	3.93	1.92
1C	29.10	4.54	1.29	4.13	1.55
Leaf removal effect[i]	$p < 0.001$	n.s.	$p < 0.05$	n.s.	n.s.
Crop removal effect	n.s.	n.s.	n.s.	n.s.	n.s.

[i] Treatment effects were listed according to two-way ANOVA test, with no interactions found.

The concentrations of malvidin-3-glucoside and kaempferol-glucoside were significantly affected by leaf removal treatments, where more leaves led to more mavidin-3-glucoside and less kaempferol-glucoside. There were high correlations between colour density per berry and malvidin-3-glucoside in skin extract samples (R^2=0.93), and between total red pigments per berry and malvidin-3-glucoside in skin extract samples (R^2=0.98).

Kaempferol and other flavonols have been shown to contributes to co-pigmentation in red wines (Boulton 2001). Spayd et al. (2002) reported that the concentration of the kaempferol-glucoside, along with two other glucosides of flavonols, was dramatically higher in sun-exposed berries than in shaded berries at harvest, while total monomeric skin anthocyanin was more in favour of sun-exposure /cooler-temperature. In this study, kaempferol-glucoside seemed to be more responsive to sun exposure (fruiting zone PAR) than malvidin-3-glucoside, so malvidin-3-glucoside could be more responsive to source to sink ratio. Or, it is possible that higher fruit temperatures resulted from less leaf area may inhibit anthocyanin production (Carbonell-Bejerano et al. 2013). However, this is speculative, as berry temperature was not measured in this study.

Diago et al. (2012) reported that basal leaf removal led to accumulation of flavonols and anthocyanins. Mazza et al. (1999) reported that sun exposure in the fruiting zone can lead to higher levels of phenolics and colour density in Pinot noir wine, which corresponded to the higher phenolics and anthocyanin content in grape skins. In this study, some basal leaf removal was conducted on all the vines, PAR value in the fruiting zone was not uniform, with quarter leaf retained (0.25L) resulting in the highest PAR value (Pasch 2014). However, increased sun exposure in fruiting zone did not result in higher phenolic and anthocyanin concentration (Figure 3.5, 3.6 and Table 3.7). Jackson & Lombard (1993) suggested that not all attempts to advance maturity or improve grape composition with leaf removal have been successful, because canopy shading is not a problem in the particular vineyard. Haselgrove et al. (2000) suggested that anthocyanin accumulation may not be improved by a high degree of cluster exposure, but that study was in a hot climate on Shiraz. A recent study showed that higher anthocyanin and phenolic content was generated by low yield and leaf removal on Cabernet Franc in Michigan (Zhuang et al. 2014). However, in a cool climate as this study, where the fruiting zone exposure was relatively good, source to sink ratio rather than light response could be the reason leading to this phenomenon (Figure 3.8).

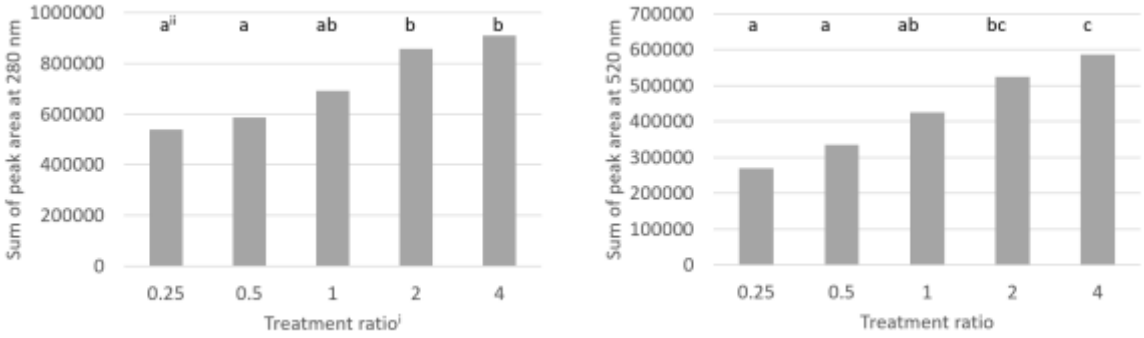

Figure 3.8 Sum of peak area at 280 nm and 520 nm of skin extract sorted by TMR

[i] TMR = level of leaf area (e.g. 0.25, 0.5, 1) / level of crop load (e.g. 0.25, 0.5, 1)

[ii] Columns with different letters were significantly different by Duncan test ($p < 0.05$)

3.3 Canonical variates analysis (CVA) and principal components analysis (PCA)

Canonical variates analysis (CVA) was conducted to test how well selected parameters (see below) could differentiate treatment effects on vines. Preliminary analyses (refer to all the data analyses listed in 3.2) revealed that the contents of starch dry weight in root, seed dry weight per seed, skin fresh weight per berry, juice °Brix, juice pH, citric acid, P, total phenolics in skin extract per berry, tannin concentration in skin extract per berry, colour density, colour hue, degree of red pigment colouration, total red pigments per berry, malvidin-3-glucoside, kaempferol-glucoside, sum of peak

area at 280 nm, and sum of peak area at 520 nm were all responsive to various treatments e.g. leaf removal and/or crop removal. However, decisions were made that if two or more parameters shared some similarities (e.g.having a high correlation between each other), then only one parameter was kept in order to avoid strengthened effects on similar parameters. Thus, colour density, total red pigments per berry, and sum of peak area at 520 nm were eliminated, and colour hue, degree of red pigment colouration, and malvidin-3-glucoside were kept. Similarly, total phenolics in skin extract per berry was kept and sum of peak area at 280 nm was removed. In conclusion, 13 parameters were selected to run a canonical variates analysis (Figure 3.9).

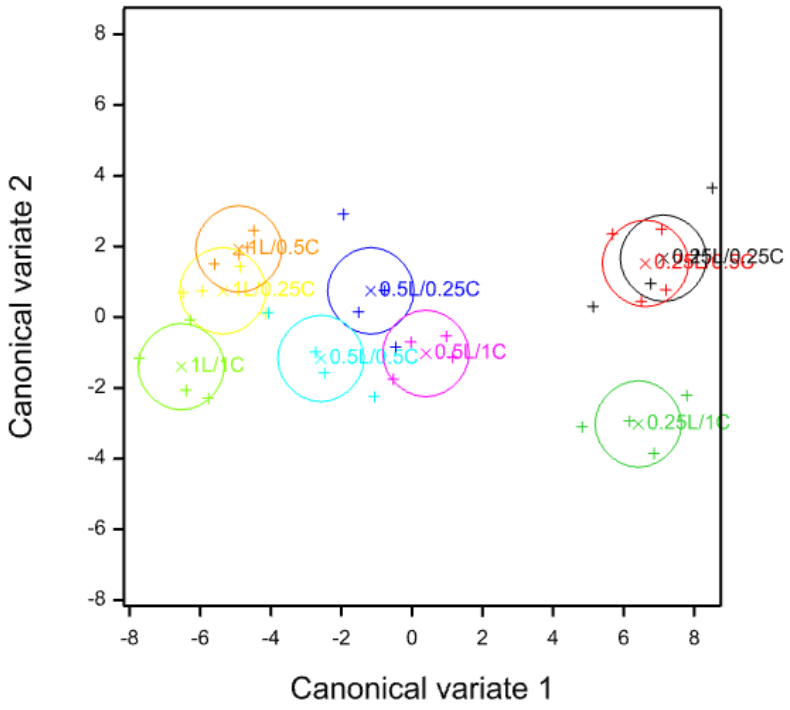

Figure 3.9 Canonical vitiates analysis by 13 selected parameters. Note: the circle means 95% confidential area. Black: 0.25L/0.25C, red: 0.25L/0.5C, green: 0.25L/1C, navy blue: 0.5L/0.25C, sky blue: 0.5L/0.5C, purple: 0.5L/1C, yellow: 1L/0.25C, orange: 1L/0.5C, yellow green: 1L/1C.

Vines from different treatments could be clearly identified with those 13 parameters provided, especially when they have different leaf levels.

The same parameters were used to generate principal components analysis (PCA). Latent vectors were used to select the most appropriate parameters from the 13 in total to differentiate vines from three leaf levels. Citric acid, seed dry weight per seed, juice pH, P, colour hue in skin extract, degree of red pigment colouration, and juice °Brix were ruled out for further analysis. Kaempferol-glucoside, malvidin-3-glucoside, starch content in root, skin fresh weight per berry, tannin concentration in skin

extract per berry, and total phenolics in skin extract per berry were reserved. The PCA result was shown on leaf levels (Figure 3.10).

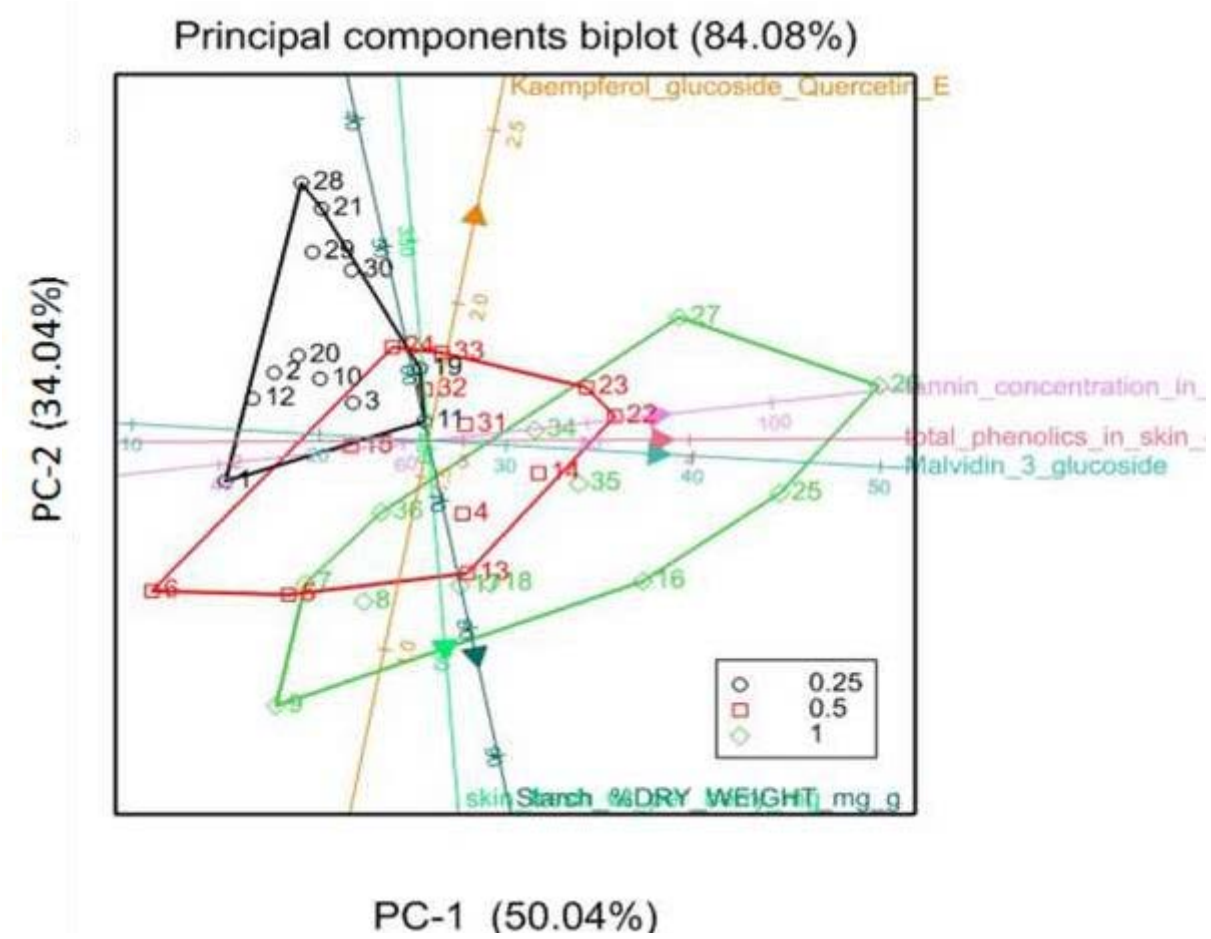

Figure 3.10 principal components analysis of selected parameters of vine and fruit by leaf levels

In general, fruit from vines with full leaf area (1L) tended to have more tannin, more malvidin-3-glucoside, higher total phenolic content, heavier skin fresh weight, and roots from full leaf area (1L) also had more starch content, compared to vines with leaf removal treatments. Though there was some overlap among vines between 1L and 0.5L, and between 0.5L and 0.25L, it is obvious that vines from 1L and from 0.25L were quite different.

3.4 Conclusion

To conclude, with leaf removal and/or crop removal, vines performed differently. Physical and chemical composition of fruit carried by those vines varied accordingly, which may result from the different vine performance caused by treatments (canopy microclimate). However, the data reveal several clues that the differences in fruit composition could result from source to sink ratio changes caused by the treatments and from photosynthate availability by leaf removal treatments. In this

study, a shading effect was not pronounced even under full leaf area (1L). On the contrary, higher leaf area led to a higher concentration of skin phenolics, grape skin fresh weight, which are often desired parameters for red wine quality (Jackson 2008; Iland et al. 2011). Higher starch concentration/CHO availability in root is also beneficial for cluster development for next season and sustainable production (Coombe 1992). Thus, in a cool climate, more leaf area in upper canopy could potentially improve fruit-derived wine sensory attributes such as red colour intensity, flavour, and mouthfeel if there is no obvious shading effect exists.

Chapter 4 Selected wine quality parameters

4.1 Introduction

The concept of wine quality is complex and lacks a clear definition. However, appearance, aroma, taste and mouthfeel are essential elements of wine quality and major drivers for expert evaluation and consumer liking (Iland et al. 2009). Appearance and mouthfeel of a red wine are derived from mainly phenolic compounds, although other compounds may alter or strengthen their impact (Cheynier, et al. 2006). The overall aroma profile of a wine consists of a common basic aromatic structure, but different concentrations and proportions of odour chemicals within that structure make the aroma system more complex. For example, the dominant aroma of a wine is generated from one or a group of aroma compounds that can break the aroma buffer (but which does not necessarily has/have the highest concentration among other aroma compounds) and hence transmit a different aroma nuance to the wine (Ferreira 2010). Major taste qualities in wine are contributed by sugars, acids, ethanol, and minerals. Iland et al. (2011) suggested that the quality of wine can be rated according to the type, intensity, complexity, length and balance of its sensory attributes. Thus in this study, compounds that may have some sensory contribution to a typical Pinot noir wine were selected to be indicators of wine quality.

The appearance, smell, taste and mouthfeel of wine originates from the grapes, the treatment of the grape juice and skins, their fermentation, and the maturation process of the wine (Clarke & Bakker 2004). In this study, and to minimise the effect of these, the treatment of the grape juice and skin and the process of fermentation along with maturation were maintained to be as identical and uniform as possible.

In this chapter, general information of the wine (pH, TA, alcohol, residual sugar as well as SO_2 content) will be firstly presented. The profile and content of phenolics, with an emphasis on tannins, anthocyanins and other colour properties, will be discussed. Finally, the aroma profile and the equilibria among the different aroma compounds will then be presented.

4.2 Results and discussion

4.2.1 General information of wine

Within leaf removal treatments, both wine pH and wine TA were significantly affected, although there were no significant differences in other wine general analytical parameters between treatments (Table 4.1). With increased leaf area, wine pH values decreased and wine TA increased. It

is worth noting that similar trends were obtained for both juice pH and wine pH, but the differences in TA between different leaf levels were more pronounced in wine (Table 3.3, Table 4.1). However, most of the research literature focuses on juice TA and rarely on wine TA. On Cabernet Sauvignon, berries from single leaf cuttings had significantly lower TA than cuttings with six leaves (Ollat & Gaudillere 1998). Reynolds et al. (1996) reported that leaf removal consistently reduced juice TA and suggested that the response of berry/juice TA was likely due to canopy microclimate, in agreement with Song et al. (2014), who also reported the juice TA from Pinot noir vines increased with increasing vine vigour. In contrast, the impact of crop removal on juice TA was minor. Keller et al. (2005) reported that TA, pH and colour of fruit at harvest were independent of crop thinning. Thus, it would be reasonable to conclude that TA of berry/juice or even wine is likely to be closely related to leaf area, or canopy microclimate generated by leaves. Meanwhile, the concentration of three organic acids in grape juice were also analysed and reported earlier (Table 3.5). It is interesting to note that the concentrations of those three individual acids showed no significant response to leaf removal treatments, and citric acid concentration was influenced by crop removal treatments.

In terms of residual sugar and alcohol, since all musts were adjusted to the same sugar concentration pre-fermentation, it is reasonable to expect there should not be any significant differences between treatments, and indeed, this was the case (Table 4.1). The concentration of free SO_2 at bottling was adjusted to 25 mg/L (refer to section 2.3 Winemaking process), so finding no significant differences in free SO_2 and total SO_2 was reasonable. However, free SO_2 levels were quite low compared to a commercial Pinot noir wine (the typical commercial rates of free SO_2 would be around 25-30 ppm), which could have an effect on other wine sensory attributes, e.g. the aroma profile.

Table 4.1 General analysis on wine

Treatment	Wine pH	Wine TA (g/l)	RS (g/l)	Alcohol (%)	Free SO_2 (mg/l)	Total SO_2 (mg/l)
0.25L						
0.25C	3.83	4.39	0.85	11.50	3.55	17.73
0.5C	3.86	4.14	1.35	11.70	5.67	12.76
1C	3.84	4.18	1.45	11.70	6.03	14.18
0.5L						
0.25C	3.78	4.56	1.55	11.60	4.61	19.14
0.5C	3.66	4.74	2.10	11.30	6.38	14.89
1C	3.63	4.76	1.85	11.80	6.38	15.95
1L						
0.25C	3.77	4.66	1.75	11.60	4.96	14.53
0.5C	3.72	4.66	1.40	11.80	6.74	15.60
1C	3.65	4.83	1.80	11.70	6.03	14.89
leaf removal effect[i]	$p < 0.01$	$p < 0.01$	n.s.	n.s.	n.s.	n.s.
Crop removal effect	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

[i] Treatment effects were listed according to two-way ANOVA test, no interactions found.

4.2.2 Wine colour properties

Wine colour hue and degree of red pigment colouration were significantly affected by leaf removal treatments although not linearly (Table 4.2). The pattern of these two parameters suggested a carry-over effect from grape skin (Table 3.7), since considerable efforts were made to keep grape and wine processing to be consistent.

Mazza et al. (1999) reported that anthocyanin concentration increased in berries of defoliated Pinot noir vines, and Castro et al. (2005) showed that both low shoot density and basal leaf removal could improve Touriga Nacional wine colour intensity. In the previous chapter, both colour density and total red pigments in skin extracts were higher with increased leaf levels, with shading not appearing to be a limiting factor for the synthesis of phenolic compounds. It was also suggested that the total amount of anthocyanin could be affected by reduced vine photosynthetic capacity and dry matter production, as per Guidoni et al. (2008). In contrast to the treatment effect in colour density and total red pigments in skin extracts, these measurements in wine were not statistically different (Table 3.7 and Table 4.2). The reason for this could be the different extraction regimes acting on grape skins, or any differences in skins becoming diminished during the winemaking and/or maturation process when the pigments undergo co-pigmentation or polymerization. However, some subjective visual differences in wine colour could be seen between treatments (Figure 4.1).

Table 4.2 Wine colour properties

Treatment	Wine colour density (a.u.)	Wine colour hue	Degree of red pigment colouration (%)	Estimate of SO_2 resistant pigments (a.u.)	Total red pigments (a.u.)
0.25L					
0.25C	2.56	1.07 ab[i]	17.12 ab	0.50	7.22
0.5C	2.16	1.06 ab	14.72 ab	0.42	7.12
1C	1.66	1.19 b	13.63 a	0.32	5.56
0.5L					
0.25C	2.61	0.94 a	18.17 b	0.49	7.52
0.5C	2.69	0.86 a	18.87 b	0.46	7.68
1C	2.26	0.92 a	17.23 ab	0.40	6.92
1L					
0.25C	2.54	0.93 a	16.57 ab	0.46	7.98
0.5C	2.80	0.89 a	16.99 ab	0.49	8.69
1C	2.35	0.88 a	17.37 ab	0.41	7.22
Leaf removal effect[ii]	n.s.	$p < 0.001$	$p < 0.01$	n.s.	n.s.
Crop removal effect	n.s.	n.s.	n.s.	n.s.	n.s.

[i] Means followed by a different letter were significantly different by Tukey test ($p < 0.05$)

[ii] Treatment effects were listed according to two-way ANOVA test, no interactions found.

Wine colour generated using CIELab showed a quite similar pattern to skin extract data, i.e. comparing the colour of Rep.3 skin extract (Figure 3.7) and wines made from replicates 2&3 (Figure 4.1). It was interesting to compare the result from Pasch (2014) on wine colour, who used the same method but measured right before bottling, and which showed a different pattern. The reason could be that even one or more red pigments increased under some treatments, but the insufficient amount of one or more groups of flavonoids could result in a non-significant increase in wine colour intensity, stability and complexity during wine aging and maturation (Sacchi et al. 2005). In other words, wine stabilizes with time. Though increasing the content of one or some red pigments could improve wine colour temporarily, insufficient amounts of compounds of any phenolics could cause the failure of improving the wine colour with aging. That is why the colour pattern changed from Pasch (2014) to what was shown below.

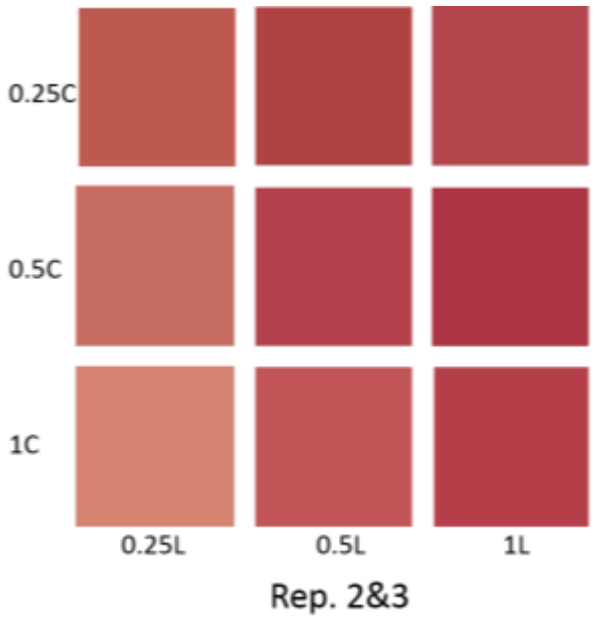

Figure 4.1 Representations of wine colour (generated by CIELab) for one of the two wine replicates

Values of L* (lightness), a* (redness/greenness) and b* (yellowness/blueness) were calculated. In this study, a* was significantly affected by leaf levels ($p < 0.01$, two-way ANOVA test). However, there was no significant difference in L* or b* between treatments, though the concentration of total flavonols was affected by crop levels, albeit not linearly (Table 4.7). Rustioni et al. (2012) suggested that the competing equilibria are important in the co-pigmentation effect, and that flavonols could be the best cofactors for this, and that the concentration of quercetin-3-O-glucoside was best correlated with the strength of co-pigmentation. An "anti-co-pigmentation" (disruption of the equilibrium and loss in colour) effect is also possible, which can be strengthened by adding phenols originating from seeds (Rustioni et al. 2012).

Pinot noir wines tested in this project were in bottle for seven months. Thus, it is difficult to tell whether polymerisation or co-pigmentation is the main effect that anthocyanins undergo, especially when there was no statistical difference observed in b*(Sacchi et al. 2005; Kennedy et al. 2006; Rustioni et al. 2012). Nevertheless, the difference in pH could be another reason that contributed to differential a* value since the form of anthocyanin is pH dependent (Cheynier, et al. 2006) (Table 4.1). Notably, wine pH was lower from the vines without leaf removal, which would favour more anthocyanins into the form of flavylium cations and improve the red colour (Cheynier, et al. 2006).

4.2.3 Wine phenolic properties (excluding anthocyanin)

Neither wine tannin concentration nor wine total phenolics was statistically different by leaf or crop removal treatments (Table 4.3). Both grape skin and seed were homogenised and then extracted

with 50 % ethanol, so values reported for berries are total potentially extractable rather than indicative of winemaking processes. Thus, it is hard to say whether a typical winemaking process would result in differential results compared to that discussed in section 3.2.3. Results from this study showed that the berry physiological status did change, i.e. total phenolics and tannin concentration in skin extracts on per berry basis were reduced by leaf removal treatments (Figure 3.5, Figure 3.6). However, the results in Table 4.3 suggest that the difference in skin phenolic, skin tannin and seed tannin may not be fully expressed by the winemaking process, or the extraction methods (homogenizing and extracted with 50% ethanol) used on berry parts was too aggressive that a typical wine solution normally could not be able to extract that much. Thus, the difference in berry composition would diminish during winemaking process, which is clearly a partial extraction especially for phenolic compounds.

Table 4.3 Total phenolics and tannin concentration in wine

Treatment	Wine tannin concentration (mg/l epicatechin equiv.)	Wine total phenolics (a.u.)
0.25L		
0.25C	386	19.7
0.5C	331	18.7
1C	289	16.6
0.5L		
0.25C	367	17.9
0.5C	385	18.3
1C	369	17.9
1L		
0.25C	354	17.3
0.5C	398	19.6
1C	366	16.8
Leaf removal effect[i]	n.s.	n.s.
Crop removal effect	n.s.	n.s.

[i] Treatment effects were listed according to two-way ANOVA test, no interactions found.

After fractionation by Solid-Phase extraction (Jeffery et al. 2008a), the absorbance at 280 nm and 520 nm of the three phenolic fractions (F1, F2, and F3, Table 4.4) were not significantly different. The recovery rates at 280 nm ranged from 81.4% to 92.9% and averaged 86.9%, similar to what was achieved by Liu (2014), who found the recovery rate in average was 83% among 86 New Zealand Pinot noir wines. The recovery rate at 520 nm was not calculated due to the absorbance of anthocyanins being pH dependent: changing the solvent from a wine solution to a different organic solvent altered the pH value unpredictably.

Table 4.4 Fractions after SPE

Treatment	F1@280 nm (a.u.)	F2@280 nm (a.u.)	F3@280 nm (a.u.)	Recovery @280nm (%)[i]	F1@520 nm (a.u.)	F2@520 nm (a.u.)	F3@520 nm (a.u.)
0.25L							
0.25C	12.84	3.97	3.83	86.92	2.24	0.57	0.39
0.5C	11.85	3.00	3.59	81.40	2.00	0.43	0.37
1C	11.39	2.52	3.96	86.75	1.86	0.33	0.34
0.5L							
0.25C	11.38	4.22	4.20	90.98	2.13	0.70	0.48
0.5C	13.09	3.62	2.67	87.02	2.66	0.46	0.28
1C	12.30	3.06	3.49	86.46	2.15	0.45	0.35
1L							
0.25C	11.25	3.98	3.66	88.66	2.53	0.70	0.45
0.5C	11.89	3.56	3.74	81.36	2.40	0.59	0.43
1C	11.40	3.20	4.32	92.88	2.25	0.51	0.48
Leaf removal effect[ii]	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Crop removal effect	n.s.	$p < 0.05$	n.s.	n.s.	n.s.	$p < 0.01$	n.s.

[i] Recovery rate was calculated following the formula (F1@280 nm+F2@280 nm+F3@280 nm)/ (wine total phenolics+4)

[ii] Treatment effects were listed according to two-way ANOVA test, no interactions found.

The absorbance of F2 at both 280 nm and 520 nm was significantly affected by crop removal treatments (Table 4.4). The original methodology paper (Jeffery et al. 2008) indicated that F2 was more hydrophilic than F3. The absorbance of F2 or F3 found at 520 nm as a percentage of the combined absorbance units at 520 nm of both F2 and F3 were listed in Table 4.5 with no significant difference found. However, the percentages listed in Table 4.5 were out of the range given by Jeffery et al. (2008) who used older wines, but still indicating the effect of wine age as the wines used in this study were very young and should have greater amount of coloured polymeric species eluting in the first polymeric fraction (F2) as per Jeffery et al. (2008) and Liu (2014). The ratio of absorbance units at 520 nm to 280 nm were also displayed in Table 4.5, with F2 having a higher proportion of coloured compared to non-coloured material relative to F3, in agreement with Jeffery et al. (2008). Other information of F2 and F3 on the organoleptic properties and original biosynthesis pathway, e.g. why F2 was affected by crop removal treatments, is needed, although Liu (2014) suggested that the F2 and F3 might have different abilities to precipitate tannin with methyl cellulose.

Table 4.5 Crop removal effect on coloured polymeric material present in wine fractions F2 and F3 measured by spectrophotometer (no significant difference)

Treatments	520 nm		F2	F3
	% in F2[i]	% in F3[ii]	520/280 (%)	520/280 (%)
0.25C	60	40	16	11
0.5C	58	42	15	11
1C	52	48	15	10

[i] % in F2=absorbance units of F2/ (absorbance units of F2+absorbance units of F3)

[ii] % in F3=absorbance units of F3/ (absorbance units of F2+absorbance units of F3)

All the HPLC identified compounds in F1 were grouped into categories, which consisted of

- Flavanol monomers, including catechin, epicatechin, gallocatechin, epigallocatechin and epicatechin gallate (Table 4.6);
- Flavanol dimers, including procyanidin 1 and procyanidin 2 (Table 4.6);
- Flavonols, including quercetin, rutin, kaempferol-glucoside, quercetin-glucuronide 1 and quercetin-glucuronide 2 (Table 4.7);
- Hydroxybenzoic acids, including gallic acid, syringic acid, protocatechuic acid, *p*-hydroxybenzoic acid and vanillic acid, and hydroxycinnamic acids, including *p*-coumaric acid, ferulic acid, caffeic acid and its ester caftaric acid (Table 4.8), and
- Malvidin-3-glucoside (Table 4.7). The total concentrations of each category were the sum of concentrations (mg/L) from the individual compounds.

Within flavanol monomers, catechin followed by epicatechin were the two most abundant compounds, but with no significant differences in concentration between treatments (Table 4.6). Previous reports showed that flavanol monomers in New Zealand commercial Pinot noir wines were related to wine show medal grades, with lower concentrations of catechin and epicatechin in Gold and Silver wines compared to those in Bronze and no-medal wines (Liu, 2014). In addition, higher levels of catechin and epicatechin are not beneficial for co-pigmentation according to Rustioni et al. (2012). However, in this study, there is no significant difference between treatments in terms of the sum of catechin and epicatechin.

Table 4.6 Concentration (mg/L) of individual phenols (flavanol monomers and dimers, excluding gallocatechin)

Treatment	Catechin	Epicatechin	Epigallocatechin	Epicatechin gallate	Procyanidin 1	Procyanidin 2	Total flavanols[i]
0.25L							
0.25C	28.73	16.47	1.28	0.80	26.97	5.02	85.23
0.5C	25.73	14.83	1.65	0.69	30.12	6.53	85.72
1C	25.09	14.19	1.55	0.59	30.79	4.61	83.91
0.5L							
0.25C	17.93	10.49	1.32	0.76	26.68	5.09	65.65
0.5C	21.13	11.76	1.50	0.92	32.25	8.57	81.69
1C	22.90	13.33	1.45	0.86	32.30	6.55	83.90
1L							
0.25C	19.61	11.99	1.96	1.08	25.92	6.18	74.40
0.5C	19.31	11.35	1.95	1.16	29.51	8.50	77.87
1C	19.27	11.82	1.71	0.90	30.20	5.65	74.23
Leaf removal effect[ii]	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Crop removal effect	n.s.	n.s.	n.s.	n.s.	$p < 0.05$	$p < 0.05$	n.s.

[i] Total flavanols was calculated following the formula concentration of (catechin + epicatechin + gallocatechin +epigallocatechin + epicatechin gallate + procyanidin 2+procyanidin 1)

[ii] Treatment effects were listed according to two-way ANOVA test.

On the other hand, the difference in gallocatechin concentration was quite unique and seemed not affected by single factors, e.g. leaf removal or crop removal (two-way ANOVA test), but significantly affected by the interactions of leaf removal and crop removal ($p < 0.05$) However, although gallocatechin concentration remained stable under 0.25L, gallocatechin concentration appeared to be affected by crop removal under 0.5L and 1L (Figure 4.2). The reason behind this remains unclear.

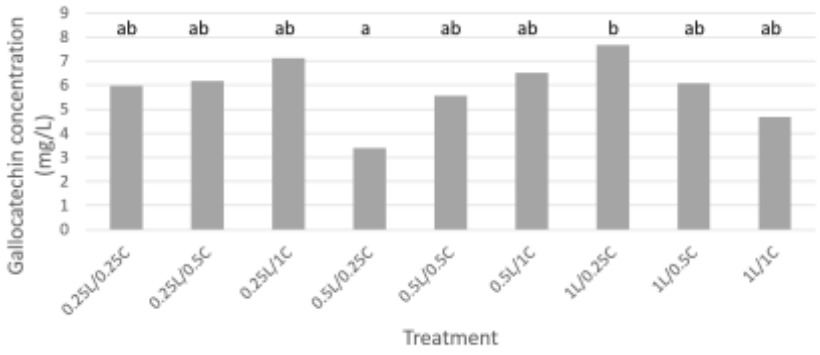

Figure 4.2 Concentration of gallocatechin affected by treatment. Columns with different letters were significantly different by Tukey test ($p < 0.05$)

Epigallocatechin and epicatechin gallate were not affected by any treatment, but the two flavanol dimers, namely procyanidin 1 and procyanidin 2 were affected by crop removal (but not linearly). Perez-Magarino & Jose (2004) reported that the dimers of flavanols tended to reach higher concentrations in the wines made from grapes collected on the later harvest dates, suggesting that flavanol polymerization progresses with the degree of grape ripening. In this study, crop removal could potentially enhance grape ripening as King et al. (2012) has reported, associated with higher amounts of flavanol polymerization accordingly. However, the concentrations of the two flavanol dimers showed different patterns from what was expected. Or, crop removal of a similar amount as 0.5C could be more appropriate than that for 0.25C, as the latter may lead to less flavanol polymerization because of end product inhibition associated with inappropriate small sink size.

In wine, malvidin-3-glucoside concentration was highly correlated with sum of peak area at 520 nm by spectrophotometer (R^2=0.999). However, the correlations between malvidin-3-glucoside concentration and a*, and between malvidin-3-glucose and total red pigments were only moderate (R^2=0.36 and R^2=0.65, respectively). On the other hand, malvidin-3-glucoside in wine remained unaffected between treatments (Table 4.7), despite its concentration being significantly different in skin extracts (Table 3.9). Thus, red colour of wine may rely on other factors as well (i.e. pH) rather than only anthocyanin concentration, while the latter failed to transfer from grape skin into wine.

Kaempferol-glucoside in skin extracts was affected by leaf removal treatments (Table 3.9), while kaempferol-glucoside in wine was shown to be affected by both leaf and crop removal treatments but not linearly (Table 4.7). In addition, the concentrations of quercetin and quercetin-glucuronide 1 were influenced by leaf removal, which corresponds to the level of cluster exposure (Price 1994), and the concentration of rutin was influenced by crop removal. Rustioni et al. (2012) pointed out that flavonols could be the best co-pigmentation co-factors. Thus, a higher degree of co-pigmentation was expected at 0.5C. Unfortunately, even if there was a higher degree of co-pigmentation (not examined in this study) in wines from less leaf but more crop vines, the wine colour was not the deepest among the three crop levels (Figure 4.1).

Table 4.7 Concentration (mg/L) of individual phenols (anthocyanin and flavonols)

Treatment	Malvidin-3-glucoside	quercetin	Rutin	Kaempferol-glucoside (quercetin equiv.)	Quercetin-glucuronide 1 (quercetin equiv.)	Quercetin-glucuronide 2 (quercetin equiv.)	Total flavonols[i]
0.25L							
0.25C	72.0	0.77	5.76	10.9 ab[ii]	5.96	2.78	26.1 ab
0.5C	77.4	0.77	9.88	14.1 b	6.52	2.32	33.6 b
1C	68.5	0.66	7.18	12.0 ab	6.24	2.06	28.2 ab
0.5L							
0.25C	72.6	0.63	5.42	9.4 a	5.64	2.79	23.8 ab
0.5C	90.8	0.72	9.04	11.8 ab	6.25	3.02	30.8 ab
1C	74.6	0.68	10.60	11.2 ab	5.35	2.55	30.4 ab
1L							
0.25C	90.7	0.50	5.00	7.9 a	4.71	3.05	21.2 a
0.5C	85.0	0.52	9.27	11.1 ab	5.70	3.15	29.7 ab
1C	78.6	0.57	8.20	10.2 ab	5.12	2.74	26.9 ab
Leaf removal effect[iii]	n.s.	$p < 0.05$	n.s.	$p < 0.01$	$p < 0.05$	n.s.	n.s.
Crop removal effect	n.s.	n.s.	$p < 0.01$	$p < 0.01$	n.s.	n.s.	$p < 0.01$

[i] Total flavonols was calculated following the formula concentration of (quercetin + rutin + Kaempferol-glucoside + Quercetin-glucuronide 1 + Quercetin-glucuronide 2)

[ii] Means followed by a different letter were significantly different by Tukey test ($p < 0.05$).

[iii] Treatment effects were listed according to two-way ANOVA test, no interactions found.

Among hydroxybenzoic acids and hydroxycinnamic acids, protocatechuic acid was only affected by leaf removal, while caffeic acid and caftaric acid were only affected by crop removal (Table 4.8). Hydroxybenzoic acid and *p*-coumaric acid were affected by both leaf removal and crop removal.

Table 4.8 Concentration (mg/L) of individual phenols (hydroxybenzoic acids and hydroxycinnamic acids & derivatives)

Treatment	Gallic acid	Syringic acid	Protocatechuic acid	*p*-Hydroxybenzoic acid	Vanillic acid	*p*-coumaric acid	Ferulic acid	Caffeic acid	Caftaric acid
0.25L									
0.25C	12.84	5.90	12.66	9.37 abc[i]	7.26	0.295 d	0.049	1.66	14.23 abc
0.5C	12.14	4.86	11.04	11.47 c	6.19	0.262 bcd	0.050	1.99	19.97 cd
1C	11.74	4.57	10.52	11.67 c	5.11	0.261 bcd	0.056	1.31	19.35 cd
0.5L									
0.25C	10.05	5.98	9.56	8.19 ab	7.32	0.272 cd	0.047	1.82	12.43 ab
0.5C	11.39	5.61	11.97	10.92 bc	7.17	0.217 a	0.034	2.33	17.71 bcd
1C	11.31	5.00	11.70	11.34 c	5.47	0.215 a	0.033	1.92	20.44 d
1L									
0.25C	10.54	5.93	8.24	7.80 a	7.56	0.278 d	0.031	1.89	11.39 a
0.5C	10.58	5.66	8.87	10.43 abc	7.09	0.233 abc	0.023	2.39	17.76 bcd
1C	10.93	5.24	8.80	10.48 abc	6.08	0.224 ab	0.036	1.87	18.85 cd
Leaf removal effect[ii]	n.s.	n.s.	$p < 0.01$	$p < 0.05$	n.s.	$p < 0.001$	n.s.	n.s.	n.s.
Crop removal effect	n.s.	n.s.	n.s.	$p < 0.001$	n.s.	$p < 0.001$	n.s.	$p < 0.05$	$p < 0.001$

[i] Means followed by a different letter were significantly different by Tukey test ($p < 0.05$).

[ii] Treatment effects were listed according to two-way ANOVA test, no interactions found.

Hydroxybenzoic acids in wine are generated either from glycosides or esters of hydroxybenzoic acids in grapes, via hydrolysis (Clarke & Bakker 2004). Dixon & Paiva (1995) suggested that canopy shading may stimulate the synthesis of some hydroxybenzoic acids, namely protocatechuic acid, *p*-hydroxybenzoic acid and syringic acid, through the stress-induced phenylpropanoid metabolism pathway. However, protocatechuic acid concentration was actually lower when more leaves obtained in this study. The reason remains unknown, but as protocatechuic acid is an antioxidant, more research in this area could be of value.

On the other hand, cinnamic acids are present in both grapes and wine, but are largely esterified in wine (Clarke & Bakker 2004). It has been recognized that *p*-coumaric acid is a key metabolite of the phenylalanine ammonia lyase pathway which is increased in response to certain stresses experienced by the vine, e.g. ultraviolet radiation (Fritzemeier & Kindl 1981; Creasy & Creasy 2003). However, Goldberg et al. (1998) reported that *p*-coumaric concentrations were not affected by climatic conditions (warm or cool climate). Nevertheless, *p*-coumaric acid is the precursor of 4-ethylphenol and 4-vinylphenol that could generate undesirable aromas and flavours in wine (Salameh et al. 2010). Both caffeic acid and caftaric acid were affected by crop removal treatment,

but with different patterns. Caffeic acid concentrations were highest under 0.5C. But caftaric aicd, which is the ester of caffeic acid, concentration was generally higher when more crop was retained (0.5C and 1C).

4.2.4 Wine aroma profile

Studies on Pinot noir wine have shown that the most odour active volatile organic compounds (VOCs) include alcohols, short chain fatty acids, and ethyl and acetate esters (Fang & Qian 2006; Kemp 2010). In this study, 31 compounds have been selected and their thresholds were listed in Table 4.9.

Table 4.9 Volatile aroma compounds in Pinot noir wines (descriptor, chemical group and odour thresholds)

Analyte	Descriptor	Chemical group	Odour perception threshold (μg/L)	Ranges# (μg/L)
Linalool	Caramel, apple-sweet [1]	Terpenoids	25.2 [5] 50 [6]	41.4-170.1
Geraniol	Floral [1]	Terpenoids	30 [7]	0-4.8
β-Damascenone	Sweet, tea, floral [1]	C_{13}-norisoprenoids	0.05 [7] 0.002 [8]	0.6-4.4
β-Ionone	Berry, violet [2]	C_{13}-norisoprenoids	0.09 [5] 90 [18]	0.1-0.7
Benzaldehyde	Nutty cherry [1]	Aldehydes/C6 compounds	350-1000 [8] 5000 [17]	5.1-66.0
Octanoic acid	Goaty rancid cheese [1]	Acids	500 [5]	665-2002
Hexanoic acid	Sweaty [1]	Acids	420 [5]	1104-1941
Butanoic acid (Butyric acid)	Sweaty [1]	Acids	173 [5]	209-755
Acetic acid	Vinegar [1]	Acids	200000 [7]	349-874 (mg/L)
3-Methyl butanoic acid (isovaleric acid)	Rancid, cheese [3]	Acids	1600 [8]	160-710
2-Methylbutanoic acid	Rancid, cheese [3]	Acids	--	--
3-Methylbutan-1-ol (isoamyl alcohol)	Nail polish [1]	Alcohols	250-770 [8] 30000 [16]	135.8-312.5 (mg/L)
Hexanol	Grape juice [1]	Alcohols/C6 compounds	8000 [7] 25.2 [5]	1.9-4.7 (mg/L)
2-Phenylethanol	Rose [1]	Alcohols	10000 [7] 14000 [5]	17.3-100.7 (mg/L)
Trans-3-hexenol	Green [1]	Alcohols/C6 compounds	1000 [9] 8000 [14]	56.9-126.5
Cis-3-hexenol	Fruity green [1]	Alcohols/C6 compounds	400 [10] 606 [5]	24.2-115.8
1-Heptanol	Green, leafy [3]	Alcohols	2500 [11]	12.2-270.3
Ethyl acetate	Sweet fruity [1]	Esters	7500 [7]	--

Ethyl butanoate	Fruity peach [1]	Esters	20 [5,7]	116.4-339.9
Ethyl hexanoate	Fruity, wine [1]	Esters	14 [5] 5 [7]	299.3-593.8
Ethyl octanoate	Cooked fruity, pleasant [1]	Esters	5 [5] 2 [14]	410.2-874.3
Ethyl decanoate	Fruity [1]	Esters	200 [5] 1100 [15]	154.2-971.3
Ethyl 2-methyl propanoate (ethyl isobutyrate)	Sweet, apple fruity [1]	Esters	15 [5] 18 [5]	103.6-113.8
Ethyl 3-methylbutanoate (ethyl isovalerate)	Ester fruity [1]	Esters	100-400 [8] 3 [5]	11.6-51.8
Ethyl pentanoate	Mint, fruity [1]	Esters	10 [11] 1.5[13]	0.9-4.3
Ethyl (S)-2-hydroxypropanoate (Ethyl lactate)	Floral, fruity, green [4]	Esters	50000-200000 [8]	--
Ethyl heptanoate	--	Esters	220 [11]	2.6-9.2
Ethyl cinnamate	--	Esters	1.1 [14]	0.8-7.2
Ethyl hydrocinnamate	--	Esters	--	--
Hexyl acetate	Sweet floral [1]	Esters	400 [12] 700 [14]	1.8-15.7
3-Methylbutyl acetate (isoamyl acetate)	Fruity banana [1]	Esters	30 [7]	148.5-377.5

[1] Fang & Qian 2005, [2] Kemp 2010, [3] Fragasso et al. 2012, [4] Mirandalopez et al. 1992, [5] Ferreira et al. 2000, [6] Buttery et al. 1969, [7] Guth 1997, [8] Clarke & Bakker 2004, [9] Moyano et al. 2002, [10] Escudero et al. 2007, [11] Maarse 1991, [12] Benkwitz et al. 2011, [13] Genovese et al. 2007, [14] Dunlevy et al. 2009, [15] Rocha et al. 2004, [16] Prida & Chatonnet 2010, [17] Zea et al. 2001, [18] Cooke et al. 2009

[#] Ranges were obtained from Tomasino (2011)

Ninety percent of the terpenes are present as non-volatile glycosides in grapes and are hydrolyzed (enzymatically or chemically) to the free form during fermentation and aging (Ebeler 2001). Moreover, linalool can be further transformed to geraniol during winemaking, while the latter can be further changed into citronellol through enzymatic reactions, which has a lower sensory threshold than the other two (Hernandez et al. 2003; Ribéreau-Gayon et al. 2000). In this study, citronellol concentration was not monitored, but linalool concentration already exceeded the odour threshold (Table 4.9, Table 4.10). The concentration of geraniol in wine was significantly affected by leaf and crop removal treatments, however, it is hard to say whether there would be any organoleptic difference caused by geraniol because its concentration was below the reported odour threshold.

It was reported that the concentration of linalool and geraniol in wines from ultra-low vigour zones were higher compared to wines from higher vigour zones (Song et al. 2014). This could be an effect of microclimate, i.e. greater fruit exposure to sunlight for low vigour vines commonly generate, or a

sink to source relationship effect, i.e. the production of terpenes were enhanced by increasing grape maturity by lower vigour vines with more dry matter being allocated into fruit (Skinkis et al. 2010; Song et al. 2014). The sink to source relationship rather than microclimate may be one factor modulating terpenoids production, although several other mechanisms could also be involved that altered terpenoid concentration. No statistical difference can be seen in linalool concentration between treatments, but higher concentrations of linalool can be generally observed with more leaves and higher crop levels (Table 4.10).

Table 4.10 Contents of selected aroma compounds (terpenoids and C_{13}-norisoprenoids)

Treatment	Linalool (μg/L)	Geraniol (μg/L)	β-damascenone (μg/L)	β-ionone (μg/L)
0.25L				
0.25C	33.7	3.89 b[i]	6.74	0.209
0.5C	34.0	3.85 ab	6.80	0.216
1C	36.9	3.51 ab	7.17	0.233
0.5L				
0.25C	48.0	3.68 ab	6.67	0.234
0.5C	46.9	3.23 ab	6.21	0.249
1C	70.8	2.60 ab	6.14	0.271
1L				
0.25C	47.1	3.25 ab	6.53	0.244
0.5C	50.9	3.57 ab	6.38	0.278
1C	59.1	2.58 a	6.38	0.285
Leaf removal effect[ii]	n.s.	$p < 0.05$	n.s.	n.s.
Crop removal effect	n.s.	$p < 0.01$	n.s.	n.s.

[i] Means followed by a different letter were significantly different by Tukey test ($p < 0.05$).

[ii] Treatment effects were listed according to two-way ANOVA test, no interactions found.

The concentrations of β-damascenone and β-ionone were both above their odour thresholds, although no significant difference was observed between treatments (Table 4.9, Table 4.10). Escudero et al. (2007) observed that the fruity aroma in red wines can be enhanced by C_{13}-norisoprenoids. β-damascenone and β-ionone, along with other C_{13}-norisoprenoids, result from carotenoid degradation during grape ripening (Ebeler 2001). Sunlight exposure by leaf removal and shoot thinning could improve both pre-veraison carotenoids accumulation and their post-veraison degradation to C_{13}-norisoprenoids (Crupi et al. 2010). In addition, Lee et al. (2007) reported that C_{13}-norisoprenoids concentration in Cabernet Sauvignon wine was affected by leaf removal treatments, in agreement with Feng et al. (2015) who found that β-damascenone concentrations were positively correlated to the increased sunlight exposure caused by fruiting zone leaf removal. However, the

responses of β-damascenone to sunlight were variable even under similar treatments. Song et al. (2014) reported that in Pinot noir wine, the concentration of β-ionone was influenced by, but β-damascenone was independent of, vine vigour. It was also reported that neither sunlight nor UV treatment resulted in any changes in the concentration of β-damascenone (Song et al. 2015). Lee et al. (2007) further suggested that leaf layer number may influence norisoprenoid concentrations negatively, independent of sunlight exposure. In this study, sunlight exposure has proved not to be the modulating factor for phenolic compounds production, and the differences in fruiting zone sunlight exposure were not severe (discussion in Section 3.2.3). Thus, neither sunlight exposure nor source to sink relationship had a significant effect on β-damascenone and β-ionone production under the circumstances of this study.

At high concentrations, fatty acids are associated with rancid, cheesy and vinegar-like aromas (Clarke & Bakker 2004). In this study, except for 3-methylbutanoic acid and 2-methylbutanoic acid (the threshold of the latter remains unknown), the rest of the acids listed in Table 4.11 exceeded their odour thresholds (Table 4.9). Clarke & Bakker (2004) suggested that excess of fatty acids concentration was most likely to have been increased by acetobacter in unhygienic conditions. However, in this study, high fatty acid concentration could result from low levels of SO_2 (Table 4.1) where the activity of the micro-organism may not have been fully suppressed (Table 4.1). On the other hand, the concentration ranges of five fatty acids in thirty-two New Zealand commercial Pinot noir wine as reported by Tomasino (2011), suggested that in this study, the acids were almost in the normal range.

Table 4.11 Contents of selected aroma compounds (acids)

Treatment	Acetic acid (mg/L)	Butanoic acid (butyric acid) (μg/L)	3-Methylbutanoic acid (μg/L)	2-Methylbutanoic acid (μg/L)	Hexanoic acid (μg/L)	Octanoic acid (μg/L)
0.25L						
0.25C	506.6 abc[i]	982.9	676.1	620.5	971.8	565.7 ab
0.5C	500.1 abc	1000.5	692.6	616.4	1045.7	651.0 ab
1C	512.1 abc	1038.5	727.7	648.5	1045.2	647.3 ab
0.5L						
0.25C	526.5 bc	933.2	768.0	738.1	958.4	562.0 ab
0.5C	442.9 a	914.7	907.3	831.0	1054.5	674.5 ab
1C	463.8 ab	923.5	816.9	749.3	1050.4	723.0 b
1L						
0.25C	571.7 c	970.3	781.7	735.9	975.3	546.7 a
0.5C	515.0 abc	923.7	831.4	811.6	1020.6	637.9 ab
1C	525.6 bc	930.9	954.6	910.5	1047.5	680.3 ab
Leaf removal effect[ii]	$p < 0.01$	n.s.	$p < 0.05$	$p < 0.01$	n.s.	n.s.
Crop removal effect	$p < 0.01$	n.s.	n.s.	n.s.	n.s.	$p < 0.01$

[i] Means followed by a different letter were significantly different by Tukey test ($p < 0.05$).

[ii] Treatment effects were listed according to two-way ANOVA test, no interactions found.

Acetic acid can act as a precursor for the synthesis of acetyl-CoA and be a redox sink for anabolic and physiological stress reactions (Ugliano & Henschke 2009). In this study, the concentration of acetic acid in wine was responsive to both leaf removal and crop removal treatments, with the lowest level in 0.5C among the three crop levels and 0.5L among the three leaf levels. Houtman et al. (1980) reported that the production of acetic acid was promoted with increased grape maturity. However, in this study, by higher °Brix (Table 3.3), was not associated with higher acetic acid concentration.

Straight-chain fatty acids (hexanoic acid and octanoic acid in this study) are by-products of saturated fatty acid metabolism of yeasts, while branched-chain fatty acids (2-methylbutanoic acid and 3-methylbutanoic acid in this study) are believed to be derived from the oxidation of the aldehydes formed from α-keto acids during amino acid metabolism, although the mechanism of regulation remains unclear (Clarke & Bakker 2004; Ugliano & Henschke 2009). Statistical analysis showed that 2-methylbutanoic acid and 3-methylbutanoic acid were positively responsive to leaf levels, while the pattern of hexanoic acid and octanoic acid concentration showed that they could be affected by crop removal treatments (although there was no statistical difference in hexanoic acid between treatments) (Table 4.11).

Unfortunately, only a few reports have addressed the influence of vineyard managements on fatty acids synthesis and concentration in finished wine, which makes it difficult to explain why some acids tested in this study had such kinds of responses. Of the few research reports available, octanoic acid was mentioned relatively frequently. Song et al. (2014) reported that the concentrations of octanoic acid in wines produced from four different vigour zones were not significantly different. On the contrary, Sun et al. (2011) reported that octanoic acid concentration in finished wine was decreased with shoot thinning in one of the two vintages, while Suklje et al. (2014) reported that octanoic acid concentration was lower with leaf and lateral shoots removal than no leaf removal in the fruiting zone. Thus, it can be concluded that fruit exposure may limit octanoic acid synthesis independent from vine vigour. However in this study, fruiting-zone sunlight exposure was adequate for all levels of leaf removal (for discussion refer to Section 3.2). This is consistent with the fact that there was no significant difference in octanoic acid concentration between leaf removal treatments. Both 3-methylbutanoic acid and butanoic acid remained unaffected by shoot thinning (Sun et al. 2011), and further research is needed.

C_6 alcohol and aldehydes, which are generally considered to be responsible for “green” characters in wine, are generated from enzyme-mediated aerobic oxidation of linoleic and linolenic acids (C18:2 and C18:3) (Tomasino 2011; Moreno & Peinado 2012). For aldehydes, the benzaldehyde concentration was higher than what was reported by Tomasino (2011). One possible reason could be the small amount of SO_2 addition and therefore more aerobic oxidation than usual (Section 2.3 Winemaking process and Table 4.1).

Table 4.12 Contents of selected aroma compounds (alcohols and aldehydes)

Treatment	Benzaldhyde (μg/L)	Trans-3-hexenol (μg/L)	Cis-3-hexenol (μg/L)	Hexanol (mg/L)	1-heptanol(μg/L)	isoamyl alcohol (mg/L)	phenethyl alcohol (mg/L)
0.25L							
0.25C	405	67.9 bc[i]	56.8 ab	2.82	10.4	185 ab	24.0 a
0.5C	506	59.2 ab	55.7 ab	2.76	11.8	190 abc	22.9 a
1C	424	44.1 a	81.1 b	2.53	12.1	183 a	23.1 a
0.5L							
0.25C	358	82.9 cd	49.5 ab	2.90	10.7	222 bcd	31.2 ab
0.5C	557	78.9 cd	46.7 ab	2.91	11.8	234 d	35.5 b
1C	549	67.0 bc	47.0 ab	2.78	12.5	234 d	33.2 b
1L							
0.25C	765	86.1 d	53.3 ab	2.92	10.3	210 abcd	30.6 ab
0.5C	680	79.4 cd	38.7 a	2.87	11.6	226 cd	34.3 b
1C	554	70.8 bcd	49.5 ab	2.93	12.3	248 d	37.7 b
Leaf removal effect[ii]	n.s.	$p < 0.001$	$p < 0.05$	n.s.	n.s.	$p < 0.001$	$p < 0.001$
Crop removal effect	n.s.	$p < 0.001$	n.s.	n.s.	$p < 0.01$	$p < 0.05$	n.s.

[i] Means followed by a different letter were significantly different by Tukey test ($p < 0.05$).

[ii] Treatment effects were listed according to two-way ANOVA test, no interactions found.

Hexan-1-ol can generate herbaceous and grassy aromas in wines when at high concentration, but concentrations were reported to decrease as a result of shoot thinning, as with trans-3-hexenol and cis-3-hexanol (Sun et al. 2011). However, hexanol concentration was independent of treatments in this study, though trans-3-hexenol and cis-3-hexenol were affected (Table 4.12).

Training system was reported to alter the concentration of 1-heptanol, and the possible reason leading to this could be the differences in crop loads and/or sun exposure caused by the trellis system (Fragasso et al. 2012), which in this study was affected by crop removal treatments. Both 3-methylbutan-1-ol (isoamyl alcohol) and 2-phenylethanol concentration were significantly affected by leaf removal treatments, though they were reported to be independent of shoot thinning but affected by harvest date (Sun et al. 2011). Song et al. (2014) also reported that 3-methylbutan-1-ol concentrations in wine was independent of vine vigour. Thus, the concentrations of some alcohols may not be affected by greater sun exposure, which normally generated by low vigour and open-canopy vines.

In all, the responses of alcohols and aldehydes to different vineyard treatments were various and complicated. The reason could be the differences in specific treatments, experimental location and

variety. However, alcohols tested in this study are most readily affected by either leaf removal or crop removal treatments, thus, the source to sink relationship could be involved in determining the precursors in grapes and later regulate alcohol production.

Esters were the major class of aroma-active compounds analysed in this study (Table 4.13). Their formation can be affected by many factors such as yeast strain, fermentation temperature, oxygen availability, grape nutrient composition, and nitrogen level during fermentation (Clarke & Bakker 2004; Ebeler & Thorngate 2009; Perestrelo et al. 2006; Vianna & Ebeler 2001). Since efforts were put into maintaining uniform winemaking process among different treatments, the composition and concentration of grape nutrient/aroma precursors could be the main factor leading to the difference in ester concentration, in this study.

Table 4.13 Contents of selected aroma compounds (esters)

Treatment	Ethyl acetate (mg/L)	Ethyl butanoate (μg/L)	Ethyl hexanoate (μg/L)	Ethyl octanoate (μg/L)	Ethyl decanoate (μg/L)	Ethyl 3-methylbutanoate (μg/L)	Ethyl isobutyrate (μg/L)	Ethyl penanoate (μg/L)	Ethyl lactate (mg/L)	Ethyl heptanoate (μg/L)	Ethyl hydrocinnamate (μg/L)	Ethyl cinnamate (μg/L)	Hexyl acetate (μg/L)	Isoamyl acetate (μg/L)
0.25L														
0.25C	68.3	184	261 ab[i]	409 abc	135	9.25	181	1.09	67.7	2.81 ab	0.726	1.42	4.24	158 a
0.5C	73.7	225	301 ab	532 bc	145	10.66	188	1.01	67.7	3.27 bc	0.786	1.12	4.63	189 ab
1C	74.9	222	313 b	572 c	158	10.80	189	1.05	65.7	3.57 c	0.975	1.06	3.90	185 ab
0.5L														
0.25C	76.4	190	246 a	358 a	122	12.27	239	1.00	77.3	2.25 a	0.670	0.91	5.64	255 abc
0.5C	62.9	193	267 ab	423 abc	148	16.02	282	0.94	75.2	2.40 a	0.715	0.87	6.10	287 bcd
1C	68.9	213	282 ab	490 abc	152	15.40	284	0.92	85.8	2.65 ab	0.792	0.94	7.23	340 cd
1L														
0.25C	75.0	182	252 a	368 ab	129	12.35	246	1.07	79.0	2.49 a	0.735	0.90	6.25	251 abc
0.5C	80.6	201	255 ab	374 ab	119	13.41	245	1.15	71.9	2.43 a	0.690	0.93	6.48	323 cd
1C	77.5	201	273 ab	398 ab	125	17.67	322	1.07	79.7	2.46 a	0.748	0.85	7.41	396 d
Leaf removal effect[ii]	n.s.	n.s.	$p < 0.05$	$p < 0.01$	n.s.	$p < 0.05$	n.s.	n.s.	$p < 0.05$	$p < 0.001$	n.s.	n.s.	$p < 0.05$	$p < 0.001$
Crop removal effect	n.s.	$p < 0.05$	$p < 0.01$	$p < 0.01$	n.s.	n.s.	n.s.	n.s.	n.s.	$p < 0.05$	n.s.	n.s.	n.s.	$p < 0.01$

[i] Means followed by a different letter were significantly different by Tukey test ($p < 0.05$).

[ii] Treatment effects were listed according to two-way ANOVA test, no interactions found.

It appears that other than ethyl acetate, ethyl decanoate, ethyl isobutyrate, ethyl pentanoate, ethyl hydroxycinnamate, and ethyl cinnamate, other esters examined in this study were affected by some treatments (Table 4.13). Generally, higher concentrations were obtained with higher crops. Further analysis (as shown in Figure 4.3) showed that ethyl hexanoate (descriptor "fruity"), ethyl octanoate (descripor "cooked fruity, pleasant") and ethyl heptanoate (descriptor not known) were different among TMRs, with the higher concentrations obtained by lower TMRs, which is the opposite pattern of trans-3-hexenol (descriptor "green"). According to Ferreira et al. (2003), the odour intensity and the concentration of aroma compounds are positively related (although not linearly). The exact contributions of these aroma compounds in the current study were not determined. However, the sensory profile of the wines made from the grapes with leaf and crop removal on the grapevines, have the potential to be different. Based on the results for the concentration of ethyl hexanoate, ethyl octanoate and ethyl heptanoate, the wine could be fruitier on nose when the TMR is low; the wine could be greener when the TMR is high for higher trans-3-hexenol concentration. However, the aroma profile of a wine is complicated and difficult to explain based on VOC concentrations. A recently developed sensory method, "Napping", could be beneficial for categorising wine aroma profile as the complement to chemistry methods (Cadoret et al. 2010).

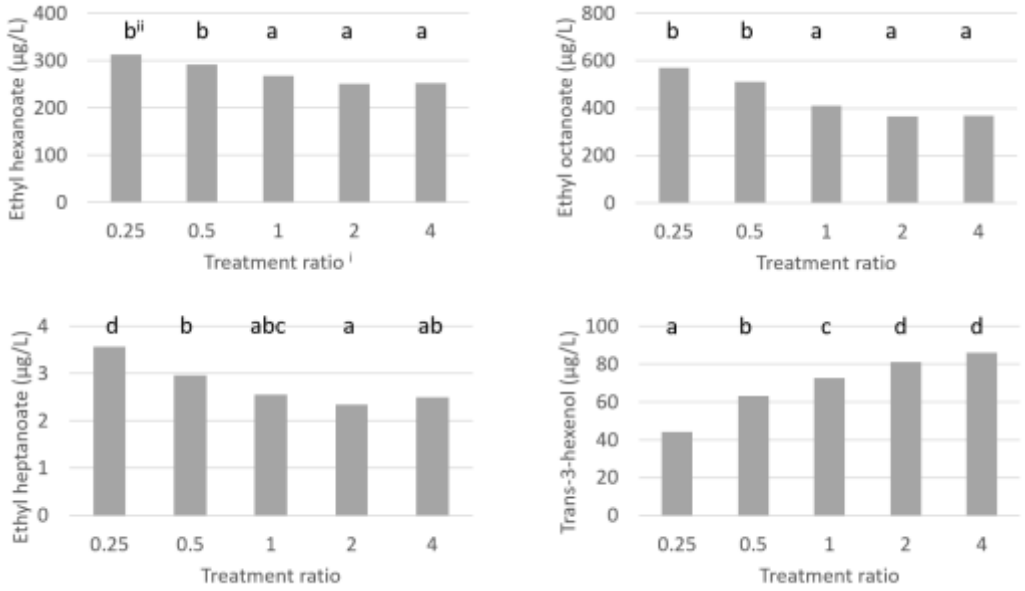

Figure 4.3 Aroma compounds that were affected by TMRs

[i] TMR = level of leaf area (e.g. 0.25, 0.5, 1) / level of crop load (e.g. 0.25, 0.5, 1)

[ii] Columns with different letters were significantly different by Duncan test ($p < 0.05$)

4.3 Canonical variates analysis (CVA) and principal components analysis (PCA)

In this section, wine quality parameters were categorized into three groups

- Colour parameters including some parameters listed in Table 4.2, Table 4.4 and Table 4.5, a*, b*, and L*;
- Palate/phenolic parameters including basic wine properties listed in Table 4.1 and phenolic properties listed in Table 4.3 through to Table 4.8;
- Aroma parameters including parameters listed in Table 4.10 through to Table 4.13.

Both canonical variates analysis (CVA) and principal components analysis (PCA) were not conducted on colour parameters because the visible colour pattern was already shown in Figure 4.1.

Preliminary analyses (not shown) revealed that wine pH, wine TA, F2@280 nm (the absorbance units of F2 at 280nm), the concentration of gallocatechin, quercetin, rutin, kaempferol-glucoside, procyanidin 1 and 2, quercetin-glucuronide 1 and total flavonols, protocatechuic acid, p-hydroxybenzoic acid, p-coumaric acid, caffeic acid, caftaric acid were all affected by either leaf removal treatments or crop removal treatments or both. Since wine pH and wine TA had high correlations (R^2=0.81), caffeic acid had high correlatons with procyanidin 2 (R^2=0.81), hydroxybenzoic acid had high correlations with caftaric acid (R^2=0.92), and total flavonol had high correlations with kaemperol-glucoside (R^2=0.87), wine TA, procyanidin 2, caftaric acid, kaemperol-glucoside were removed for CVA (Figure 4.4).

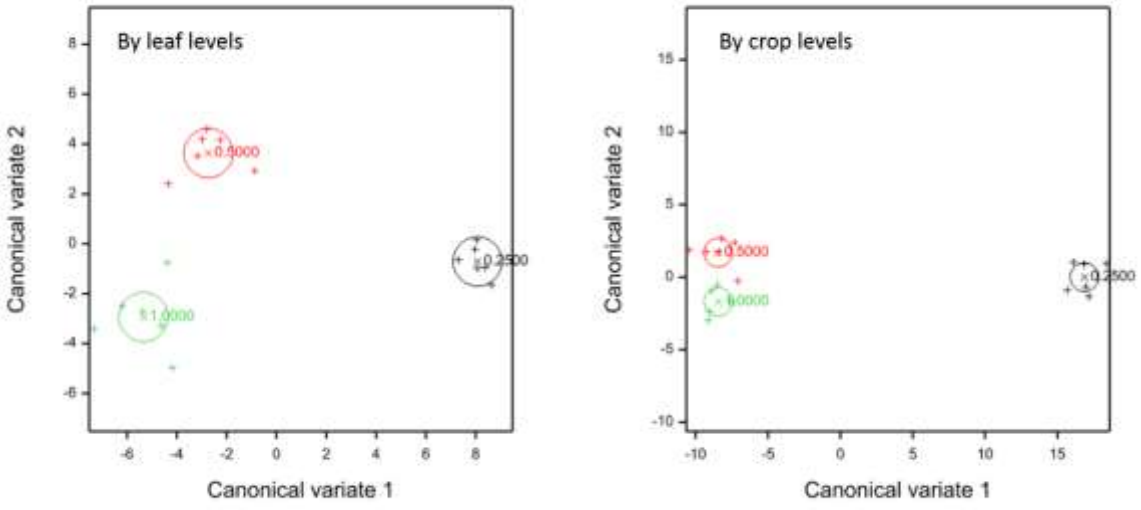

Figure 4.4 Canonical variates analysis of wine palate/phenolic properties by 12 selected parameters. Note: the circle means 95% confidential area. Black: 0.25L (left) or 0.25C (right), red: 0.5L (left) or 0.5C (right), green: 1L (left) or 1C (right).

It is clear that wines made from the vines with leaf and crop manipulation can be identified with the 12 palate/phenolic parameters provided, especially when they have different leaf levels and when the crop removal treatment was severe.

Later, some parameters were removed because of the low latent root value and only eight parameters were chosen for further analysis by PCA. F2@280 nm, gallocatechin, quercetin-glucuronide 1, protocatechuic that were used for canonical variates analysis were removed (Figure 4.5).

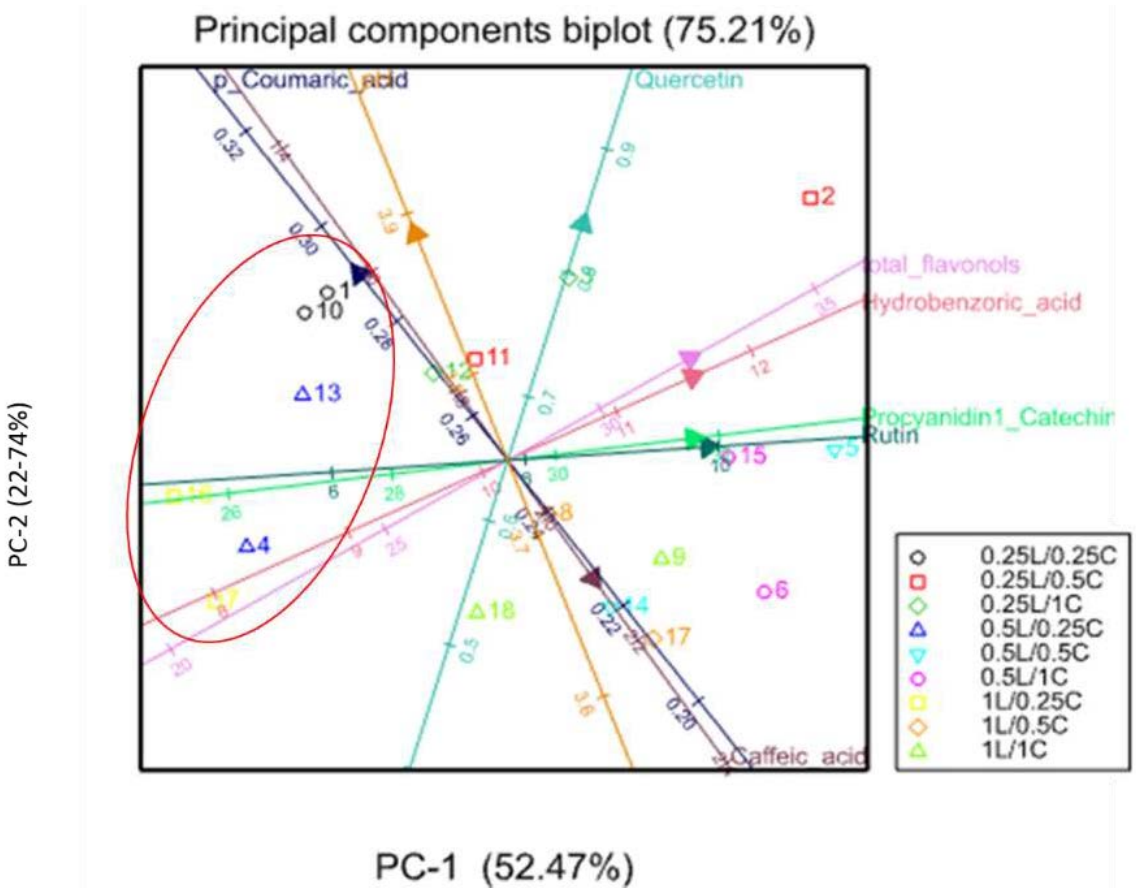

Figure 4.5 Principal components analysis of wine palate/phenolic properties by 8 selected parameters. Note: Black: 0.25L/0.25C, red: 0.25L/0.5C, green: 0.25L/1C, navy blue: 0.5L/0.25C, sky blue: 0.5L/0.5C, purple: 0.5L/1C, yellow: 1L/0.25C, orange: 1L/0.5C, yellow green: 1L/1C; red circle groups the dots standing for 0.25C.

Though certain variables can be seen between the two replicated wines, wines made from vines with 0.25C treatment (red circle in Figure 4.5) were high in *p*-coumaric acid and pH but low in rest of the parameters compared to heavier crop loads. The wine palate/phenolic properties are complicated and can be affected by both leaf removal and crop removal treatments, sometimes by the treatment interactions, e.g. gallocatechin.

The same parameter selection method was applied to analyse the aroma profile. Correlations between parameters were identified. In detail, 3-methylbutanoic acid had high correlation with 2-methylbutanoic acid (R^2=0.91) and with ethyl-3-methylbutanoate (R^2=0.83), ethyl butanoate had correlations with ethyl hexanoate (R^2=0.76) and with ethyl octanoate (R^2=0.60), ethyl heptanoate had high correlation with trans-3-hexenol (R^2=0.72), and isoamyl alcohol had high correlations with phenylethanol (R^2=0.90) and with isoamyl acetate (R^2=0.83). As a result, geraniol, acetic acid, 3-methylbutanoic acid, octanoic acid, cis-3-hexenol, 1-heptanol, isoamyl alcohol, ethyl butanoate, ethyl lactate, ethyl heptanoate and hexyl acetate were kept for CVA (Figure 4.6).

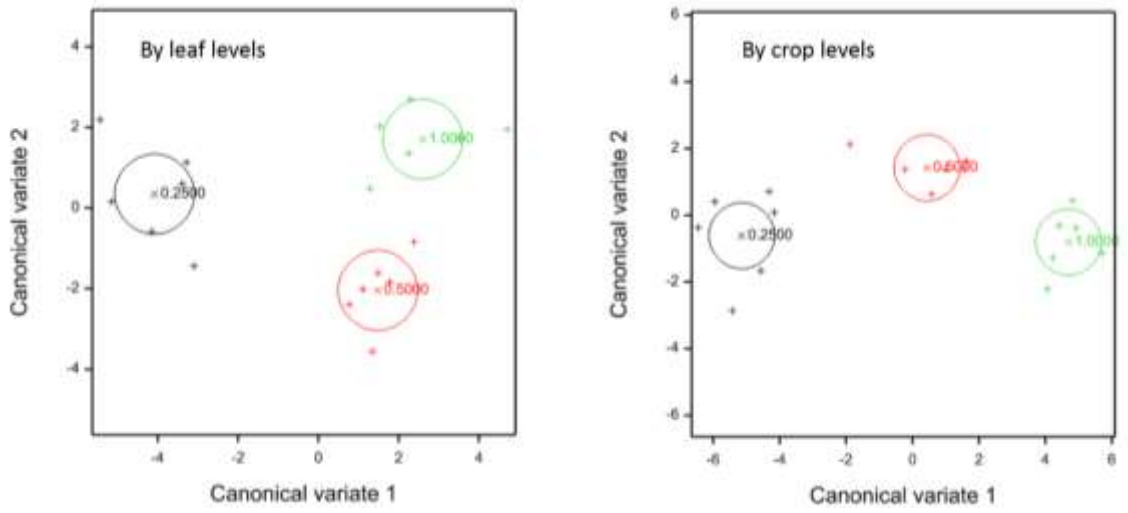

Figure 4.6 Canonical vitiates analysis of wine aroma properties by 11 selected parameters. Note: the circle means 95% confidential area. Black: 0.25L (left) or 0.25C (right), red: 0.5L (left) or 0.5C (right), green: 1L (left) or 1C (right).

It is clear that by the 11 selected aroma parameters, wine aroma profile can be statistically categorised by both leaf removal treatments and crop removal treatments.

The parameters chosed for PCA includes geraniol, octanoic acid, cis-3-hexenol, isoamyl alcohol, ethyl heptanoate, and hexyl acetate (Figure 4.7). It is clear that wines made from vines with higher leaf area, e.g. 1L/1C and 1L/0.5C, tend to have high concentrations of hexyl acetate and isoamyl alcohol, which also have high correlations with phenylethanol (R^2=0.90) and with isoamyl acetate (R^2=0.83). As a result, from an organoleptic point of view, wines made from vines with higher leaf area could be fruitier due to higher ester concentrations compared to vines with lower leaf area. On the other hand, wines made from 0.25L/1C and 0.25L/0.5C were higher in cis-3-hexenol which is generally considered to have “fruity green” characteristic (Fang & Qian 2005). Nevertheless, wines made from low crop loads, e.g. 1L/0.25C, 0.5L/0.25C and 0.25L/0.25C were high in geraniol and low in octanoic acid, which could be floral and less cheesy.

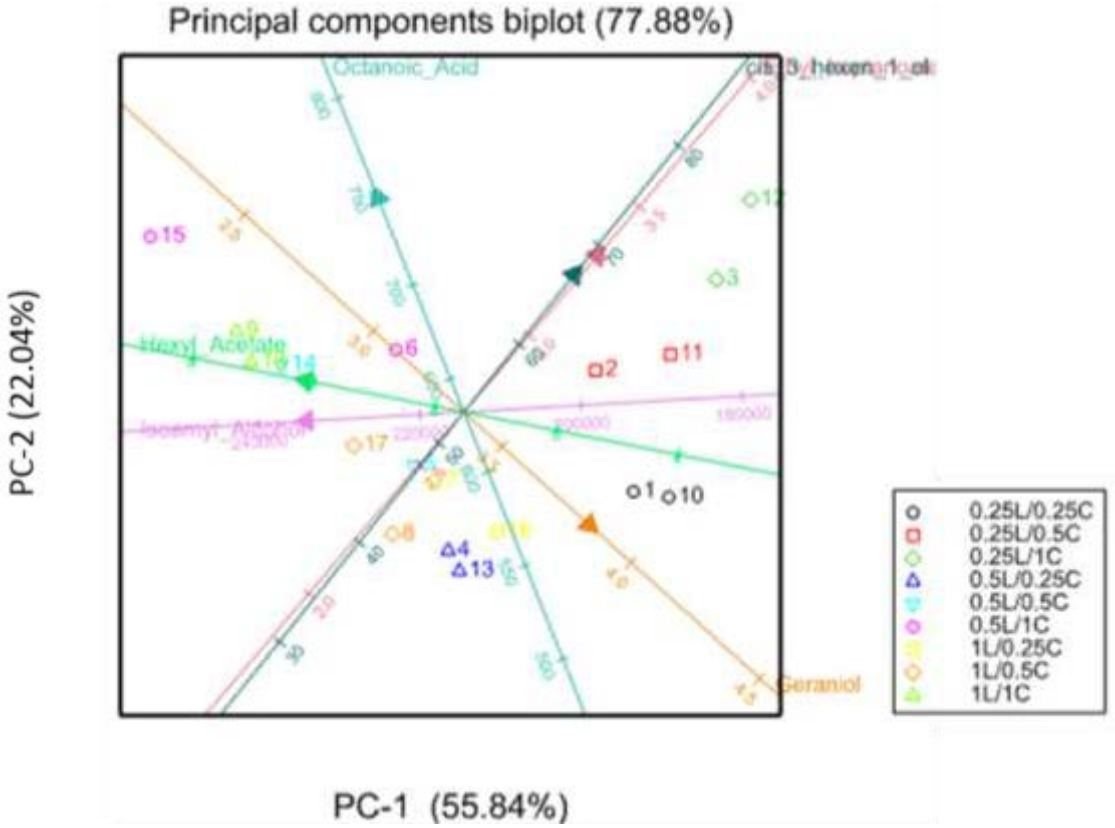

Figure 4.7 Principal components analysis of wine aroma properties by 6 selected parameters. Note: Black: 0.25L/0.25C, red: 0.25L/0.5C, green: 0.25L/1C, navy blue: 0.5L/0.25C, sky blue: 0.5L/0.5C, purple: 0.5L/1C, yellow: 1L/0.25C, orange: 1L/0.5C, yellow green: 1L/1C.

4.4 Conclusions

In general and from statistical point of view, wines made from vines with more leaf area tends to be richer in caffeic acid and possibly fruitier in sensory characteristic. In contrast, with extremely limited leaf area, wines were high in pH, *p*-coumaric acid and quercetin concentration, and tended to have more green aromas. It is also interesting to note that wines made from vines with extremely low crops had low concentrations of total flavonols, rutin, hydroxybenzoic acid and procyanindin 1.

To conclude and in relations to section 3.3, a shading effect was not pronounced even under full leaf area, but wines made from vines with higher leaf area expressed an aroma profile that could be presented as being fruitier on nose and that were deeper in colour. However, extremely low crop loads may not beneficial for wine quality, for they are generally low in phenolic monomers and dimers, and esters.

Chapter 5 Summary

The effect of grapevine leaf area (LA) relative to crop load (CL) was investigated in the cool climate growing region of Canterbury, New Zealand, using Pinot noir (clone 10/5) Vertical Shoot Positioning (VSP) trained vines. Vine shoot leaf number were standardized and two of the four basal leaves were removed three weeks after fruit-set. LA was adjusted to 12 leaves (1L), 6 leaves (0.5L) or 3 leaves (0.25L). CL was adjusted to full crop (1C), half crop (0.5C) or quarter crop (0.25C), and in turn, produced five different treatment ratio (TMR, "0.25", "0.5", "1", "2", and "4"). Leaf area to crop load ratio (LA/CL, cm^2/g) was calculated from leaf area per vine divided by total yield per vine after harvest.

LA/CL was responsive to both leaf removal and crop removal treatments, but the change was not entirely synchronous with TMR (R^2=0.86, Figure 3.1). The reason could be that for the same TMR, vines have different compensation abilities with different actual leaf areas. For example, both 0.25L/0.5C and 0.5L/1C treated vines shared the same TMR "0.5", but the latter vines had bigger leaves, higher single leaf photosynthetic rates (even higher than 1L/1C vines), and resulted in higher LA/CL than 0.25L/0.5C vines. This indicated that 0.5L/1C vines had the ability to produce more photosynthates in relation to their limited leaf area and therefore compensated somewhat for the loss in leaf area. On the other hand, pruning weight was greater with increased TMR except when TMR was at its highest, "4" (Figure 3.2), indicating that the vegetative growth of current season, especially the winter cane weight, was altered by manipulating TMR. In addition, carbohydrate status in grapevine roots was disturbed, largely by changes to the starch dry weight caused by leaf, but not crop removal treatments (Table 3.1).

There were no significant differences in physical characteristics of berry parts (Table 3.2), but juice °Brix and juice pH were affected especially by leaf removal treatments (Table 3.3). In terms of organic acids and minerals, citric acid and phosphorus (P) were affected by some treatments (Table 3.5, Table 3.6), but the reason remains unknown. Tannin concentration and total phenolics in seed extracts remained stable under different treatments, however, phenolic and colour properties of skin extracts were affected, especially by leaf removal treatments (Figure 3.5, Figure 3.6, and Table 3.7). Because of the dilution and inherent properties of skin phenolics (e.g. lack of phenolic monomers and dimers but being rich in polymers), some phenolic monomers were not measured by the current HPLC analysis method (Table 3.8). Malvidin-3-glucoside concentration was positively, but kaempferol-glucoside concentration was negatively, related to leaf treatments.

Thus, in this study, some of the parameters analysed on grapevines and their fruit were affected by leaf removal, and to a less extent, by crop removal. Keller et al. (2005) reported that carrying out crop removal on field-grown Cabernet Sauvignon, Riesling, and Chenin blanc vines on a high-capacity site significantly reduced harvest yields, but had little effect on vegetative growth, fruit ripening, and fruit composition. In this study, anthocyanins, tannin and phenolic content in skin extracts and skin fresh weight were higher from vines with full leaf area (1L) compared to other vines with leaf removal treatments. Although phenolic synthesis was reported to be affected by canopy microclimate, e.g. sun exposure and temperature (Mazza et al. 1999; Ristic et al. 2010; Cortell & Kennedy 2006), shading effects were not pronounced in this study among the range of leaf removal treatments. Thus, the differences in fruit composition could result from photosynthate availability as affected by leaf removal treatments.

Wine pH and wine TA were significantly affected by leaf removal treatments (Table 4.1), likely a carry-over effect from fruit composition (Table 3.3). For wine colour parameters, the statistical differences in colour density and total red pigments were diminished from skin extracts into wine (Table 4.2, Table 3.7). However, some subjective visual differences in wine colour can be observed (Figure 4.1). Further analysis showed that a*(redness/greenness) was affected by leaf removal treatments, but b*(yellowness/blueness) was unaffected by treatments, indicating that there were no obvious colour shifting caused by, for example co-pigmentation. Thus, co-pigmentation in young Pinot noir wines may not be influenced by crop removal and/or leaf removal under the conditions found in this study.

The statistical differences in tannin concentration and total phenolics were also diminished from skin extracts into wine (Table 4.3, Figure 3.5), although phenolic monomers and dimers were responsive to leaf removal and/or crop removal treatments (Table 4.6, Table 4.7 and Table 4.8). Among them, the behaviour of gallocatechin was interesting (responsive to the interaction of LA and CL, but not affected by single factors) and needs further study (Figure 4.2). Two procyanidins dimers, rutin, caffeic acid and caftaric acid were affected by crop removal only, while quercetin, quercetin-glucuronoide 1 and protocatechuic acid were affected by leaf removal only. Kaempferol-glucoside, *p*-hydroxybenzoic acid and *p*-coumaric acid were affected by both leaf removal and crop removal treatments. It is also interesting to note after fractionation of phenolic compounds, the absorbance of the second fraction (F2) at 280 nm and 520 nm were affected by crop removal treatments. Thus, the physiochemical properties of F2 need to be explored.

Based on chemical analysis, the sensory profile of the wines made from the grapes with leaf and crop removal on the grapevines could be different. For grape-derived terpenoids and C_{13}-

norisoprenoids, there were no significant differences between treatments except for geraniol, the concentration of which however was below its odour threshold. The concentrations of some fatty acids were higher when more leaves or more crop were retained. For esters, some were found at greater concentration when more leaves retained, some showed the opposite tendency. The wine aroma, therefore, could be "greener" when the TMR is high because C_6 alcohol concentrations were greater. However, although concentrations of primary aroma compounds were not affected much, the secondary aroma compounds were affected by treatments; these were generated during winemaking and maturation from grape-derived aroma precursors and the overall grape must matrix.

Although efforts were put in to maintaining a uniform winemaking process, there were many possibilities for differences to occur. In this situation, there are still some carry-over effects identified in this project, including pH, colour hue, degree of red pigment colouration, kaempferol-glucoside, along with the visual colour pattern generated by CIELab method for both grape berries and wines. Thus, the characteristics of fruit, which were influenced by vine physiological and physical characteristics, can be transferred into wine.

In a cool climate such as New Zealand, with Pinot noir and a VSP training system, shading effects were not so pronounced after two of the four basal leaves were removed after fruit-set to generate an acceptable fruiting zone sunlight exposure. Based on this research, maintaining a higher leaf area in the upper canopy could potentially improve phenolics and colour in wine. Though some parameters of grape berries were only affected by leaf removal treatments (e.g. kaempferol-glucoside, malvidin-3-glucoside), these and other parameters (largely due to these not being detected in skin extracts) in wine were also responsive to crop removal treatments (e.g. kaempferol-glucoside, *p*-hydroxybenzoic acid), indicating that not only the photosynthate availability for the whole vine, but also the relative availability of photosynthate to every cluster (sink) is important for final wine composition.

Generally in this study, more leaves in the upper canopy (high LA) and/or high LA/CL could improve some parameters in grape berries and wine (e.g. starch dry weight in root, gallocatechin concentration in wine), but this was not always true. Sometimes, the highest value of those parameters were not obtained in 1L or 1L/0.25C treated vines, and sometimes, there was not a significant difference in vines with 0.5L or 1L treatments. For example, the highest pruning weight was not gained by 1L/0.25C (Figure 3.2), possibly due to end-product inhibition of photosynthesis. Moreover, there was no significant difference in skin extracts on the parameter of sum of peak area at 280 nm (by HPLC) between TMR "2" and "4" (Figure 3.8). In wine, the deepest colour density, and

the highest concentrations of *p*-hydroxybenzoic acid, *p*-coumaric acid and caftaric acid were not obtained in the 1L/0.25C treatment (Table 4.2, Table 4.8). Additionally, the most desirable aroma profile may not be found in the 1L/0.25C wines for they lack fatty acid-based aroma compounds and some esters. Thus, the most appropriate LA/CL or TMR to produce quality wines was at the extremes (e.g. the highest or lowest LA/CL, or TMR ="0.25" or "4"), though it would be good to assess the overall liking of the wines (e.g. through sensory evaluation) when considering the wine matrix (wine quality) as a whole, as a complement for chemical analysis.

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