# Effect of reduced irrigation on grapevine physiology, grape characteristics and wine composition in three Pinot noir vineyards with contrasting soils

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Patricio Mejias-Barrera

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# Publications and research posters presented from this thesis

## Publications

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**Mejias-Barrera, P.**, Creasy, G.L., Harrison, R., and Hofmann, R. 2015. Links between vine water stress indicators: measured versus visual. *In*: Proceedings of the 19th International Meeting of Viticulture GiESCO (Vol. 1). pp. 99-103. Groupe international d'Experts en Systèmes vitivinicoles pour la CoOpération (GiESCO).

## Posters

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Abstract of a thesis submitted in partial fulfilment of the requirements for the Degree of Doctor of Philosophy.

# Effect of reduced irrigation on grapevine physiology, grape characteristics and wine composition in three Pinot noir vineyards with contrasting soils

by

Patricio Mejias-Barrera

The effect of water stress on grapevine performance has been extensively studied in different wine producing regions around the world, but little has ocurred in New Zealand. Pinot noir is the second most planted variety in the country and the most planted in Waipara. An improved understanding of the physiological responses of Pinot noir vines growing in different soils under a water restricted scenario is crucial for winegrowers, because vineyard irrigation is commonly practiced in Waipara and water is expected to become scarcer in future seasons.

Three Pinot noir vineyards having similar characteristics, but planted in three of the most representative types of soil of the Waipara region were selected to investigate the effect of reducing irrigation by about 50% under commercial conditions. Control (CON) vines corresponded to those receiving the irrigation applied according to the viticulture manager's criteria, and a reduced irrigation (RI) treatment was implemented by modifying the drippers spacing and flow rate. The experiment was carried out during the 2013-2014 and 2014-2015 seasons.

Edapho-climatic characteristics were compared within the region and among the three sites. "Terroir" provides the link between wine composition and place of origin. Thus, soil and climatic conditions, were characterised to understand the uniqueness of Pinot noir wines produced in Waipara. Differences in soil profile available water were found between the three types of soil. Also, variations in temperatures, wind speed and evapotranspiration, among other parameters were found within the region as well as between sites.

A range of analyses was used to identify differences in grapevine physiology between vines under RI and those normally irrigated. Primary leaf area abscission and stomatal closure were short-term responses to water stress, which together with the lack of differences in stem water potential

suggested the isohydric behaviour of Pinot noir under the conditions of this study. Other parameters like carbon isotope ratio, leaf proline content and root carbohydrates were little affected by RI.

Berry weight was reduced by the treatment, but this varied depending on the site and season. Seed water content, seed fresh and dry weight were unaffected by RI which may suggest that seeds remain “isolated” from the rest of the berry from veraison onwards, even under moderate water stress. Taurine was found in berry juice, the first time that this nitrogen compound is described in *Vitis vinifera* L.

Wines produced during the first season showed differences in wine titratable acidity (TA), colour and aroma profile by GCMS only at the site having the lowest profile available water, while wines from those sites with high and very high profile available water did not report differences between CON and RI for most of the parameters evaluated.

This study demonstrated the edapho-climatic variability within the Waipara region, as well as the adaptive responses to water stress site by site, confirming irrigation as one of the main factors modifying “terroir” expression. From a practical perspective, the findings suggest merit for the use of reduced irrigation in vineyard management, as a means to save cost whilst maintaining grape quality.

**Keywords:** Pinot noir, Waipara, North Canterbury, water stress, reduced irrigation, terroir, soil, climate, grapevine physiology, grape characteristics, wine, amino acids, taurine, carbon isotope ratio, GCMS, stem water potential, proline, wine aroma compounds, seed weight, tannin.

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# Chapter 1

# Introduction

Unlike Europe where *Vitis vinifera* L. cultivation is traditionally non-irrigated (Lovisolo *et al.* 2010), vineyards in New Zealand are mostly grown using irrigation. Worldwide, wine-producing regions experience seasonal drought (Chaves *et al.* 2010), which in most cases coincides with the grapevine growing season, meaning water stress is one of the most important factors limiting grapevine growth (Hochberg *et al.* 2013b).

Irrigation practices, especially in "new world" wine countries (like New Zealand), have put pressure on the water resources in most areas where grapevines are cultivated. Medrano *et al.* (2015) described the high water requirements that are necessary to complete the growth cycle of grapevines, and which become critical during the dry summer in most of the wine region. As reported by Chaves *et al.* (2010), the frequency of heat waves and heavy rains is predicted to increase, with the cool climate wine regions being no exception to this.

Water deficit during the growing season does not imply exclusively negative effects. A regulated water stress balances vine vegetative and reproductive growth with the objective of regulating berry quality (Lovisolo *et al.* 2010). However, the combined effect of drought with periods of high air temperature, and therefore, high evaporative demand could have a negative effect not only on grapevine productivity, but also berry and wine quality (Tomás *et al.* 2014). This research set out to investigate the effects of a 50% reduction in irrigation on grapevine physiology, grape characteristics and wine composition across three sites and over two seasons, aiming to evaluate the consequences that a lower water availability scenario could have on Pinot noir vineyards in Waipara.

Pinot noir is the most planted variety in Waipara (New Zealand Winegrowers 2015), and therefore, understanding the physiological effects of water stress under field conditions is crucial as water for irrigation is becoming more scarce. Soil acts as a reservoir for nutrients and plant-available water and is an important factor in grape and wine production, not only because it determines nutrient and water availability, but also for its implication in the "terroir" effect in viticulture (Tramontini *et al.* 2014). Definitions of "terroir" can implicate soil, but also includes climate and other factors. A feature of the Waipara region is the diversity of soils, with many different types planted to vineyards (Tonkin *et al.* 2014). Thus, in addition to investigating the effect of reduced irrigation on vine, berry and wine parameters, this diversity of soils provided an opportunity to study the effect of soil variations (particularly in relation to water-holding capacity) within a reasonably uniform mesoclimate.

## Thesis structure

Including this introduction, this thesis contains six chapters. Chapter 1 includes the introduction, literature review, and research objectives and hypotheses. Chapter 2 sets the scene for this specific study with an analysis of data from local weather stations, and a description of the sites in terms of soils, microclimate and the experimental design. Chapters 3, 4 and 5 contain the results of the relating to grapevine physiology, berry ripening and wine composition, respectively. Finally, chapter 6 provides the overall conclusions and is followed by the appendices containing supporting information for the different chapters. The list of references is included at the end of the document. The chapters containing the results follow a sequence, starting with the soil and climatic characterisation of the sites, followed by the grapevine physiology, grape compositional factors and finally wine-based evaluations. This is summarised in Figure 1

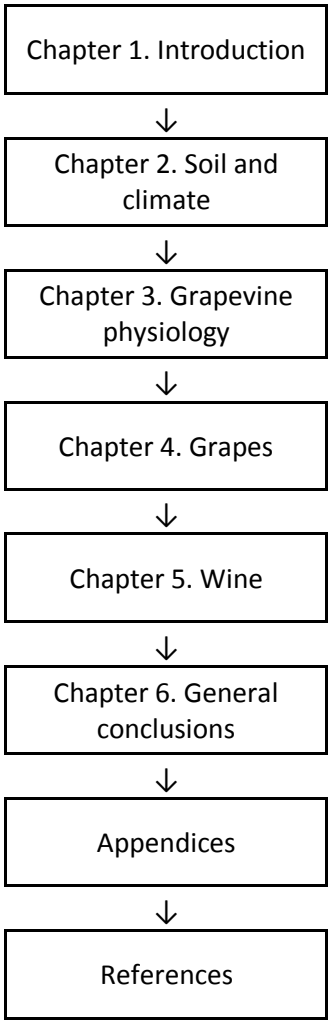


**Figure 1 Thesis structure**

## 1.2 Literature review

### New Zealand Pinot noir statistics

According to New Zealand Winegrowers (2015), the New Zealand productive vineyard area is over 35,000 hectares and nearly 326,000 tonnes of grapes were harvested during the 2015 vintage. The industry is dynamic, with the 2015 vintage being more than 250% of that in 2002. In terms of volume, 66% of the wine produced in New Zealand in 2015 was Sauvignon blanc, followed by Chardonnay with 8.3%, and Pinot noir with 8%, though the latter is the second most planted variety in New Zealand. The country exported 212 million litres of wine valued at $1.54 billion in 2015, which is nine times in volume and six times in value than the exports in 2002. The three major markets by value for the New Zealand wine in 2015 were USA (NZ$372 millions), Australia (NZ$ 362.1 million), and the UK (NZ$354 millions).

In the Waipara region there are 1,254 hectares of vineyards, which represents about 3.6% of the national producing area (New Zealand Winegrowers 2014). As reported by New Zealand Winegrowers (2014), nationwide there are 20,266 hectares planted with Sauvignon blanc (56.4%), followed by Pinot noir with 5,563 hectares (15.5%). In Waipara, the most planted variety is Pinot noir, where until 2014, 344 hectares of this variety were registered by the New Zealand Winegrowers (2014). The second and third most planted varieties in the region correspond to Sauvignon blanc and Riesling, respectively.

### Grapevine water stress physiology

Although grapevines are well adapted to semi-arid climates due to their large root system and mechanisms to deal with water scarcity; high evapotranspirative water loss and restricted water supply in many of the New Zealand wine regions have made vineyard irrigation an even more important viticultural practice. Irrigation prevents excessive canopy temperature, contributes to grapevine growth and guarantees plant survival in more extreme cases (Chaves *et al.* 2010). However, there has been an intense debate on the effect of water deficits on grapevine physiology, evidenced in the high variability of the results found in the literature (Lovisolo *et al.* 2010).

There are a series of factors influencing the grapevine responses to water deficit. Firstly, the timing and intensity of these are highly genotype-dependent (Chaves *et al.* 2010), caused difficulty when grapevine physiology under water stress since, especially as there are an estimated 10-20,000 cultivars of *Vitis vinifera* L. grown from 50° North latitude, through tropical to Mediterranean-type climates (Schultz 2003). In general, the grapevine is considered a “drought-avoiding” species, with efficient stomatal control over transpiration (Chaves *et al.* 2010, Schultz 2003), but varieties vary in

their ability to control stomata aperture under water stress. Thus, isohydric varieties (drought avoiders or "pessimistic") are those that would modify their physiology to conserve the current resources (Schultz 2003). These cultivars keep their leaf water potential steady, regardless of soil water availability or atmospheric water demand through a tight regulation of stomatal conductance (Hochberg *et al.* 2013a, Schultz 2003). In addition, Lovisolo *et al.* (2010) indicated that in isohydric grapevines, leaf water potential rarely drops to be more negative than -1.5 MPa, which is considered close to the threshold for severe cavitation. On the other hand, anisohydric varieties ("optimistic") use all the resources available, expecting more to be arriving as needed (Schultz 2003). These normally show lower control over stomatal aperture under water stress (Chaves *et al.* 2010), which has as a consequence a decrease of daytime leaf water potential (Poni *et al.* 2014). However, Lovisolo *et al.* (2010) showed that the same cultivar can behave as iso- or anisohydric depending on the environmental conditions. For example, Pinot noir behaves as anisohydric when water stress is applied pre-veraison and as isohydric when it is applied post-veraison (Lovisolo *et al.* 2010 and literature therein). In Chapter 3, the isohydric behaviour of Pinot noir under the conditions of this study will be discussed.

Abscisic acid (ABA) plays an important role in grapevine water stress. This is synthesised in the roots in response to water stress and transported through the xylem into the leaves, where it controls stomatal conductance (Lovisolo *et al.* 2010). Recently, Ferrandino and Lovisolo (2014) have indicated that ABA also plays a role on secondary metabolism and berry quality. Thus, exogenous ABA treatments at veraison have demonstrated that ABA plays a role in berry ripening by enhancing soluble solids and anthocyanin accumulation and decreasing organic acid concentration (Ferrandino and Lovisolo 2014, Medrano *et al.* 2015). In addition, Tramontini *et al.* (2014) described that ABA is involved in stimulating the synthesis of flavonoids, including anthocyanins, which are significant contributors to wine quality.

Proline is another organic solute accumulated in grapevines under abiotic stress. This compound acts as osmotic regulator between the cytoplast and vacuole, protecting membrane integrity and stabilizing antioxidant enzymes (Ozden *et al.* 2009). Additionally, proline has other physiological functions, acting as antioxidant and energy source (Deluc *et al.* 2009). However, inconsistent results are reported in the literature with respect to the importance of proline in osmotic adjustment in grapevines under water stress. For example, Hochberg *et al.* (2013b) in grapevine leaves and Deluc *et al.* (2009) in berries have reported that although proline concentration increased under water deficit, its contribution to osmotic adjustment compared to inorganic ions was relatively small. This was confirmed by Patakas *et al.* (2002), who found that the osmotic adjustment in stressed plants was due to the accumulation of inorganic ions such as $Ca^{2+}$, $K^{+}$, and $SO_4^{2-}$, instead of proline and other amino acids. The authors also indicated that the energetic cost of osmotic adjustment using inorganic

ions is much lower than that of synthesising organic molecules in the cell for the same physiological function. This might be the reason why grapevines prioritize the use of inorganic ions instead of organic molecules to regulate osmotic adjustment under abiotic stress. Although proline has little role in osmotic adjustment, its concentration in leaves and berries will be used as an indicator to detect physiological changes under reduced irrigation.

On the other hand, photosynthesis in grapevines has been shown to be quite resilient to water stress and dependent on the diffusion pathways of $CO_2$ (Medrano *et al.* 2015 and literature therein). As previously described, water stress induces stomatal closure which, in theory, should decrease photosynthesis rate, but the results found in the literature are not conclusive. Water use efficiency (WUE) refers to the balance between production (kg of biomass produced or moles of $CO_2$ assimilated) and water cost ($m^3$ of water used or moles of water transpired) (Tomás *et al.* 2014). This has been widely used to quantify the effect of different irrigation strategies on grapevine physiology, but since this does not describe the whole canopy stomatal conductance behaviour during an entire day, this may be used only as a reference (Medrano *et al.* 2015 and literature therein). For this reason, a more integrated measurement to evaluate long-term effect of water stress on carbon assimilation has been proposed. Carbon isotope discrimination ($\delta^{13}C$) constitutes a good integrative parameter that provides information about grapevine water status through the season, instead of a snapshot as other vine water stress indicators such as stem and leaf water potential (Santesteban *et al.* 2015). This is well explained in Van Leeuwen *et al.* (2010, p. 94):

> *"Ambient atmospheric $CO_2$ contains 98.9% of $^{12}C$ isotope and 1.1% of $^{13}C$ isotope.$^{12}C$ is more easily used by the enzymes of photosynthesis for hexose production. Therefore, the sugar produced by photosynthesis contains a higher proportion of the $^{12}C$ isotope than ambient $CO_2$. This process is called "isotope discrimination". When plants face water deficit conditions, isotope discrimination is reduced because of stomatal closure. Therefore, the $^{13}C/^{12}C$ ratio in photoassimilates provides a signature of plant water status over the period in which they were synthesised. When measured on grape sugar at ripeness, the $^{13}C/^{12}C$ ratio (so-called δ13C) indicates average vine water status during grape ripening."*

Both leaf and berry sugar $\delta^{13}C$ will be used here to determine long term effects of water stress on Pinot noir vine physiology.

Water stress can induce a series of other physiological changes that can directly or indirectly alter grape characteristics, and therefore, wine composition. Pellegrino *et al.* (2014) indicated that among other physiological effects, reduced irrigation slows canopy development and decreases berry size, as well as causing a reduction in total leaf area and photosynthesis rate. The same authors also described that water deficit combined with high temperatures may induce leaf senescence, and a reduction in the photosynthesis rate, thus resulting in a reduction in carbohydrate supply. Part of

those carbohydrates are accumulated in the berries, which are a strong carbohydrate sink after veraison (Hale and Weaver 1962, Williams 1996). Therefore, a reduction in photosynthesis rate should slow berry sugar accumulation. For example, Ginestar *et al.* (1998) attributed the lower Brix of berries under water stress found in their research to a reduction in photosynthesis rate.

Well-exposed bunches, which can occur as a consequence of defoliation in the cluster zone due to water stress, may induce changes in grape phenolic compound concentrations. For example, Rustioni *et al.* (2011) reported that grapes in a fully-exposed treatment had higher anthocyanin concentrations than those that were shaded. However, the relationship between bunch exposure to sunlight and grape tannin concentration is not linear. A higher cluster exposure may increase the berry temperature to levels at which some metabolic processes are inhibited (Spayd *et al.* 2002). Thus, anthocyanin production increases up to an optimum berry temperature of 30°C, whereas this is inhibited above 35°C (Kliewer 1977, Spayd *et al.* 2002). Therefore, any treatments that influence defoliation and therefore fruit exposure, may be altering fruit composition.

In addition, bunch exposure to sunlight, either by canopy manipulation or water stress, has shown to affect aroma compound synthesis, which has a direct impact on wine sensory characteristics. Although Chaves *et al.* (2010) indicated that little research has been developed in this area, some data can be found in the literature. For example, reduced vine water status has been described as affecting carotenoid and norisoprenoids (precursors to aroma compounds) in cv. Touriga nacional (Oliveira *et al.* 2003). Similar results are reported in the reviews of Chaves *et al.* (2010) and Robinson *et al.* (2014), where the results of a series of studies show an increase of norisoprenoids concentration in grapes grown under water deficit. The literature is not always in agreement, however, with Qian *et al.* (2009) indicating that deficit irrigation had no effect on esters and terpenes concentration in cv. Merlot. Water deficit was also not well correlated with levels of 3-isobutyl-2-methoxy pyrazine in Cabernet Sauvignon wine (Robinson *et al.* 2014 and literature therein).

Literature about how water stress affects grapevine physiology shows it to be variety-dependent, as well as highly related to the intensity and timing of the stress. However, the place where the vineyard is planted also has an influence on the intensity of this effect. This will be reviewed below.

## Influence of place of origin on wine characteristics

As previously reviewed, water stress plays a key role in determining grape and wine characteristics, but the place where the vineyard is planted also has an influence. Regional and local differences have been described in the literature, showing the significance of the place of origin (mainly regarding soil type), even under similar climatic conditions. Among the wine producing countries, Canada highlights as being prolific in generating information about regional differences among its wines. Thus, Cliff and Dever (1996) evaluated the sensory and compositional profiles of Chardonnay and Pinot noir wines

from British Columbia, concluding that under the methodology used in their research, it is possible to differentiate wines from different parts of the same region and different vintages. Douglas *et al.* (2001) characterised Riesling wines from the Niagara Peninsula. They were able to, using univariate and multivariate statistics, distinguish between wines produced in two different locations. Wines from Niagara Peninsula were also characterised by Schlosser *et al.* (2005), who differentiated Chardonnay wines produced in three different places within that region. They found differences among the wines in parameters like TA, pH, colour intensity, aroma compounds, flavour and mouthfeel. Those differences, according to the authors, were sufficient to propose sub-appellations within Niagara Peninsula. Cabernet franc wines from that wine region were also analysed by Hakimi-Rezaei and Reynolds (2010), who found that wines from Harbour and Georges sites (both situated on the Lake Ontario shore-line) were clearly different from other wines using sensory and chemical analyses. From the same region, 41 Bordeaux-style wines were characterised by Kontkanen *et al.* (2005) to try to support the designation of three sub-appellations in the Niagara Peninsula. Results of the chemical and sensory analyses established significant regional differences among the wines. These experiences demonstrate the importance of the place where the vines are growing in and the significance of characterising the differences between them. They also demonstrate an opportunity for the Waipara region, where the area has not been so well characterised.

In Australia, Bramley and Hamilton (2004) and Bramley (2005) evaluated vineyard variability in yield and quality over several vintages in Coonawarra, South Australia. They found marked differences in yield through time and space at all the three sites evaluated. Also, parameters like pH, TA, anthocyanins, phenolics, and berry weight showed considerable inter-annual variations, with phenolics being one of the most variable season by season. In this study, spatial variations between three sites, as well as differences between seasons will be analysed.

In France, specifically in the Rhone Valley, Sabon *et al.* (2002) evaluated the volatile compound profile in Grenache wines in relation with the place where the grapes were grown. Their findings suggested that volatile composition may be an indicator of the origin of those wines. In other research, Van Leeuwen *et al.* (2004) determined the influence of climate, soil, and cultivar on vine development and grape composition of cv. Merlot, Cabernet franc, and Cabernet Sauvignon in Saint-Émilion. Data obtained in that study indicated that the effect of climate was greatest on most parameters evaluated, followed by soil and cultivar. The authors also reported that the effect of climate and soil on vine development and grape composition can be explained in large part by their influence on vine water status.

In Italy, Costantini *et al.* (2012) characterised the "Vino Nobile di Montepulciano" wine territory to try to understand the relationship between the soil and the viticultural and oenological behaviour of

the Sangiovese variety. After a detailed soil survey, the study determined that there is a strong link between the grape characteristics and the type of soil where they grew. The type of soil influenced parameters like berry weight and the organoleptic characteristics of the wines. Constantini *et al.* (1996) also reported similar results when they characterised the cv. Prugnolo gentile from the same wine region. The soils analysed in that research had large differences in yield components like cluster number and cluster weight. In addition, wines from different types of soil showed differences in wine organoleptic profiles. In the Cembra and Adige valleys of Italy, Falcetti and Iacono (1996) reported differences in sugar content, titratable acidity and organoleptic profile among Chardonnay wines produced in different locations. Also, the study reported that differences in canopy development and yield per plant, which may directly or indirectly influence wine characteristics, were altered by the soil type the vines were growing in.

The U.S. has also some examples of wine characterisation by site. Thus, Guinard and Cliff (1987) described the differences between Pinot noir wines from Carneros, Napa, and Sonoma in California by descriptive analysis. The results showed that Carneros wines differed from Napa and Sonoma wines using a principal component analysis. Andrews *et al.* (1990) studied sixteen Missouri Seyval blanc wines (a French-American hybrid grape variety) and showed significant differences among the wines using chemical and sensory analyses. Burns (2012) showed marked differences between two Pinot noir wines produced on two different soils located in Willamette Valley, Oregon. The one from Jory soil series (basalt) produced a wine that was light red in colour, strong bouquet, and flavours of red cherries, raspberries, red plums, and red currants, whereas Willakensie soil series (marine sediment) produced a wine dark red in colour, strong finish, and fruit flavours of dark cherries, blackberries, and black plums. This demonstrated the influence of soil characteristics on Pinot noir wine composition, even under similar climatic conditions.

Some efforts to try to understand New Zealand wine production as influenced by the site have been made in the past. For instance, Imre and Mauk (2009), using geological, climatic, and production data, reported valuable information for the understanding of most the wine regions of the country. The study highlighted that regions such as Marlborough, Central Otago, Waipara and Wairarapa are climatically comparable to regions that produce premium Pinot noirs such as Beaune, Burgundy, Côte d'Or and most Pinot noir regions in North America. However, they concluded that soil needs further research to understand its influence on New Zealand Pinot noir quality. In other research, Hawke's Bay Cabernet Sauvignon was characterised to try to identify differences among a series of locations within that wine region. The study reported differences in vegetative development, yield components, fruit ripening, and wine organoleptic profile, establishing differences between sub-regions within Hawke's Bay (Tesic *et al.* 2002a, Tesic *et al.* 2002b). Trought and Bramley (2011) and Bramley *et al.* (2011) used some tools of precision viticulture to characterise vineyard variability in

Marlborough, where soil electrical conductivity surveys were found to be a good tool to determine vineyard variability due to the values of soil electric conductivity being closely correlated with vine trunk circumference, and juice °Brix, TA and pH. Imre (2011) quantified soil characteristics and viticultural parameters in Central Otago and Waipara Pinot noir vineyards. The research demonstrated a link between spatial variations in soil electrical conductivity and trunk circumference. Tomasino (2011) found organoleptic and chemical differences among commercial Pinot noir wines from Central Otago, Martinborough, Marlborough and Waipara by canonical variate analysis (CVA). Such methodology will be adapted here to differentiate wines between treatments and sites. Imre *et al.* (2012) studied the influence of soil geochemistry on the chemical and aroma profiles of Pinot noir wines produced at three different vineyards in Central Otago, finding differences in tannin content and concentrations of volatile aroma compounds in wines made during the 2008 season. Recently, Rutan *et al.* (2014) characterised the aroma composition of Central Otago Pinot noir reporting differences between wine categories and vintages for a series of volatile aroma compound concentrations. However, they concluded that, overall, Central Otago Pinot noir wines do not depend on few key aroma compounds for their aromatic complexity, but instead on the interaction of many aromatic compounds.

Literature reporting aroma compound concentrations in New Zealand Pinot noir is not abundant, therefore, the results in Tomasino (2011), Imre *et al.* (2012) and Rutan *et al.* (2014) will be used as a reference to compare the findings reported here. The information of these three studies is summarised in Table 1.

**Table 1 Range of concentrations for aroma compounds recently found in Pinot noir wine from different regions in New Zealand. All results are reported in µg/L**

| Aroma compound | Perception threshold | Olfactory description | Waipara | Central Otago | | | Marlborough | Martinborough |
|---|---|---|---|---|---|---|---|---|
| | | | Tomasino (2011) | Imre *et al.* (2012) | Rutan *et al.* (2014) | Tomasino (2011) | Tomasino (2011) | Tomasino (2011) |
| *Acids* | | | | | | | | |
| 2-Methylbutanoic acid | 3,000 [a] | Cheese | nr | nr | nr | nr | nr | nr |
| Acetic acid | 200,000 [a] | Vinegar | 349,000 - 702,000 | nr | nr | 415,000 - 690,000 | 553,000 - 874,000 | 516,000 - 707,000 |
| Butanoic acid | 10,000 [a] | Cheese | 290 - 716 | nr | 1,026 – 1,845 | 209 - 755 | 314 - 562 | 325 - 715 |
| Hexanoic acid | 3,000 [a] | Sweat, cheese | 1,141 - 1,941 | 640 - 680 | 712 – 1,217 | 1,104 - 1,744 | 1,142 - 1,497 | 1,169 – 1,700 |
| Isobutyric acid | 2,300 [b] | Rancid | nr | nr | 389 - 895 | nr | nr | nr |
| Isovaleric acid | 33.4 [c] | Parmesan, sweat | nr | nr | 275 - 665 | nr | nr | nr |
| Octanoic acid | 500 [b,c] | Fatty, rancid | 665 – 2,002 | 1,300 – 1,700 | 911 – 1,302 | 760 – 1,157 | 724 – 1,092 | 726 – 1,067 |
| *Alcohols* | | | | | | | | |
| 1-Heptanol | 2,500 [h] | Herbal, leafy, green | 19.3 – 270.3 | nr | nr | 25.0 – 246.8 | 30.0 – 115.9 | 12.2 – 171.0 |
| *cis*-3-Hexen-1-ol | 400 [a, b, c] | Cut grass, leafy | 39.2 – 115.8 | 35.7 – 42.4 | 22 - 43 | 24.2 – 82.4 | 30.0 – 64.6 | 33.6 – 65.4 |
| Hexanol | 8,000 [a, b, c] | Toasted, green | 2,000 – 4,700 | 568 - 607 | 809 – 1,272 | 2,400 – 3,700 | 2,300 – 3,300 | 1,900 – 3,500 |
| Isoamyl alcohol | 30,000 [b, c] | Fusel, alcoholic | nr | nr | 104,295 – 150,538 | nr | nr | nr |

| Aroma compound | Perception threshold | Olfactory description | Waipara | Central Otago | | | Marlborough | Martinborough |
|---|---|---|---|---|---|---|---|---|
| | | | Tomasino (2011) | Imre *et al.* (2012) | Rutan *et al.* (2014) | Tomasino (2011) | Tomasino (2011) | Tomasino (2011) |
| Phenylethyl alcohol | 14,000 [c] | Floral, rose | nr | nr | 68,719 – 134,980 | nr | nr | nr |
| *trans*-3-hexen-1-ol | 8,000 [f] | Vegetable | 56.9 – 107.9 | 9.6 – 12.2 | 18 - 35 | 66.1 – 126.5 | 72.0 – 123.6 | 60.5 – 92.6 |
| *Esters* | | | | | | | | |
| 2-Phenylethyl acetate | 250 [a] | Fruity, floral, honey | nr | 11.8 – 12.3 | 11.6 – 18.1 | nr | nr | nr |
| Ethyl acetate | 12,270 [b] | Sweet fruity | nr | nr | nr | nr | nr | nr |
| Ethyl butanoate | 20 [a, b, c] | Fruity, strawberry | 134.9 – 271.0 | 30.0 – 32.4 | 75 - 153 | 116.4 – 289.4 | 164.8 – 286.2 | 165.8 – 339.9 |
| Ethyl cinnamate | 1.1 [b, c] | Fruity, cherry, plum | 0.8 – 3.0 | 0.36 – 0.71 | 1.6 – 4.1 | 1.8 – 3.1 | 0.8 – 2.8 | 1.2 – 7.2 |
| Ethyl decanoate | 200 [b,c] | Fruity, waxy | 171.9 – 940.3 | 17 - 23 | 164 - 207 | 190.3 – 971.3 | 259.0 – 518.5 | 154.2 – 629.5 |
| Ethyl heptanoate | 220 [g] | Fruity, pineapple | 3.1 – 9.2 | nr | nr | 3.6 – 8.1 | 2.6 – 4.0 | 3.2 – 5.9 |
| Ethyl hexanoate | 14 [b, c] | Fruity, strawberry | 299.3 – 559.4 | 41 - 45 | 312 - 372 | 320.7 – 557.4 | 334.9 – 409.9 | 339.2 – 593.8 |
| Ethyl hydrocinnamate | 1.6 [b, c] | Fruity, balsamic | nr | 10.3 – 11.5 | 1.11 – 2.31 | nr | nr | nr |
| Ethyl isobutyrate | 15 [c] | Fruity, sweet | nr | nr | 25 - 54 | nr | nr | nr |
| Ethyl isovalerate | 3 [c] | Fruity, sweet | nr | nr | 27 - 49 | nr | nr | nr |
| Ethyl lactate | 154,000 [b] | Lactic, raspberry | nr | nr | 134,921 – 191,724 | nr | nr | nr |
| Ethyl octanoate | 580 [b] | Sweet, fruity | 442.6 – 874.3 | 60.1 – 69.5 | 318 - 384 | 415.6 – 763.6 | 437.8 – 598.0 | 410.2 – 642.5 |
| Ethyl pentanoate | 1.5 [e] | Fruity, orange | 1.6 – 3.1 | nr | nr | 1.1 – 4.3 | 1.3 – 3.1 | 1.4 – 3.4 |
| Hexyl acetate | 1,500 [d] | Fruity, green apple | nr | 9.4 – 9.9 | 10.6 – 18.6 | nr | nr | nr |

| Aroma compound | Perception threshold | Olfactory description | Waipara | Central Otago | | | Marlborough | Martinborough |
|---|---|---|---|---|---|---|---|---|
| | | | Tomasino (2011) | Imre *et al.* (2012) | Rutan *et al.* (2014) | Tomasino (2011) | Tomasino (2011) | Tomasino (2011) |
| Isoamyl acetate | 30 [b, c] | Banana, pear | 148.5 – 244.4 | nr | 189 - 254 | 160.0 – 377.5 | 216.7 – 370.2 | 151.3 – 297.9 |
| *Monoterpenes, norisoprenoids and aldehydes* | | | | | | | | |
| Citronellol | 100 [a, b] | Citronella | nr | 1.7 – 2.3 | 6.9 – 11.1 | nr | nr | nr |
| Geraniol | 20 [b] – 30 [a, c] | Floral, fruity, citrus | 0 – 4.8 | 13 - 26 | 12.4 – 16.2 | 0 – 3.3 | 0 – 2.4 | 0 – 2.1 |
| Linalool | 15 [a], 25 [b], 25.2 [c] | Citrus, orange, floral | 77.1 – 170.1 | 1.2 – 1.4 | 2.25 – 5.37 | 41.4 – 146.6 | 84.5 – 167.2 | 62.5 – 142.8 |
| β - Damascenone | 0.05 [a, b, c] | Rose | 0.7 – 3.3 | 4.8 – 5.6 | 4.0 – 5.4 | 1.0 – 4.4 | 1.6 – 3.4 | 0.6 – 3.7 |
| β - Ionone | 0.09 [b, c] | Berry, violets | 0.1 – 0.5 | 0.19 – 0.21 | 0.29 – 0.42 | 0.3 – 0.6 | 0.3 – 0.7 | 0.1 – 0.6 |
| Benzaldehyde | 2,000 [i] | Almond, sweet | 10.8 – 66.0 | nr | 10.2 – 18.6 | 5.1 – 11.0 | 7.7 – 39.3 | 10.2 – 32.5 |

nr: not reported

[a] Guth (1997); [b] Escudero *et al.* (2007); [c] Ferreira *et al.* (2000); [d] Li *et al.* (2008); [e] Genovese *et al.* (2007); [f] Dunlevy *et al.* (2009); [g] Zea *et al.* (2001); [h] Ferreira *et al.* (2000) from Tomasino (2011); [i] Escudero *et al.* (2007) from Rutan *et al.* (2014).

## 1.3 Research objectives and hypotheses

It was expected that soil characteristics, especially soil profile available water, would vary among the three sites chosen for this research. Also, mesoclimatic differences would be found within the Waipara area, as well as microclimatic differences among the sites. Thus, the objectives of this research were:

- To identify mesoclimatic variations within the Waipara region, and also to characterise microclimatic differences across the three sites selected for this research.

- To evaluate the physiological effects of reducing irrigation about 50% in three commercial Pinot noir vineyards in Waipara over two seasons.

- To quantify the impact of a 50% reduction in the irrigation applied by the viticulture managers' on berry characteristics during two seasons in three commercial Pinot noir vineyards in Waipara.

- To characterise the differences between wines made from grapes harvested from vines under reduced irrigation and those normally irrigated in three Pinot noir vineyards in Waipara over the 2013-2014 season.

Specifically, the following hypotheses were formulated in after consideration of an appropriate experimental design:

It was hypothesised that a 50% reduction of the irrigation normally applied to the vineyards would affect plant water status variables, such as stem water potential and stomatal conductance, as well as indirect plant water status indicators, like leaf proline content, leaf osmotic potential and leaf carbon isotope ratio ($\delta_{13}C$). Canopy structure and leaf area, and therefore fruit exposure, were also hypothesised to be affected by reduced irrigation.

Reduced irrigation was also hypothesised to affect fruit parameters, such as berry weight, Brix, pH, titratable acidity (TA), as well as skin and seed phenolics. Changes in berry carbon isotope ratio ($\delta_{13}C$) and amino acids content were also hypothesised to be found due to the soil and reduced irrigation.

The differences in berry characteristics due to reduced irrigation were hypothesised to drive changes in wine composition. Thus, it was expected to find differences in wine pH, TA, total phenolics and tannin concentration, wine colour and aroma compounds concentration.

Finally changes in grapevine physiology, grape ripening and wine composition attributable to differences in water availability and observed as a result of a reduction in irrigation were

hypothesised also to be reflected in differences in these same parameters between sites in line with the differing water-holding characteristics (i.e. soil water content and soil water potential) of the soils.

# Chapter 2

# Study sites and experimental design

## 2.1 Introduction

Worldwide, the main wine regions have been carefully characterised aiming to understand the differences between them (Jones *et al.* 2009). However, studies comparing climatic differences within a specific region are more difficult to find in the literature. One of the few examples of this is the study conducted by Dumas *et al.* (1997), who characterised the variations within the Alsatian region in France, finding marked climatic differences across the region. Their conclusions indicated that the differences in altitude and exposure of the hills constituted the main factors to explain the climatic differences within the region.

Some of the effects of the different climatic parameters on grapevine physiology is known. Ubalde *et al.* (2007) evaluated the influence of edapho-climatic factors (such as soil characteristics, temperatures, rainfall, and solar radiation) on parameters like crop load, grape pH and total acidity, and anthocyanin content in Catalonia, Spain. Their results indicated that among all the factors evaluated, climate appeared to be the most important one. Several authors have also underlined the importance of the role of climate in characterising a terroir, indicating that a specific terroir is mainly defined by its soils and climatic characteristics (Bohmrich 1996, Dougherty 2012, Jones 2006, Van Leeuwen 2009).

Prior to this study, a series of Pinot noir vineyards in Waipara were visited and three were selected where vines were grown under similar viticultural conditions, but in different soils types. In New Zealand, spatial climatic variations have received little study. Only the main regions of the country have been characterised as macro-climatic zones by National Institute of Water and Atmospheric Research (NIWA) Ltd. (2013), with a clear lack of information for the South Island. So, information about Waipara is almost non-existent in the literature.

The purpose of this chapter is to present mesoclimatic variations within the Waipara region, and also to characterise edapho-climatic differences across the three sites selected for this research. Furthermore, the sites chosen were on the three most representative soil families in the Waipara region. They allowed a detailed study on the effects of water availability at each site to be carried out. The climate and soil water data will be referenced in later chapters to explain potential variations in plant physiology, grape composition and wine composition among the three vineyards. In addition, by comparing and contrasting the results from irrigation treatments at each site, which

encompass both temperature, wind and especially soil water differences, it was possible to obtain a broad integration of the contribution of various factors which affect vine performance and grape ripening, often loosely combined in the term terroir, and hence on wine composition.

This chapter does not contain statistical analyses as its main objective was to characterise the soil and environmental characteristics of Waipara over the two seasons of study.

## 2.2 Study sites

### 2.2.1 Viticulture

Three commercial blocks of Pinot noir (*Vitis vinifera* L.) located in Waipara, North Canterbury, were selected for study during the 2013-2014 and 2014-2015 seasons. These were The Mound vineyard, owned by Waipara Hills (WH), Greystone block 5 (GB5) and block 10 (GB10), which belong to Greystone Wines. A brief description of each block is presented in Table 2.

**Table 2 Viticultural parameters relevant to each study site**

| | Waipara Hills | Greystone block 5 | Greystone block 10 |
|---|---|---|---|
| Clone | 115 | 115 | 115 |
| Rootstock | 3309 | 101-14 | 101-14 |
| Row spacing | 3 m | 2.5 m | 2.5 m |
| Vine spacing | 1.8 m | 1.6 m | 1.6 m |
| Year of plantation | 2003 | 2004 | 2005 |
| Trellis system | Vertical Shoot Positioned (VSP) | VSP | VSP |
| Pruning system | Three canes | Spurs | Spurs |
| Elevation m.a.s.l. (metres above sea level) | 79 | 100 | 157 |
| Location | 43°04’29.32” S 172°44’14.89” E | 43°03’30.14” S 172°47’11.26” E | 43°03’22.11” S 172°47’44.88” E |
| Row orientation (by GPS) | North | North, 15° West | North, 19° East |

### 2.2.2 Soils

The soil at WH belongs to the Glasnevin soil family. This is a typic immature Pallic soil according to the New Zealand Soil Classification (NZSC) with an alluvial parent material origin (Landcare Research New Zealand 2015). Glasnevin is a common rounded stony soil that comprises rounded stones of greywacke sandstone with a rare glauconitic sandstone and rare weathered ghosts of calcareous mudstone (marl) and limestone, as described in Tonkin *et al.* (2014). This soil does not have a significant rooting barrier within 1 m. The profile available water at different depths has been

described as moderate, with 42.7 mm of available water being reported from 0-30 cm, 64.3 mm from 0-60 cm, and 81.4 mm from 0-100 cm (Landcare Research New Zealand 2015). The data reported in Tonkin *et al.* (2014) describe Glasnevin soil as having pH near 5.3 from 0 to 50-55 cm depth, which gradually increases up to pH 6.1 at 1.2 m depth. The cation exchange capacity (CEC) (me/100 g) also tends to increase toward the deeper horizons, starting at about 5.2 at 15 cm depth and reaching nearly 7.9 at 1 m depth.

GB5 was planted on an Omihi soil, a mottled-calcareous Vertic Melanic soil, described as having a clayey texture and developed from soft calcareous rocks (Landcare Research New Zealand 2015). Omihi soils have developed in alluvium derived from limestone, marls, calcareous sandstone, and glauconitic sandstone. This type of soil has a clay loam horizon of about 30 cm depth that is strongly structured, overlying a clay loam to clay textured argillic horizon that contains swelling clays. In the lower part of the soil profile, there is an accumulation of secondary and nodular calcium carbonate (Tonkin *et al.* 2014). As for Glasnevin soils, Omihi has been described as having no significant barrier within 1 m. The profile of available water in this soil has been reported as high and very high depending on the depth. Thus, soil available water from 0-30 cm was reported as high with 68 mm, whereas from 0-60 cm this was described as very high with 121 mm, and from 0-100 this is high with 178 mm (Landcare Research New Zealand 2015). It is important to highlight that the profile of available water reported in the literature for this type of soil represents more than double as that described at WH, especially those from 0-60 and 0-100 cm. As part of a soil study carried out by the Lincoln University Soils department at Greystone vineyard in 2014, a pit was dug near GB5 and samples from different horizons were collected and chemically analysed. Soil pH was near 6.5 up to 60 cm depth, gradually increasing to between 8.0 at 80 cm depth and 8.5 at 140 cm depth. The total CEC (me/100 g) also showed higher values at deeper depth (ranging from 29 at 10 cm to 46 at 140 cm depth), following the same trend as soil pH (Tonkin *et al.* 2014).

As described in the report prepared by the Lincoln University soil resources class (2014), the soil at GB10 was similar to an older soil series known as Hui Hui, but the morphological contrasts between the soil found at GB10 and those previously described for the Hui Hui series, established a new family designated Greystone, now updated in the New Zealand soil database (Landcare Research New Zealand 2015). The Greystone soils are classified as Weathered Rendzic Melanic soils originated from soft calcareous rocks (Landcare Research New Zealand 2015). This family of soils consist in very dark, well drained Melanic soils with an argillic horizon overlying a shallow to moderately deep paralithic limestone contact (Lincoln University soil resources class 2014) As reported by Landcare Research New Zealand (2015) this type of soil has a potential rooting depth between 35 and 55 cm, due to a rooting barrier of fracturing rock. This has also similar values of profile available water as those at GB5, with values classified as high and very high. From 0-30 cm this has 69 mm of soil available

water, whereas this is 115 mm from 0-60 cm, which is classified as very high. As for GB5, the values of soil available water are higher than those at WH. Only a small amount of data are available about the chemistry of this new family of soils. Data reported by Niklas Lehto (Lincoln University Soil Department, unpublished data) describe some values of pH and other chemical analyses up to 80 cm depth for this plot. His results indicate that soil pH ranged from 5.6 to 6.0 in the first 40 cm, whereas this was near 5.8 from 40 to 80 cm depth. The cation exchange capacity reported from a composite sample was 12 me/100 g, which is lower than that at GB5, but higher than that found at WH.

### 2.2.3 Mesoclimate

#### Weather stations

Data from three weather stations were used to characterise climatic differences within the Waipara region. The "Waipara West EWS" weather station was selected from NIWA database (NIWA, 2014) as a reference. During the time in which this research was carried out, this was the only weather station of the national climatic network permanently registering information in the Waipara region.

Each vineyard also has its own weather station, which were part of a private network managed by Harvest Electronics Ltd. (www.harvest.com). The two weather stations selected were "The Mound", located in the same vineyard as the Waipara Hills plot, and "Muddy Water", which was in the next vineyard located further north of Greystone and owned by the same company.

#### Analyses

A mesoclimatic analysis was carried out to determine the differences between the West (Waipara West EWS), middle (The Mound), and East part of the valley (Muddy Water). A five year average (5YA) was calculated monthly from September to April (deemed the growing season), based on data retrieved from the national climatic database from 2009 to 2013 (NIWA, 2014) for the Waipara West EWS weather station.

The first analysis consisted in calculating the monthly maximum, minimum, and mean temperatures, as well as reference evapotranspiration (ETo) (Allen *et al.* 1998) for each season, from data retrieved from Waipara West EWS only. These were compared to the 5YA, to identify seasonal variations with respect to the average of the 5 seasons prior to the study. Due to technical issues with the temperature sensor in October 2014, Waipara West EWS did not registered temperature data for that month, and therefore, maximum, minimum, mean temperatures, as well as GDD accumulation could not be obtained. However, for comparative purposes, ETo for October in 2014 was calculated as the average of those registered in September and November.

The Mound and Muddy Water could not be included in the previous analysis due to differences in the methodology used to calculate the maximum and minimum temperatures of the month by harvest.com. This company reported the maximum and minimum temperature of the month as the higher and lower absolute values of the period, which differs from the method used by NIWA. Moreover, the lack of radiation sensor of The Mound and Muddy Water weather stations did not allow the calculation of ETo from these.

The second analysis corresponded to the comparison between the West, middle, and East part of Waipara, for parameters like: Growing degree days (GDD), which were calculated using the equation proposed by Winkler *et al.* (1974), with a base temperature equal to 10°C for all the weather stations, as follows:

$$GDD= \left(\frac{Tmax-Tmin}{2}\right)-10$$

Monthly rainfall (mm/month) and mean wind speed (km/h) were also calculated for all the weather stations and compared.

### Maximum temperature

Differences between seasons, and also with respect to the 5YA were found for monthly mean maximum temperature (Figure 2). Overall, compared to the 5YA, 2013-2014 was characterised by higher maximum temperatures from September to February, decreasing towards the end of the season. Although technical issues did not allow Waipara West EWS to collect temperature data in October 2014, a clear trend can be observed showing that until December the maximum temperature remained close to the 5YA, this being higher during the ripening period (January to April). These were also higher than the maximum temperatures registered in 2013-2014 for the same period. Both 2013-2014 and 2014-2015 reached their highest maximum temperature of the season in January and February.

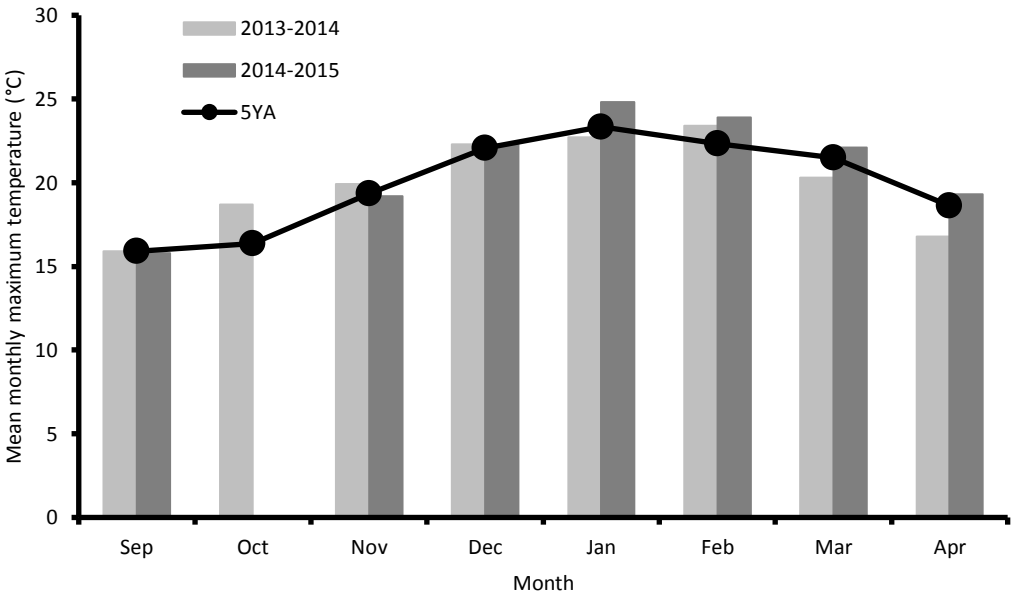


**Figure 2 Mean maximum temperature in Waipara during the 2013-2014 and 2014-2015 seasons, compared to the 5YA. Data from Waipara West EWS (NIWA)**

Furthermore, the 5YA was calculated for the entire growing season, and used for comparisons. The mean maximum temperature of the previous five seasons was 19.9°C, or 0.1°C lower than the mean registered in 2013-2014. Due to the lack of information in October 2014, a comparison of the last season was not possible.

## Minimum temperature

2013-2014 showed a higher mean minimum temperature than the 5YA until November, which dropped below the 5YA afterwards, with a slight rise in April (Figure 3). On the other hand, 2014-2015 minimum temperatures were below or near the 5YA until January, with an increase after, showing a similar trend as maximum temperature. For example, the minimum temperature in March 2014-2015 was 1°C higher than the 5YA, and 1.6° more than that registered in 2013-2014 for the same month.

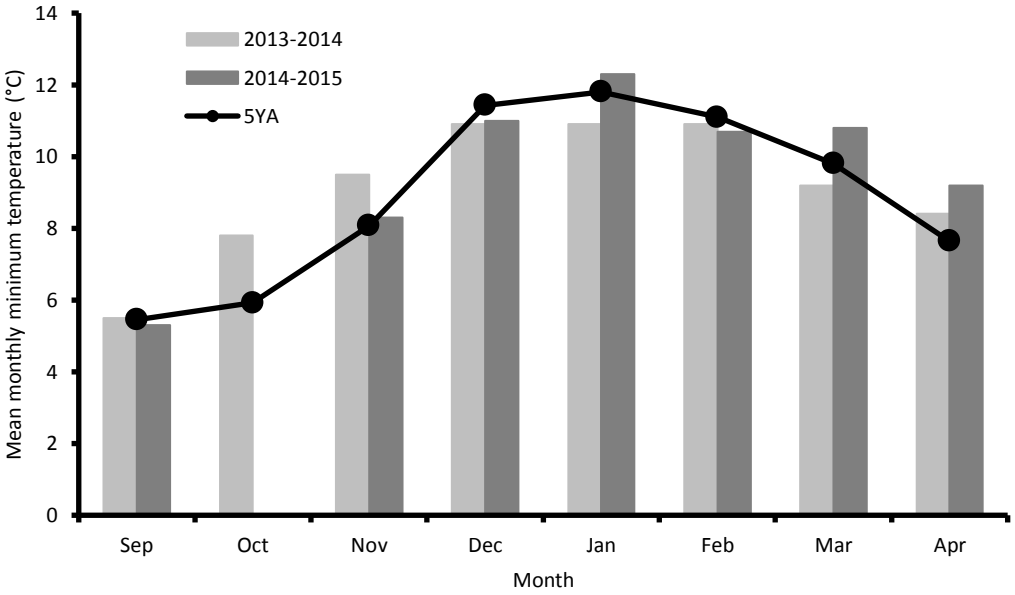


**Figure 3 Mean minimum temperature in Waipara during the 2013-2014 and 2014-2015 seasons, compared to the 5YA. Data from Waipara West EWS (NIWA)**

The mean minimum temperature of the 2013-2014 season was 9.1°, or 0.2°C higher than the 5YA minimum temperature.

## Mean temperature

The 2013-2014 season showed a warmer start than the 5YA, but was cooler than the 5YA from January to April. In contrast, 2014-2015 started near the 5YA until December, with larger mean temperatures from January to April (Figure 4).

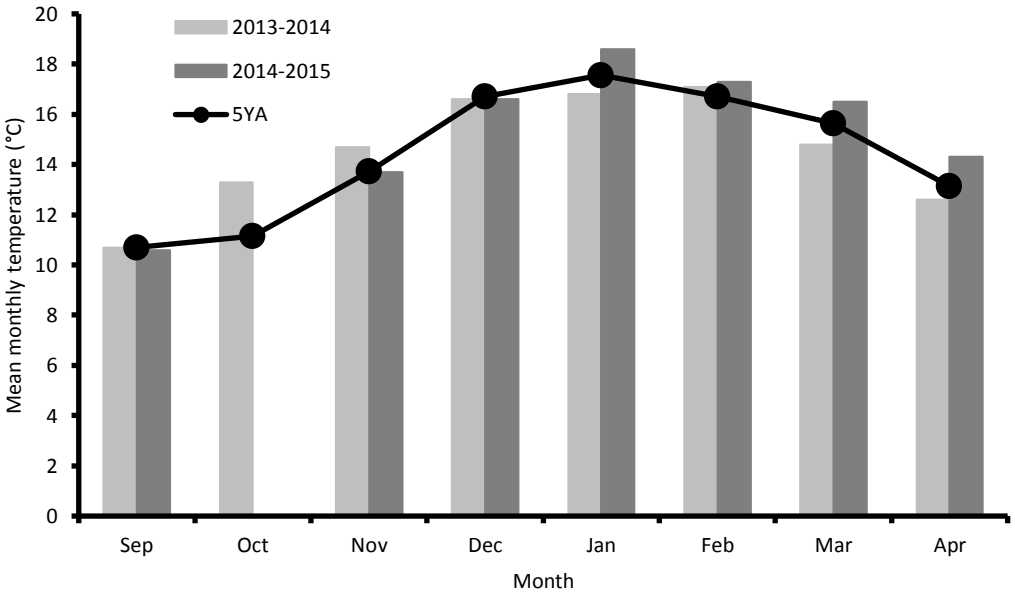


**Figure 4 Mean temperature in Waipara during the 2013-2014 and 2014-2015 seasons, compared to the 5YA. Data from Waipara West EWS (NIWA)**

January 2015 registered the highest mean temperature over the experimental period, 1°C higher than the 5YA and 1.8°C higher than January 2014. March and April were also warmer in 2014-2015 than the 5YA or 2013-2014

The ripening period (January to April) in 2014-2015 was one of the warmest registered in Waipara in the last 7 years.

### Reference evapotranspiration

As can be seen in Figure 5, the 5YA of reference evapotranspiration followed a similar pattern as the temperature data. Overall, 2013-2014 showed an ETo either lower or near the 5YA, except for October reporting 125 mm/month, or 16 mm more than the 5YA.

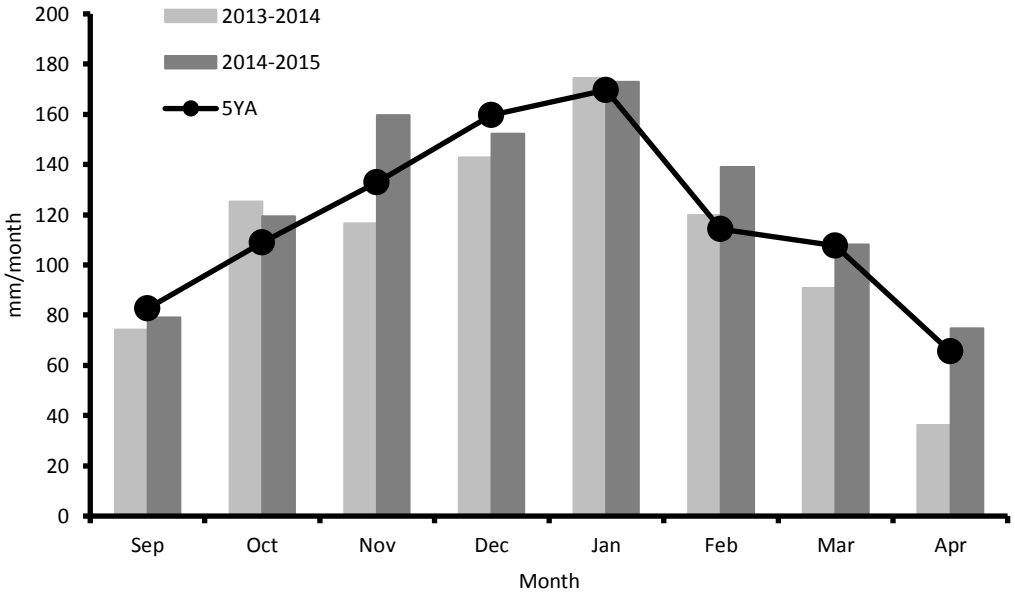


**Figure 5 Potential evapotranspiration in Waipara during the 2013-2014 and 2014-2015 seasons, compared to the 5YA. Data from Waipara West EWS (NIWA)**

From November to April in 2014-2015, ETo was much higher than the 5YA, which was also larger than the registered in 2013-2014 for the same period. These differences were reflected on the differentiated irrigation regimes applied by the viticulture's managers in both seasons (Table 4, Table 5, and Table 6).

A 5YA ETo of 941 mm/season was calculated from the data retrieved from NIWA from 2009 to 2013. The 2013-2014 reported an accumulated ETo of 881 mm/season, which is about 7% lower than the 5YA. On the other hand, the 2014-2015 season registered an ETo of 1005 mm/season, which is 64 mm higher than the 5YA.

## GDD accumulation

Figure 6 shows the seasonal variation in GDD accumulation across the three stations.

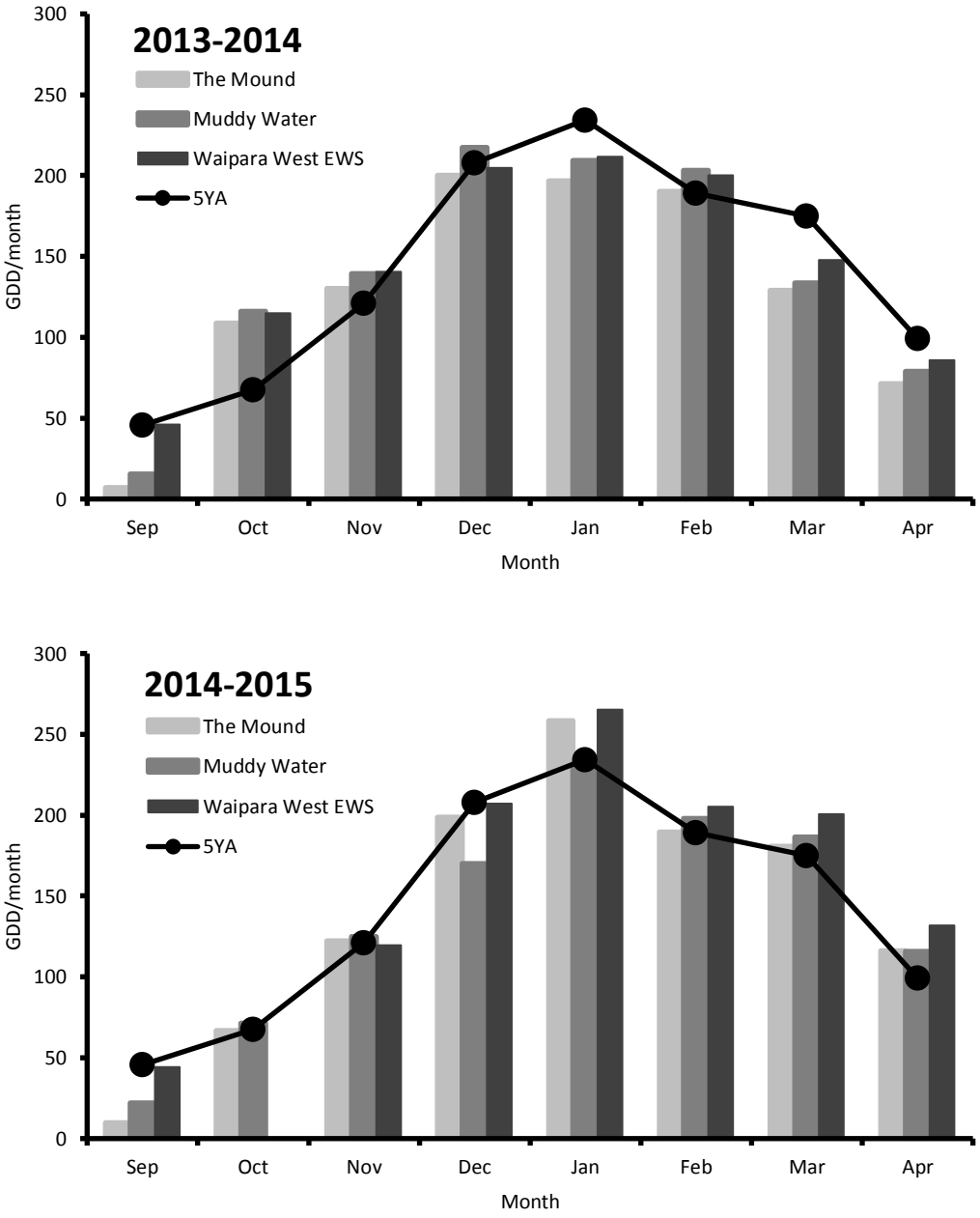


**Figure 6 Monthly GDD accumulation in Waipara during the 2013-2014 and 2014-2015 seasons, compared to the 5YA. Data from Waipara West EWS, the Mound, and Muddy Water**

In 2013-2014, September showed the first big difference between locations, in which the West part of Waipara accumulated an average of 37 GDD more than the middle and East parts. October and November were warmer than the 5YA, whereas in December only Muddy Water was higher. The Mound weather station (located near the Waipara Hills site) registered the lowest GDD accumulation

of the three weather stations during the entire period, lower even than the 5YA from December to April.

GDD accumulation remained either near or lower than the 5YA from September to December in 2014-2015 for all the weather stations. September in 2014-2015 also showed a larger GDD accumulation in the Waipara West EWS weather station, but the difference in 2015 was narrower. The period between December and January in 2015 showed clear differences between places, seasons, and with respect to the 5YA. The 265 GDD accumulated in December 2015 at Waipara West EWS was the highest registered in both seasons for all the weather stations, and about 30 GDD more than the 5YA for that month. The Mound (middle part of Waipara), as it did in 2014, showed the lower GDD accumulation during the ripening period (February to April).

The 5YA reported a total of 1139 GDD accumulated over the growing season. Waipara West accumulated 13 more GDD in 2013-2014 than the 5YA, while the other two stations registered lower numbers, with The Mound in excess of 100 GDD/season lower. GDD accumulation in 2014-2015 was closer to the 5YA at the Mound and Muddy Water.

## Rainfall

Figure 7 provides an overview of the variations in precipitations in Waipara. 2013-2014 was characterised by high amounts of rain registered in December, March, and April, the latter being about four times larger than the rainfall normally observed at that time of the year.

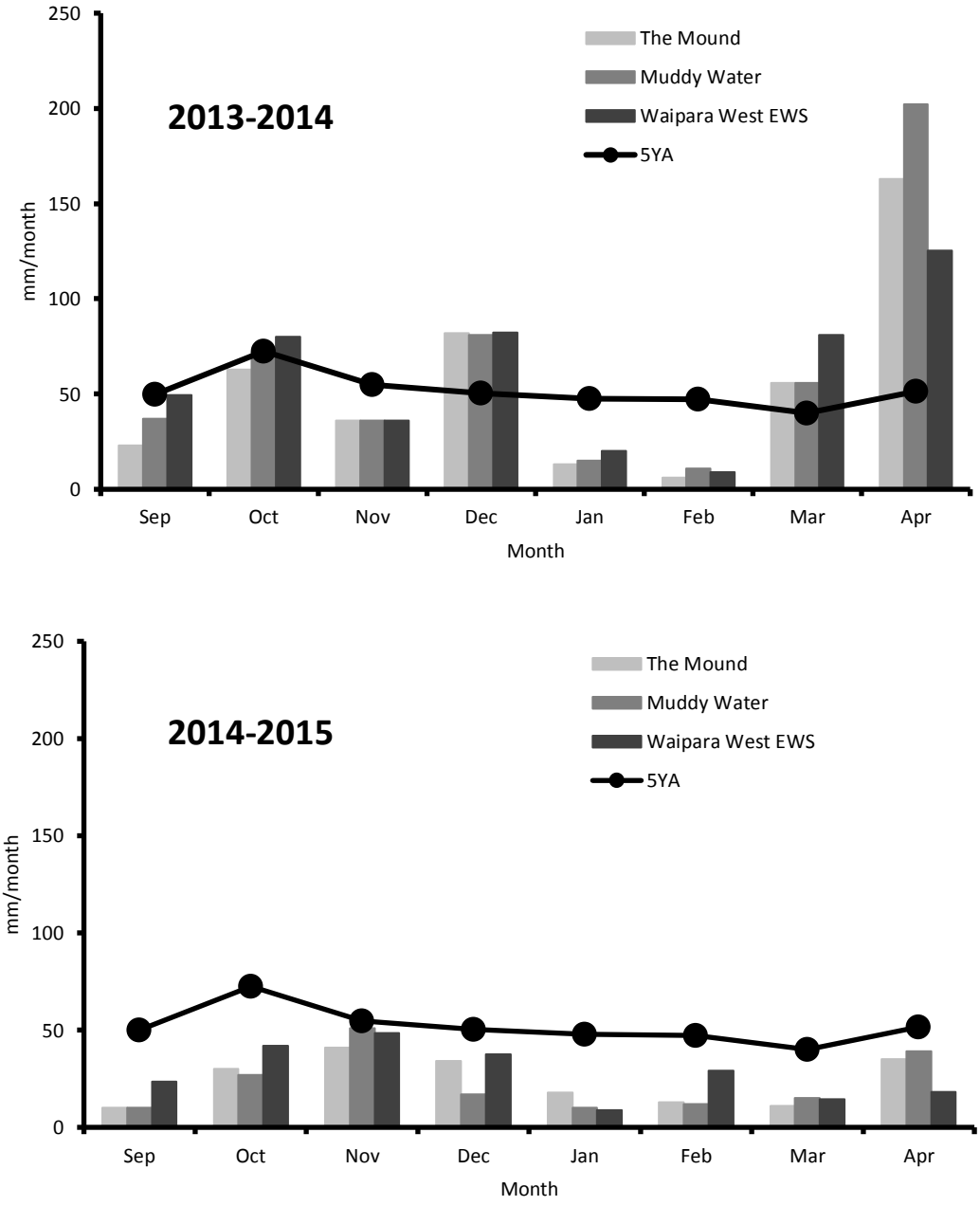


**Figure 7 Monthly rainfall in Waipara during the 2013-2014 and 2014-2015 seasons, compared to the 5YA. Data from Waipara West EWS, the Mound, and Muddy Water**

In contrast, 2014-2015 was drier than the 5YA, and 2013-2014. Only November showed an accumulated precipitation close to the 5YA, whereas the rest of the season remained below this.

The growing seasonal rainfall (September-April) calculated from the 5YA corresponded to 414 mm/season. Thus, in 2013-2014 the 442 mm accumulated at The Mound, 510 mm at Muddy Water, and 483 mm at Waipara West, demonstrated that it was a very wet end to the season. The average of 200 mm accumulated by all the weather stations in 2014-2015, contrasted with the 5YA at about 50% of the average.

No clear patterns were identified to characterise the spatial distribution of the precipitation in Waipara. In both seasons, the rainfall was accumulated indistinctly within the region, with only differences between seasons being observed.

## Wind speed

Interesting differences between seasons, and also within the region were identified for mean wind speed (Figure 8). For both 2013-2014 and 2014-2015, Waipara West EWS showed the highest average for each month, while Muddy Water reported always the lowest.

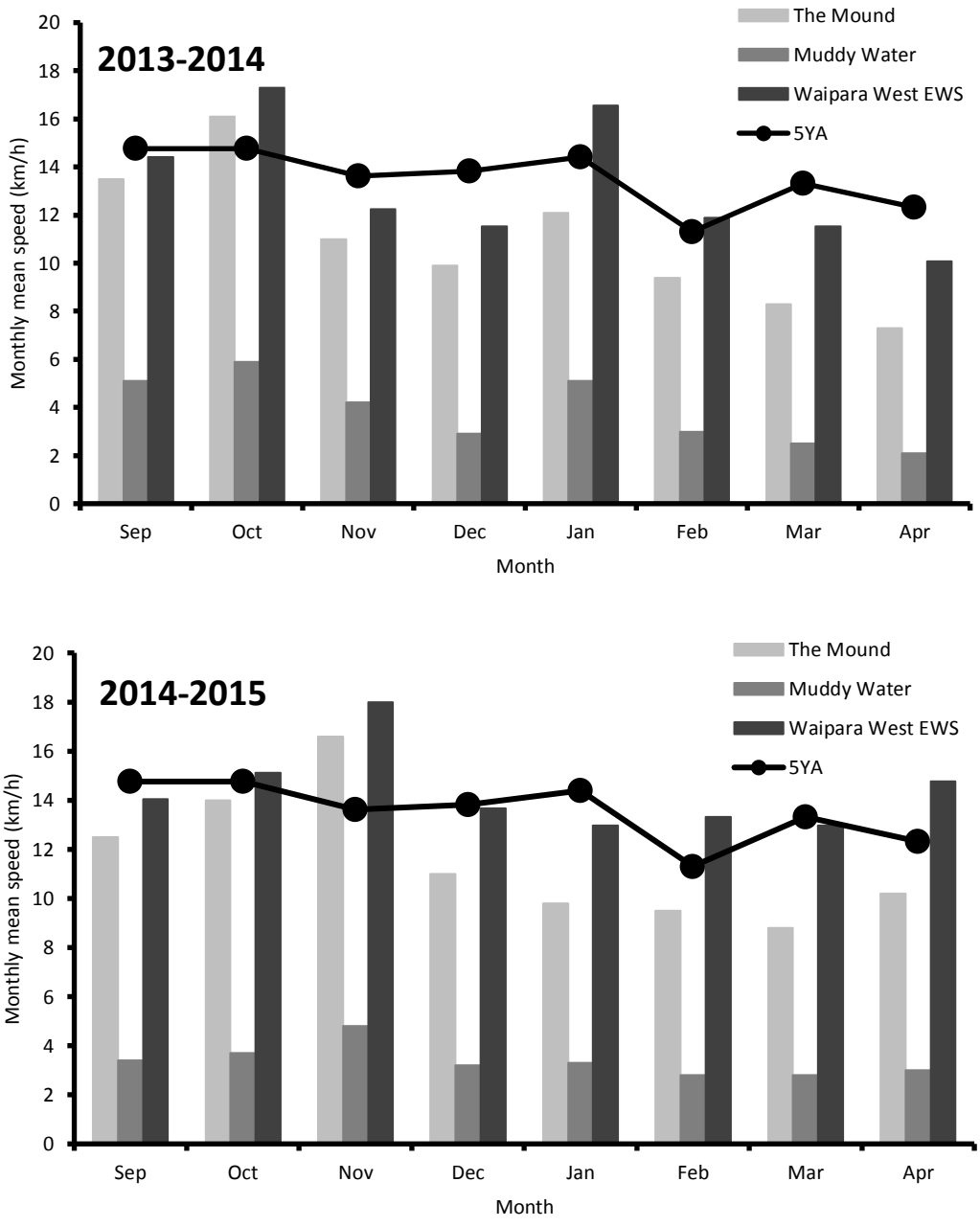


**Figure 8 Monthly mean wind speed in Waipara during the 2013-2014 and 2014-2015 seasons, compared to the 5YA. Data from Waipara West EWS, the Mound, and Muddy Water**

Overall, wind speed was below the 5YA most of the time in both seasons, with a few exceptions in which mainly Waipara West EWS exhibiting larger values.

To highlight the variations in wind speed across the region, the difference between the places where the higher and the lower wind speed were registered will be used for comparisons. Thus, in October 2014, the windiest month of the 2013-2014 season, the difference between the East and West parts of the valley was an average of 11 km/h more in the West, whereas this difference was about 8 km/h in April, the month with the lower wind speed observed in 2013-2014.

2014-2015 showed a similar tendency, with November being the windiest month of the season. During that month, the difference between Waipara West EWS and Muddy Water was more than 13 km/h, while the smaller differences were about 4 km/h in January, February, and March.

13.5 km/h was calculated as the 5YA for wind speed of the growing season. So, the mean wind speed obtained at the Mound and Waipara West EWS in 2013-2014 showed a slightly lower seasonal average than the 5YA (11 and 13.2 km/h, respectively), whereas the mean wind speed of the season in Muddy Water was more than three times lower than the 5YA, demonstrating the differences within the region.

2014-2015 registered a higher seasonal average than 2013-2014 at The Mound and Waipara West EWS, this being even higher than the 5YA at Waipara West EWS (14.4 km/h). In contrast, Muddy Water reported a seasonal average even lower than 2013-2014.

## 2.3 Experimental design

The experiment was laid out as a completely randomised design, comprising a control treatment (normally irrigated vineyard) and about 50% reduced irrigation treatment. Control (CON) corresponded to the irrigation applied by the viticulture manager's criteria, and the reduced irrigation (RI) was implemented by modifying the drippers spacing and flow rate. Four replicates per treatment were randomly distributed in rows of each plot. Each replicate consisted in a group of five contiguous plants within the same row, with about two metres of buffer zone before and after each replicate. The irrigation frequency was the same for both treatments, only the amount of water delivered to the vines varied due to the adjustment to the irrigation system. Theoretical calculations of the magnitude of these reductions were done for each site (Table 3).

**Table 3 Irrigation reduction calculated for each site**

| Site | Control | | Treatment | | Reduction (%) |
|---|---|---|---|---|---|
| | Flow rate (L/h) | Drippers spacing (m) | Flow rate (L/h) | Drippers spacing (m) | |
| WH | 1.2 | 0.6 | 1.3 | 1.2 | 46 |
| GB5 | 1.2 | 0.6 | 1.3 | 1.2 | 46 |
| GB10 | 4 | 1.6 | 2 | 1.6 | 50 |

A schematic representation of the experimental design in each vineyard is presented in Figure 9, Figure 10, and Figure 11.

North

| Bay | Vine position | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 10 | 39 | | | | | | | | | |
| 10 | 38 | | | | | | | | | |
| 10 | 37 | | | | | | | | | |
| 9 | 36 | | | | CON | | | | | |
| 9 | 35 | | | | CON | | | | | |
| 9 | 34 | | | | CON | | | | | |
| 9 | 33 | | | | CON | CON | | | | |
| 8 | 32 | | | | CON | CON | | | | |
| 8 | 31 | | | | | CON | | | | |
| 8 | 30 | | | | | CON | | | | |
| 8 | 29 | | | RI | | CON | | | | |
| 7 | 28 | | | RI | | | | | | |
| 7 | 27 | | | RI | | | | | | |
| 7 | 26 | | | RI | | | | | | |
| 7 | 25 | | | RI | | | RI | | | |
| 6 | 24 | | RI | | | | RI | | | |
| 6 | 23 | | RI | | | | RI | | | |
| 6 | 22 | | RI | | | | RI | | | |
| 6 | 21 | | RI | | CON | | RI | | | |
| 5 | 20 | | RI | | CON | | | | | |
| 5 | 19 | | | | CON | | | | | |
| 5 | 18 | | | | CON | | | | | |
| 5 | 17 | | | | CON | | | | | |
| 4 | 16 | | | | | | | | | |
| 4 | 15 | | | | | | | | | |
| 4 | 14 | | | | | | | | | |
| 4 | 13 | | | | | | | RI | | CON |
| 3 | 12 | | | | | | | RI | | CON |
| 3 | 11 | | | | | | | RI | | CON |
| 3 | 10 | | | | | | | RI | | CON |
| 3 | 9 | | | | | | | RI | | CON |
| 2 | 8 | | | | | | | | | |
| 2 | 7 | | | | | | | | | |
| 2 | 6 | | | | | | | | | |
| 2 | 5 | | | | | | | | | |
| 1 | 4 | | | | | | | | | |
| 1 | 3 | | | | | | | | | |
| 1 | 2 | | | | | | | | | |
| 1 | 1 | | | | | | | | | |
| | Row | 633 | 632 | 631 | 630 | 629 | 628 | 627 | 626 | 625 |

South

**Figure 9** **Schematic representation of the experimental design in Waipara Hills vineyard. CON represents control and RI reduced irrigation.**

South

| Bay | Vine position | 25 | 24 | 23 | 22 | 21 | 20 | 19 | 18 | 17 | 16 | 15 | 14 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 1 | | | | | | | | | | | | |
| 1 | 2 | | | | | | | | | | | | |
| 1 | 3 | | | | | | | | | | | | |
| 1 | 4 | | | | | | | | | | | | |
| 1 | 5 | | | | | | | | | | | | |
| 2 | 6 | | | | | | | | | | | | |
| 2 | 7 | | | | | | | | | | | | |
| 2 | 8 | | | | | | | | | | | | |
| 2 | 9 | | | | | | | | | | | | |
| 2 | 10 | | | | | | | | | | | | |
| 3 | 11 | | | | | | | | | | | | |
| 3 | 12 | | | | | | | | | | | | |
| 3 | 13 | | | | | | | | | | | | |
| 3 | 14 | | | | | | | | | | | | |
| 3 | 15 | | | | | | | | | | | | |
| 4 | 16 | | CON | | | | | | | | | | |
| 4 | 17 | | CON | | | | | | | | | | |
| 4 | 18 | | CON | | | | | | | | | | |
| 4 | 19 | | CON | | | | | | | | | | |
| 4 | 20 | | CON | | | | | | | | | | |
| 5 | 21 | | | | | RI | | | | | | | |
| 5 | 22 | | | | | RI | | | | | | | |
| 5 | 23 | | | | | RI | | | | | | | |
| 5 | 24 | | | | | RI | | | | | | | |
| 5 | 25 | | | | | RI | | | | | | | |
| 6 | 26 | | | | | | | | | | | | |
| 6 | 27 | | | | | | | | | | | | |
| 6 | 28 | | | | | | | | | | | | |
| 6 | 29 | | | | | | | | | | | | |
| 6 | 30 | | | | | | | | | | | | |
| 7 | 31 | | | | | | | | | | | | |
| 7 | 32 | | | | | | | | | | | | |
| 7 | 33 | | | | | | | | | | | | |
| 7 | 34 | | | | | | | | | | | | |
| 7 | 35 | | | | | | | | | | | | |
| 8 | 36 | | | | | | | | | | | | |
| 8 | 37 | | | | | | | | | | | | |
| 8 | 38 | | | | | | | | | | | | |
| 8 | 39 | | | | | | | | | | | | |
| 8 | 40 | | | | | | | | | | | | |
| 9 | 41 | | | | | | | | | | | | |
| 9 | 42 | | | | | | | | | | | | |
| 9 | 43 | | | | CON | | | | | | | | |
| 9 | 44 | | | | CON | | | | | | | | |
| 9 | 45 | | | | CON | | | | | | | | |
| 10 | 46 | | | RI | CON | | | | | | RI | | CON |
| 10 | 47 | | | RI | CON | | | | | | RI | | CON |
| 10 | 48 | | | RI | | | | | | | RI | | CON |
| 10 | 49 | | | RI | | | | | | | RI | | CON |
| 10 | 50 | | | RI | | | | | | | RI | | CON |
| 11 | 51 | | | | | CON | | | | | | | |
| 11 | 52 | | | | | CON | | | | | | | |
| 11 | 53 | | | | | CON | | | | | | | |
| 11 | 54 | | | | | CON | | | | | | | |
| 11 | 55 | | | | | CON | | | | | | | |
| 12 | 56 | | | | | | | | | | | | |
| 12 | 57 | | | | | | | | | | | | |
| 12 | 58 | | | | | | | | | | | | |
| 12 | 59 | | | | | | | | | | | | |
| 12 | 60 | | | | | | | | | | | | |
| 13 | 61 | | | | | | | | | | | | |
| 13 | 62 | | | | | | | | | | | | |
| 13 | 63 | | | | | | | | | | | | |
| 13 | 64 | | | | | | | | | | | | |
| 13 | 65 | | | | | | | | | | | | |
| 14 | 66 | | RI | | | | | | | | | | |
| 14 | 67 | | RI | | | | | | | | | | |
| 14 | 68 | | RI | | | | | | | | | | |
| 14 | 69 | | RI | | | | | | | | | | |
| 14 | 70 | | RI | | | | | | | | | | |
| 15 | 71 | | | | | | | | | | | | |
| 15 | 72 | | | | | | | | | | | | |
| 15 | 73 | | | | | | | | | | | | |
| 15 | 74 | | | | | | | | | | | | |
| 15 | 75 | | | | | | | | | | | | |
| 16 | 76 | | | | | | | | | | | | |
| 16 | 77 | | | | | | | | | | | | |
| | **Row** | 25 | 24 | 23 | 22 | 21 | 20 | 19 | 18 | 17 | 16 | 15 | 14 |

North

**Figure 10** **Schematic representation of the experimental design in Greystone block 5. CON represents control and RI reduced irrigation.**

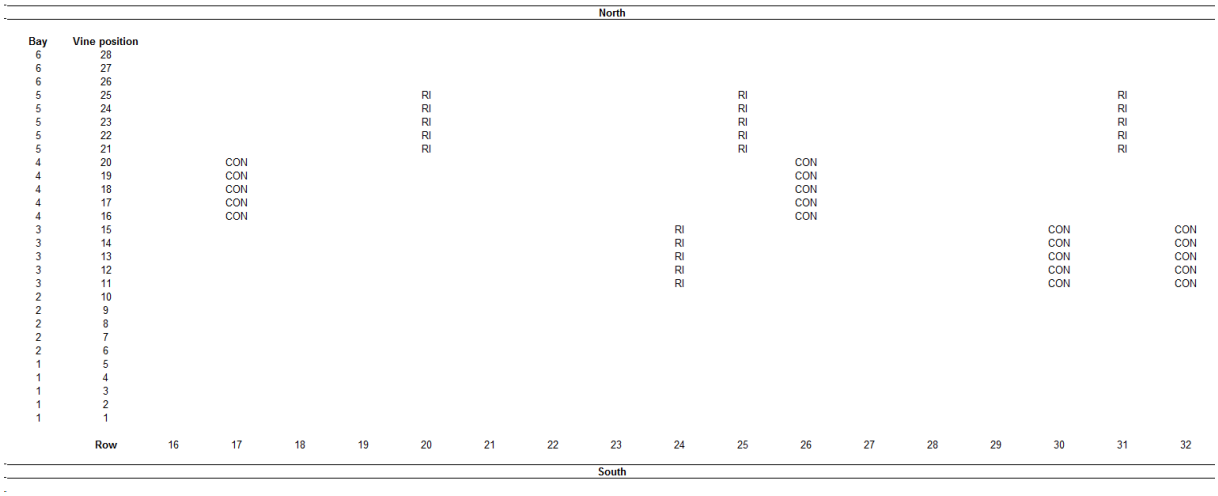


**Figure 11 Schematic representation of the experimental design in Greystone block 10. CON represents control and RI reduced irrigation.**

## 2.3.1 Microclimate

### Equipment

Temperatures from each plot were registered using a Tinytag Transit datalogger (Gemini Data Loggers, UK), protected by a Stevenson-type solar radiation shield. One logger per plot was hung from the fruiting wire at about 80 cm from the ground. All the dataloggers were tested to check their accuracy prior to installing them in the field. Each datalogger remained in the same location and was used in the same block in both seasons. The loggers were set up to record the temperature at 30 minute intervals and the data were retrieved every month using Tinytag Explorer 4.7 (Gemini Data Loggers (UK) Ltd.).

Wind speed was measured over two different weeks, during the second season only. The weeks from the 13$^{th}$ to the 18$^{th}$ of December 2014, and from the 22$^{th}$ to the 27$^{th}$ of January 2015 were chosen to evaluate the differences in wind speed among the sites. An anemometer (Type A100M; Vector instrument, Rhyl, UK) was installed in each site at about 2.3m from the ground. Data were recorded at 10 minute intervals using two CR1000 and one CR10 dataloggers (Campbell Scientific Ltd., Logan, UT). Data were downloaded at the end of each week using LoggerNet 3.4.1 (Campbell Scientific Ltd., Logan, UT).

### Analyses

Monthly averages of maximum, minimum, mean temperatures, as well as GDD accumulation were obtained and compared among all the plots of the trial for both seasons. All these parameters were calculated on a daily basis.

The results of wind speed obtained over two weeks from each site are reported as daily averages (km/h).

Finally, the water balance was calculated for each site using the climatic information retrieved from The Mound and Waipara West EWS stations and complemented with the records of monthly irrigation provided by the viticulture managers for both seasons. Irrigation and rainfall were added to obtain the total water. Then, this was subtracted from the ETo to calculate the water balance, separated by treatments for each site. As previously described, due to a technical issue the value for ETo could not be obtained for October 2014, so for practical purposes this was calculated as the average of those registered in September and November in 2014.

**Maximum temperature**

As can be observed in Figure 12, GB5 registered the highest monthly mean maximum temperatures in both seasons. WH and GB10 shared a similar pattern.

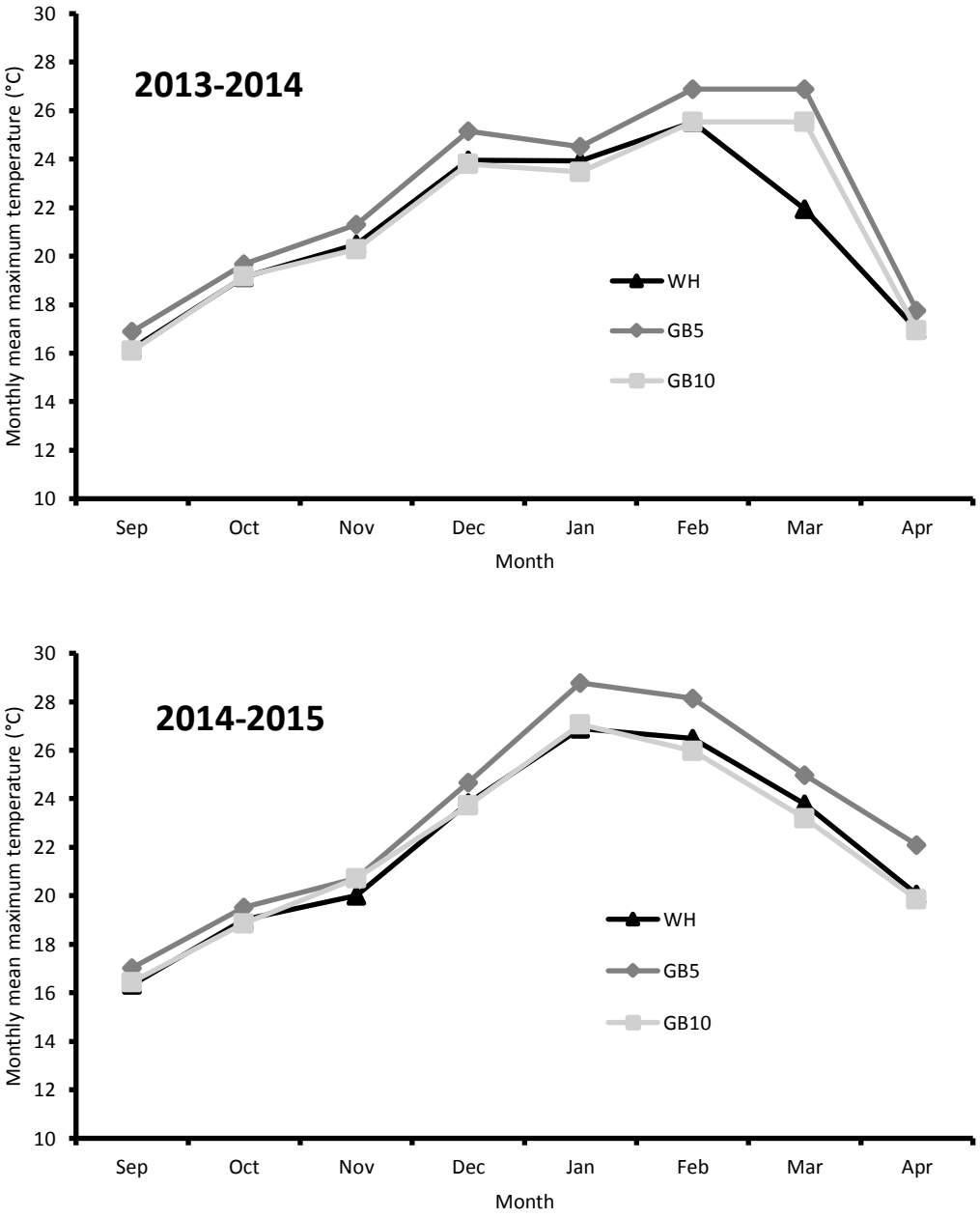


**Figure 12 Monthly mean maximum temperature in Waipara Hills (WH), Greystone block 5 (GB5), and block 10 (GB10) during the 2013-2014 and 2014-2015 seasons**

2013-2014 at GB5 reported a higher mean maximum temperature in February and March, with 26.9°C in both months. GB10 registered the lower values during most of the season, either being

equal to WH or just few tenths lower than WH. Interestingly, the extreme differences were found between the two sites located in the same property (GB5 and GB10), whereas WH and GB10 were very similar.

Higher maximum temperatures were observed through the 2014-2015 season, especially in January, where the monthly mean maximum temperature reached a peak of 28.8°C at GB5.

Furthermore, the mean maximum temperature of the season showed the differences between sites in both 2013-2014 and 2014-2015. WH registered the lowest average maximum temperature in 2013-2014 with 21.0°C, followed by GB10 with 21.4°C, and GB5 being the highest with 22.4°C. In general, the mean maximum temperature of the season was higher in 2014-2015 for all the sites, compared to 2013-2014. In 2014-2015, the lowest mean maximum temperature was observed in GB10 (22.0°C), whereas the 22.4°C registered in GB5 was the highest of the season.

### Minimum temperature

Figure 13 shows that from January onwards, WH reported the lowest minimum temperatures of all the sites in both seasons.

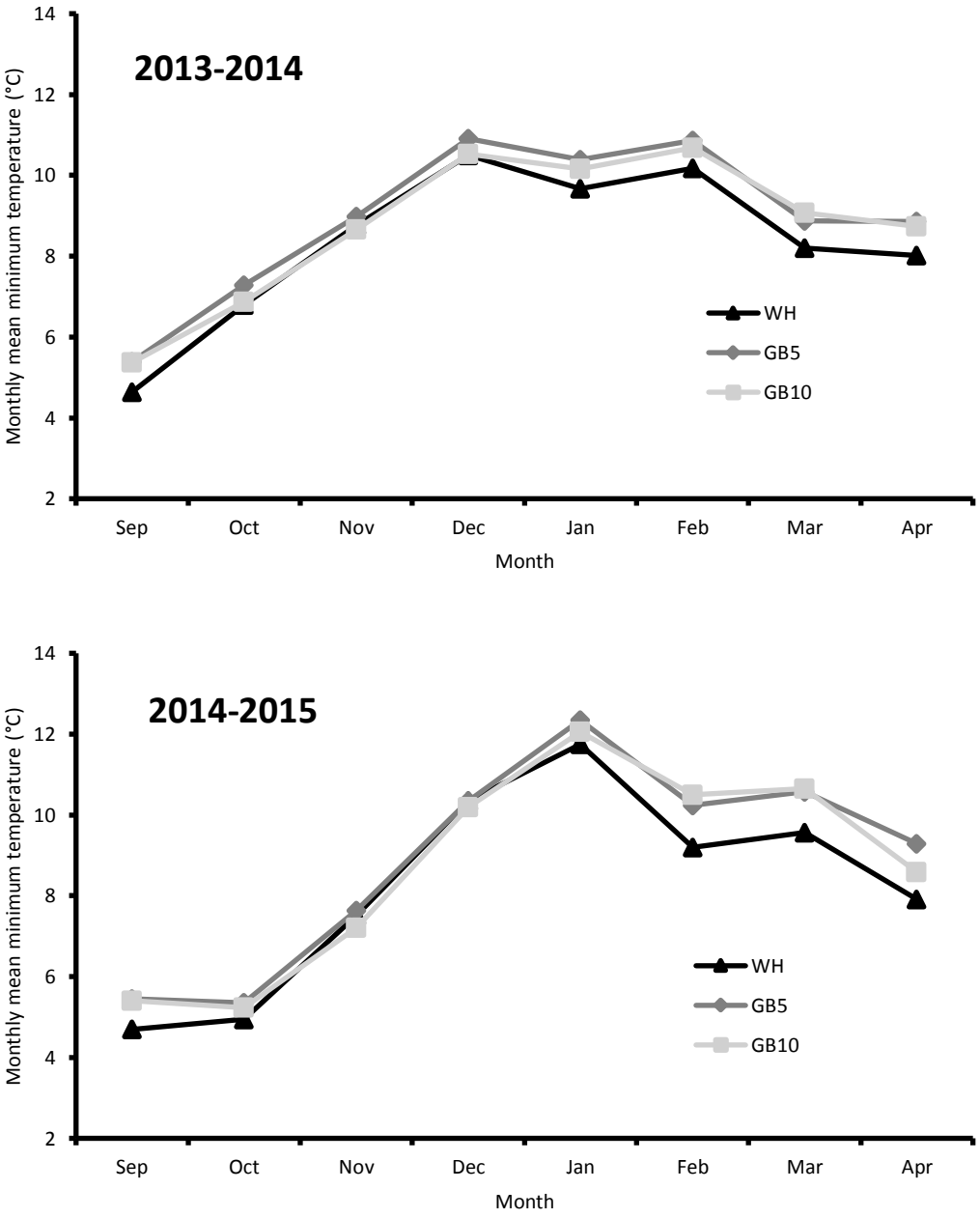


**Figure 13 Monthly mean minimum temperature in Waipara Hills (WH), Greystone block 5 (GB5), and block 10 (GB10) during the 2013-2014 and 2014-2015 seasons**

GB5 in 2013-2014 registered the highest minimum temperature of the three sites during most of the season, but less than 1°C of difference existed between the lower and the higher values until December. From January to April, this difference increased and WH became the site registering the lowest minimum temperature.

Similar results were found in 2014-2015. Minimum temperatures were higher than found in 2013-2014 from January to April. Overall, the higher minimum temperatures were reported in both GB5 and GB10, while the lower values corresponded to WH after January.

Overall, the mean minimum temperature in both seasons were very similar, but with marked differences between sites. Thus, WH showed the lowest minimum temperature of the two seasons, while GB5 the highest. For this parameter, GB10 registered temperatures more similar to GB5 rather than WH. The mean minimum temperatures of the season corresponded to: WH (8.3°C in 2013-2014 and 8.2 in 2014-2015), GB5 (8.9°C in both seasons), and GB10 (8.8°C in 2013-2014 and 8.7°C in 2014-2015).

### Mean temperature

As can be seen in Figure 14, GB5 registered the highest mean temperatures in both seasons. In 2013-2014 the highest mean temperature of the season was observed in February, whereas January was the warmest month in 2014-2015.

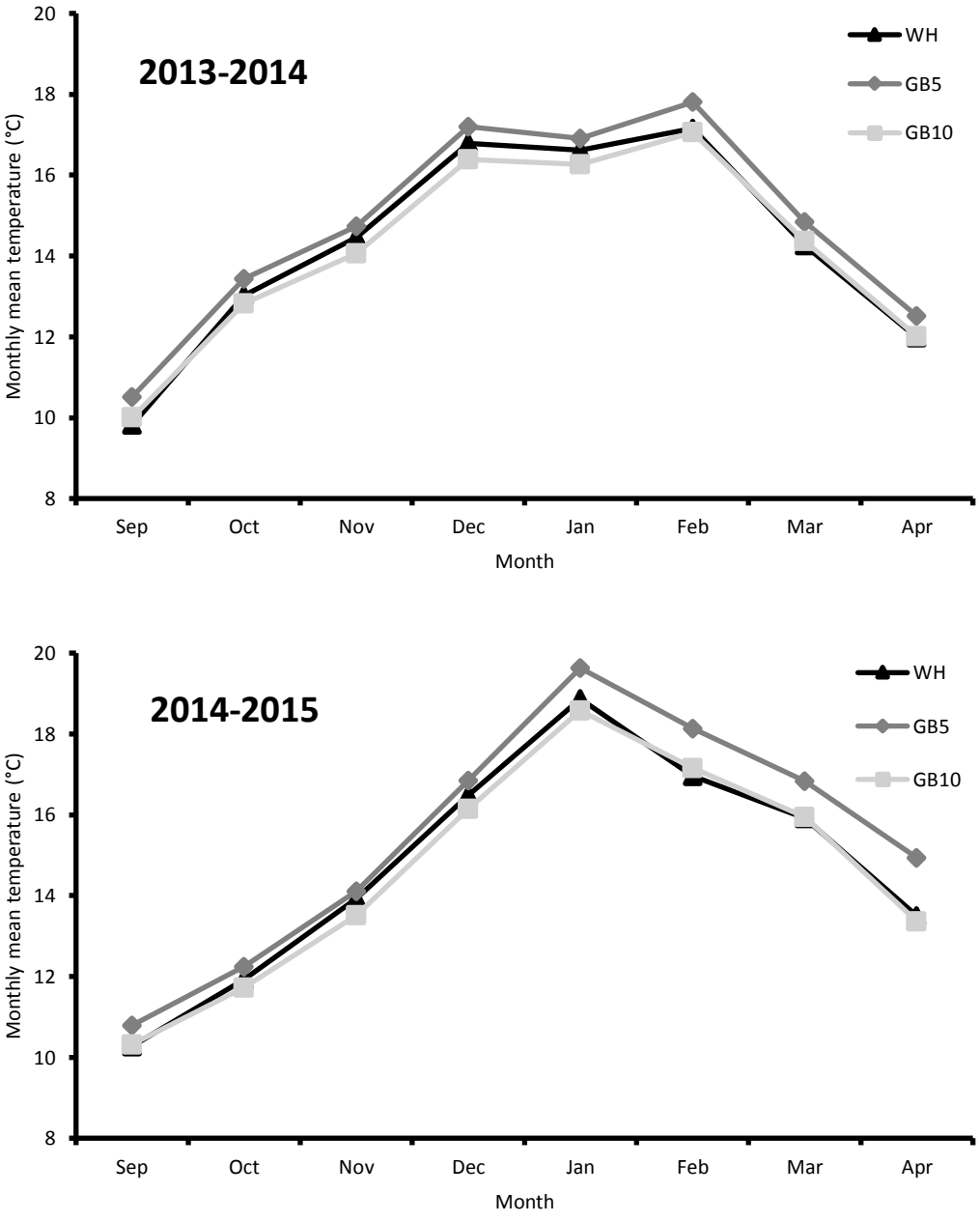


**Figure 14 Monthly mean temperature in Waipara Hills (WH), Greystone block 5 (GB5), and block 10 (GB10) during the 2013-2014 and 2014-2015 seasons**

In 2013-2014, very similar mean temperatures were observed between WH and GB10 during the entire season, whereas GB5 proved to be the warmest site. Despite the differences between sites

relating to minimum and maximum temperatures, the average temperature highlighted the similarities between WH and GB10, and also the particular conditions of GB5.

In general, the mean temperatures observed in 2014-2015 was higher than in 2013-2014, especially after December. Although the similarities between WH and GB10 were confirmed, the differences between GB5 and the other two sites were more evident in this warmer season. The mean temperature during the warmest month (January) in GB5 was 19.6°C, while in GB10 was 1°C lower, and WH 0.7°C lower.

When the average temperature of the growing season was calculated, the lowest of these was obtained from GB10 in both seasons (14.1°C in 2013-2014 and 14.6°C in 2014-2015). GB5 was the site reporting the highest mean temperature in the two seasons with 14.7°C in 2013-2014 and 15.4°C in 2014-2015, the latter being also the highest of both seasons and in all the sites. WH remained in an intermediate point.

### GDD monthly accumulation

The microclimatic analysis also showed the differences in GDD monthly accumulation among the sites of the study. GB5 accumulated more GDD than the other two sites, a similar pattern as for the other parameters described (Figure 15).

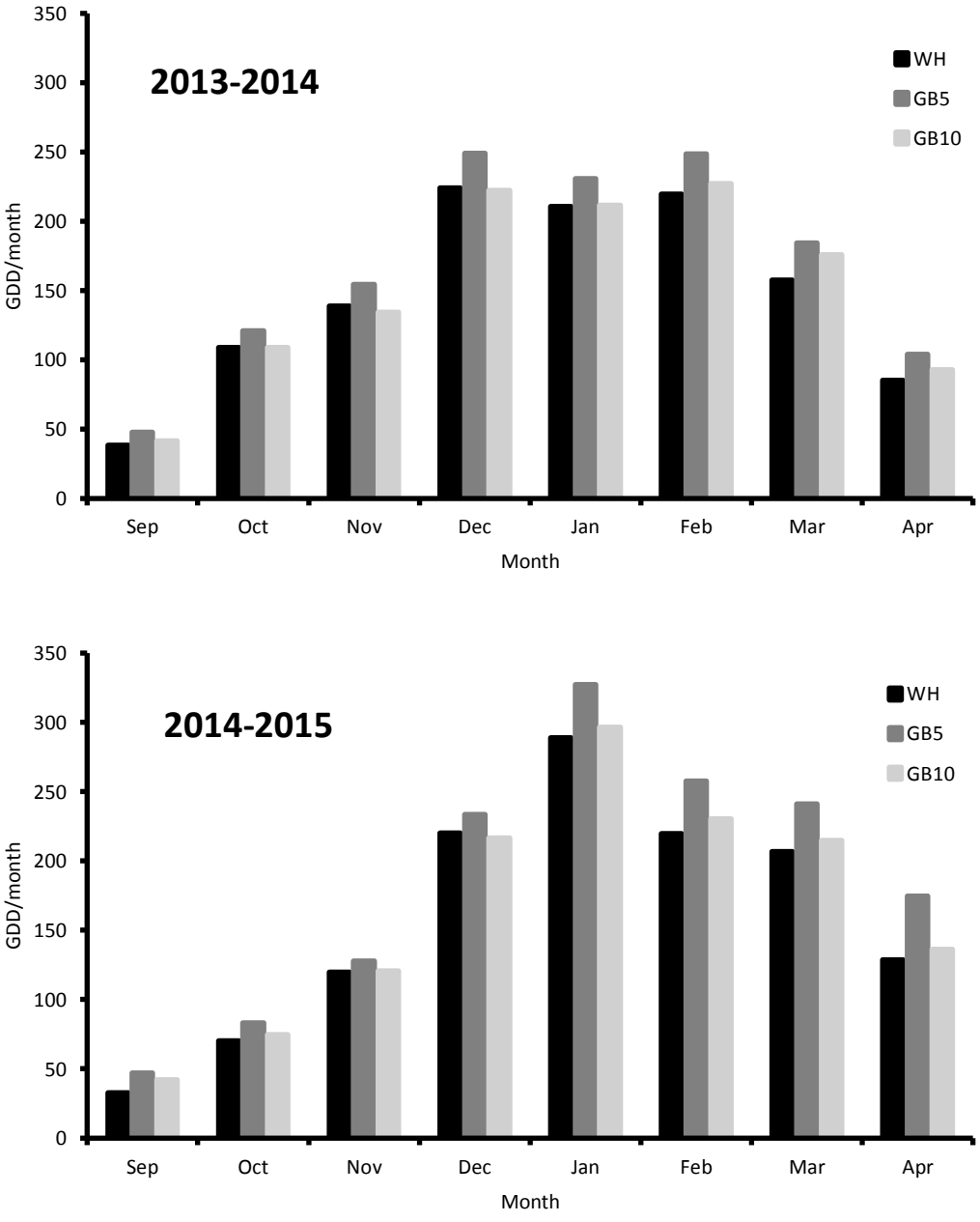


**Figure 15 GDD/month accumulated in Waipara Hills (WH), Greystone block 5 (GB5), and block 10 (GB10) during the 2013-2014 and 2014-2015 seasons**

As can be observed in Figure 15, the majority of the GDD of the season were recorded between December and March, but differences between sites were observed in 2013-2014 with 22 GDD of

difference between GB5 and GB10 observed in December, but there was a larger difference (28 GDD) between WH and GB5 in February.

In 2014-2015, heat accumulation reached its highest level in January, where GB5 accumulated 327 GDD, which was also the maximum in either season over all the sites. This represented 38 GDD more than that accumulated in GB10 and WH in that month.

The total GDD accumulated over the season also showed the same tendency in both 2013-2014 and 2014-2015, where WH accumulated the lowest GDD and GB5 the highest.

### Wind speed

Differences in mean wind speed were also found among sites. During the two weeks of the second season in which the sensors were installed, except for few days, GB10 showed the highest wind speeds, whereas WH had the lowest. GB5 remained in an intermediate point (Figure 16).

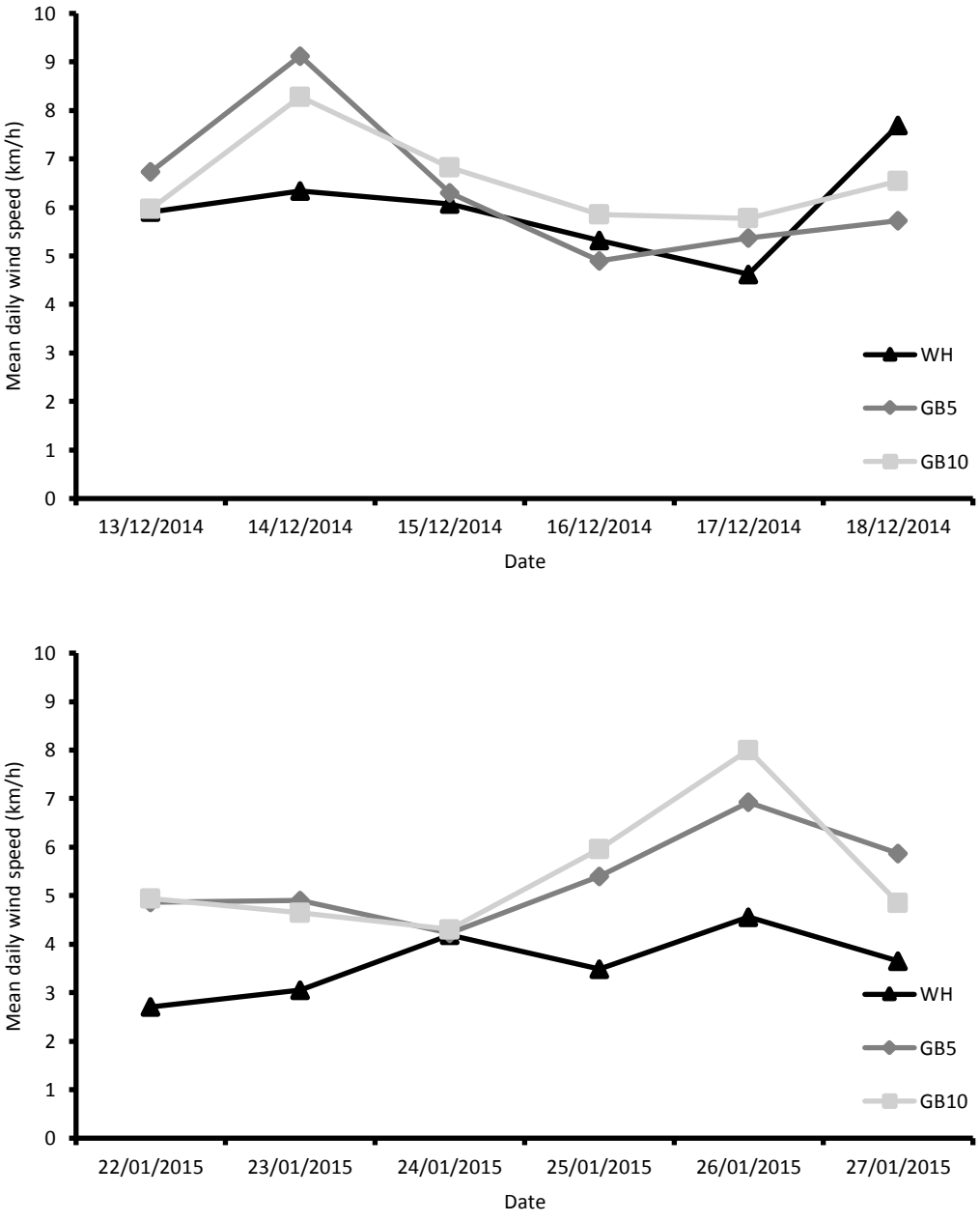


**Figure 16 Daily wind speed in Waipara Hills (WH), Greystone block 5 (GB5), and block 10 (GB10) during two weeks of the 2014-2015 season**

### 2.3.2 Water balance

As can be observed in Table 4, the higher hydric deficit was registered in January in both seasons at WH. This month also reported the higher ETo of the season in both seasons. In 2013-2014, April registered a surplus of water due to the high amount of rainfall, with this being the only month during the two years of study registering a total water higher than the ETo, and therefore, a positive water balance. The total water balance of the 2013-2014 season was two times lower than in 2014-2015 due to the higher ETo and lower rainfall.

As same as for WH, January reported the higher water deficit of the season either in 2013-2014 or 2014-2015 at GB5 (Table 5). This was also coincident with the higher ETo of the season. Also, April 2013-2014 reported a positive water balance due to the high amount of rainfall and no irrigation in that month. In this site, the water balance was also more than double in 2014-2015 compared to 2013-2014 due to the higher ETo and lower rainfall observed during the second season.

GB10 also showed a higher total water balance (more negative) in 2014-2015 compared to 2013-2014, mainly due to the higher ETo and lower precipitation reported in the second season (Table 6). Also, the higher ETo of the season was found in January in both seasons. April in 2013-2014, as well as in WH and GB5, registered a positive water balance due to the high amount of precipitation in that month.

**Table 4** **Irrigation applied, rainfall, total water, reference evapotranspiration (ETo), and water balance (ETo – total water) for each season at Waipara Hills. All values are shown in millimetres (mm).**

| Season 2013-2014 | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Sep | | Oct | | Nov | | Dec | | Jan | | Feb | | Mar | | Apr | | Total | |
| | CON | RI | CON | RI | CON | RI | CON | RI | CON | RI | CON | RI | CON | RI | CON | RI | CON | RI |
| Irrigation | 0 | 0 | 0 | 0 | 16.7 | 9.0 | 31.5 | 17.0 | 43.0 | 23.2 | 38.9 | 21.0 | 5.6 | 3.0 | 0 | 0 | 135.7 | 73.2 |
| Rainfall | 23.0 | | 63.0 | | 36.0 | | 82.0 | | 13.0 | | 6.0 | | 56.0 | | 163.0 | | 442.0 | |
| Total water | 23.0 | 23.0 | 63.0 | 63.0 | 52.7 | 45.0 | 113.5 | 99.0 | 56.0 | 36.2 | 44.9 | 27.0 | 61.6 | 59.0 | 163.0 | 163.0 | 577.7 | 515.2 |
| ETo | 74.3 | | 125.3 | | 116.6 | | 142.9 | | 174.5 | | 120.0 | | 90.8 | | 36.3 | | 880.7 | |
| Water balance | -51.3 | -51.3 | -62.3 | -62.3 | -63.9 | -71.6 | -29.4 | -43.9 | -118.5 | -138.3 | -75.1 | -93.0 | -29.2 | -31.8 | 126.7 | 126.7 | -303.0 | -365.5 |
| Season 2014-2015 | | | | | | | | | | | | | | | | | | |
| | Sep | | Oct | | Nov | | Dec | | Jan | | Feb | | Mar | | Apr | | Total | |
| | CON | RI | CON | RI | CON | RI | CON | RI | CON | RI | CON | RI | CON | RI | CON | RI | CON | RI |
| Irrigation | 0 | 0 | 11.1 | 6.0 | 22.2 | 12.0 | 22.2 | 12.0 | 22.2 | 12.0 | 25.9 | 14.0 | 16.7 | 9.0 | 14.8 | 8.0 | 135.1 | 73.0 |
| Rainfall | 10.0 | | 30.0 | | 41.0 | | 34.0 | | 18.0 | | 13.0 | | 11.0 | | 35.0 | | 192.0 | |
| Total water | 10.0 | 10.0 | 41.1 | 36.0 | 63.2 | 53.0 | 56.2 | 46.0 | 40.2 | 30.0 | 38.9 | 27.0 | 27.7 | 20.0 | 49.8 | 43.0 | 327.1 | 265.0 |
| ETo | 79.0 | | 119.4* | | 159.7 | | 152.2 | | 172.9 | | 139.1 | | 108.2 | | 74.8 | | 1005.3 | |
| Water balance | -69.0 | -69.0 | -78.3 | -83.4 | -96.5 | -106.7 | -96.0 | -106.2 | -132.7 | -142.9 | -100.2 | -112.1 | -80.5 | -88.2 | -25.0 | -31.8 | -678.2 | -740.3 |

* Correspond to the average of the ETo reported in September and November 2014

**Table 5** **Irrigation applied, rainfall, total water, reference evapotranspiration (ETo), and water balance (ETo – total water) for each season at Greystone block 5. All values are shown in millimetres (mm).**

| Season 2013-2014 | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Sep | | Oct | | Nov | | Dec | | Jan | | Feb | | Mar | | Apr | | Total | |
| | CON | RI | CON | RI | CON | RI | CON | RI | CON | RI | CON | RI | CON | RI | CON | RI | CON | RI |
| Irrigation | 0 | 0 | 0 | 0 | 2.4 | 1.3 | 2.4 | 1.3 | 4.8 | 2.6 | 16.8 | 9.1 | 0 | 0 | 0 | 0 | 26.4 | 14.3 |
| Rainfall | 37.0 | | 72.0 | | 36.0 | | 81.0 | | 15.0 | | 11.0 | | 56.0 | | 202.0 | | 510.0 | |
| Total water | 37.0 | 37.0 | 72.0 | 72.0 | 38.4 | 37.3 | 83.4 | 82.3 | 19.8 | 17.6 | 27.8 | 20.1 | 56.0 | 56.0 | 202.0 | 202.0 | 536.4 | 524.3 |
| ETo | 74.3 | | 125.3 | | 116.6 | | 142.9 | | 174.5 | | 120.0 | | 90.8 | | 36.3 | | 880.7 | |
| Water balance | -37.3 | -37.3 | -53.3 | -53.3 | -78.2 | -79.3 | -59.5 | -60.6 | -154.7 | -156.9 | -92.2 | -99.9 | -34.8 | -34.8 | 165.7 | 165.7 | -344.3 | -356.4 |

| Season 2014-2015 | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Sep | | Oct | | Nov | | Dec | | Jan | | Feb | | Mar | | Apr | | Total | |
| | CON | RI | CON | RI | CON | RI | CON | RI | CON | RI | CON | RI | CON | RI | CON | RI | CON | RI |
| Irrigation | 0 | 0 | 10.4 | 5.7 | 9.6 | 5.2 | 9.6 | 5.2 | 7.2 | 3.9 | 0 | 0 | 2.4 | 1.3 | 0 | 0 | 39.2 | 21.3 |
| Rainfall | 10.0 | | 27.0 | | 51.0 | | 17.0 | | 10.0 | | 12.0 | | 15.0 | | 39.0 | | 181 | |
| Total water | 10.0 | 10.0 | 37.4 | 32.7 | 60.6 | 56.2 | 26.6 | 22.2 | 17.2 | 13.9 | 12.0 | 12.0 | 17.4 | 16.3 | 39.0 | 39.0 | 220.2 | 202.3 |
| ETo | 79.0 | | 119.4* | | 159.7 | | 152.2 | | 172.9 | | 139.1 | | 108.2 | | 74.8 | | 1005.3 | |
| Water balance | -69.0 | -69.0 | -82 | -86.7 | -99.1 | -103.5 | -125.6 | -130.0 | -155.7 | -159.0 | -127.1 | -127.1 | -90.8 | -91.9 | -35.8 | -35.8 | -785.1 | -803.0 |

* Correspond to the average of the ETo reported in September and November 2014

**Table 6** **Irrigation applied, rainfall, total water, reference evapotranspiration (ETo), and water balance (ETo – total water) for each season at Greystone block 10. All values are shown in millimetres (mm).**

| Season 2013-2014 | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Sep | | Oct | | Nov | | Dec | | Jan | | Feb | | Mar | | Apr | | Total | |
| | CON | RI | CON | RI | CON | RI | CON | RI | CON | RI | CON | RI | CON | RI | CON | RI | CON | RI |
| Irrigation | 0 | 0 | 0 | 0 | 6.0 | 3.0 | 30.0 | 15.0 | 9.0 | 4.5 | 15.0 | 7.5 | 0 | 0 | 0 | 0 | 60.0 | 30.0 |
| Rainfall | 37.0 | | 72.0 | | 36.0 | | 81.0 | | 15.0 | | 11.0 | | 56.0 | | 202.0 | | 510.0 | |
| Total water | 37.0 | 37.0 | 72.0 | 72.0 | 42.0 | 39.0 | 111.0 | 96.0 | 24.0 | 19.5 | 26.0 | 18.5 | 56.0 | 56.0 | 202.0 | 202.0 | 570.0 | 540.0 |
| ETo | 74.3 | | 125.3 | | 116.6 | | 142.9 | | 174.5 | | 120.0 | | 90.8 | | 36.3 | | 880.7 | |
| Water balance | -37.3 | -37.3 | -53.3 | -53.3 | -74.6 | -77.6 | -31.9 | -46.9 | -150.5 | -155 | -94.0 | -101.5 | -34.8 | -34.8 | 165.7 | 165.7 | -310.7 | -340.7 |

| Season 2014-2015 | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Sep | | Oct | | Nov | | Dec | | Jan | | Feb | | Mar | | Apr | | Total | |
| | CON | RI | CON | RI | CON | RI | CON | RI | CON | RI | CON | RI | CON | RI | CON | RI | CON | RI |
| Irrigation | 3.0 | 1.5 | 7.5 | 3.8 | 20.0 | 10.0 | 15.0 | 7.5 | 15.0 | 7.5 | 9.0 | 4.5 | 9.0 | 4.5 | 0 | 0 | 78.5 | 39.3 |
| Rainfall | 10.0 | | 27.0 | | 51.0 | | 17.0 | | 10.0 | | 12.0 | | 15.0 | | 39.0 | | 181.0 | |
| Total water | 13.0 | 11.5 | 34.5 | 30.8 | 71.0 | 61.0 | 32.0 | 24.5 | 25.0 | 17.5 | 21.0 | 16.5 | 24.0 | 19.5 | 39.0 | 39.0 | 259.5 | 220.3 |
| ETo | 79.0 | | 119.4* | | 159.7 | | 152.2 | | 172.9 | | 139.1 | | 108.2 | | 74.8 | | 1005.3 | |
| Water balance | -66.0 | -67.5 | -84.9 | -88.6 | -88.7 | -98.7 | -120.2 | -127.7 | -147.9 | -155.4 | -118.1 | -122.6 | -84.2 | -88.7 | -35.8 | -35.8 | -745.8 | -785.0 |

* Correspond to the average of the ETo reported in September and November 2014

## 2.4 Discussion

Worldwide, some attempts have been made to analyse the climatic conditions of different viticulture regions, like those of Jones *et al.* (2005) and Jones *et al.* (2009) however New Zealand has not often been considered. Among those including New Zealand, the analysis of Fitzharris and Enducher (1996) compared the climatic conditions for wine grape growing between Central Europe and New Zealand, but only data from Auckland, Gisborne, Napier, Blenheim, Alexandra, and Queenstown were used for comparisons. Also, Shaw (2012) made a climatic analysis of the wine regions growing Pinot noir around the world, in which from the Southern hemisphere, South Africa, Tasmania, Australia, and New Zealand were included, but only Wairarapa, Marlborough and Central Otago were considered as Pinot noir growing regions from New Zealand. Jackson (2001) contrasted a few representative climates from France, Germany, USA, Canada, Australia and New Zealand, with Christchurch and Napier (Hawkes Bay, North Island) selected for comparison. The author stated that Christchurch has a longer growing season than Champagne in France, which means Christchurch is potentially a good zone for growing Pinot noir.

Only the main regions of New Zealand have had detailed climatic analyses. For example, NIWA, the main source of climatic information in the country, has released regional climatological reports for the main areas of the North Island (Auckland, Bay of Plenty, among others), yet only for Southland in the South Island until this study was carried out (NIWA, 2013accessed on the 27/07/2015). A good example of regional characterisation in New Zealand is the GROWOTAGO project (http://growotago.orc.govt.nz/), which provides detailed long and short term climatic information, easily available for the users. A similar project, but with a scientific focus, has been developed by the Centre of Atmospheric Research of the University of Canterbury for Marlborough and Waipara (http://wineclimate.co.nz/). The project has generated growing degree days (GDD) maps, using theoretical models developed from data registered by a weather station installed in Waipara for the project, but the data is not available for public access, and the outcomes are not easily interpretable by the winegrowers of the region.

However, some published information can be found in which Waipara region has been characterised. Schuster *et al.* (2002) and Gladstones (2011) underlined the particular characteristics of Waipara, showing the importance of the coastal hills in protecting the region from the cool marine winds. Also, the latter pointed out that Waipara is much warmer than the Canterbury plains near Christchurch. Leathwick *et al.* (2002) described the climate in North Canterbury as: “dry and mild with high solar radiation, reflecting its protection from prevailing winds by mountain ranges to the West”. The same authors reported 10.7°C as mean annual temperature for the region. Imre and Mauk (2009)

described Waipara region as having a mean annual temperature of 11.3°C, slightly higher than the reported by Leathwick *et al.* (2002).

A few results from this research can be compared to published information. Anderson *et al.* (2012) have reported an average temperature of 14.7°C, obtained from a theoretical model based on data retrieved from NIWA for the period 1971-2000. Unfortunately, these values can be taken only as a reference because they considered the growing season as being from October to April, which is different than from this study, where to growing season ran from September to April, based on the field observations. Imre and Mauk (2009) reported 14.9°C as the average growing season temperature, a value that is slightly higher than that reported by Anderson *et al.* (2012) or in this study. However, Imre and Mauk (2009) did not state the period of time considered as a season, as well as the number of seasons used to calculate the average, so once more the values can be taken only as a reference.

A comparison of GDD accumulation was also difficult to make. Anderson *et al.* (2012) calculated a median of 993 GDD/season, ranging between 683 GDD as minimum and 1097 GDD as maximum, based on gridded information for the period 1971-2000. Schuster *et al.* (2002) reported 950 GDD for the Canterbury region, pointing out that Waipara normally accumulates 100 or more GDD than the Canterbury plains. The 5YA calculated for this study indicated that September accumulates about 45 GDD, month that was not considered in the data of Anderson *et al.* (2012). 1033 GDD/season were reported by Imre and Mauk (2009), but again, the months considered for the calculation were not reported. In all cases, either due to a higher GDD accumulation or differences in methodologies, the values obtained in this research are larger than those reported in the literature, when calculated on a monthly basis.

The mesoclimatic analysis presented here highlighted the differences between seasons in Waipara region. In general, 2013-2014 showed temperatures slightly higher than the 5YA. 2014-2015 had a mean temperature 1°C larger than the 5YA. Interestingly, in both seasons the ETo was lower than the 5YA, despite higher temperatures being recorded. Other factors considered in the Penman-Monteith equation proposed by Allen *et al.* (1998), such as wind speed, solar radiation, and relative humidity, are also relevant to determine the evaporative losses for a region. Rainfall showed a significant seasonal variation, with almost 3-fold difference between 2013-2014 and 2014-2015. The precipitation accumulated during 2013-2014 was larger than the 5YA average at all locations, whereas 2014-2015 proved to be very dry compared either to the 5YA or the 2014 season. It is important to highlight that the main climatic differences between locations and seasons were observed during ripening, which may have an impact on the physiological parameters that will be analysed in the next chapters.

Differences were also found for GDD accumulation within the region and between seasons, but with no clear patterns observed. When wind speed was compared within the region, the West and middle part of Waipara consistently registered the higher average values. In contrast, the wind speed in the East part of the valley was about four times lower than the other areas, which as described earlier, could have an important impact on calculating the ETo values.

The microclimatic analysis revealed important differences across the three sites selected for this research. GB5 registered the highest temperatures, and therefore, the higher GDD accumulation across all the sites in either season. Contrary to expectations, WH and GB10 were found to have similar climatic conditions, despite being about 5.4 km apart, and there were large differences between two plots located about 770 m apart (GB5 and GB10). Although a comparison between the closest weather station and the sites was not possible for maximum, minimum, and mean temperature due to the differences in methodologies previously explained, GDD accumulation, which was calculated in the same manner in both sets of data, showed an approximation to the differences between the data registered by the weather stations and the real conditions of each plot. Thus, in both 2013-2014 and 2014-2015 all the dataloggers reported higher GDD accumulation than the registered by the weather stations, ranging these between 101 and 227 more GDD in 2013-2014, and between 145 and 372 more GDD in 2014-2015. The largest difference was found when GB5 and Muddy Water weather station were compared.

The results of water balance, which include monthly and total irrigation, rainfall, total water, and ETo, reported marked differences between seasons as well as differences among sites. First of all, in both seasons WH was the site registering the higher amount of irrigation, mainly due to the lower profile available water, which is less than half than that in either GB5 or GB10. Glasnevin soils have textures often described as gravelly sandy loam or gravelly silt loam.

Between the two sites located at Greystone vineyard, GB10 received in both seasons about twice the volume of irrigation water compared to GB5. These sites had soils with heavier textures than the WH site, described as silt loam and clay loam, respectively. Thus, the large difference in irrigation water applied may be due to other factors, such as the higher wind speed previously described for GB10, promoting a higher evaporative demand, which evidenced a premature stress, conditioning the decision of irrigating this plot more often than GB5, although the latter showing higher temperatures. As reported in results, the soil at GB10 has a rooting barrier of fracturing rock at about 55 cm depth, which also influence the decision of irrigating this plot more often than GB5 due to its limited capacity of holding water.

As previously discussed, the end of the first season registered an exceptional amount of rainfall, which contributed to alter the total water balance of the season by reducing the water deficit (less

negative water balance) to a values less than half than those in 2014-2015 for all sites. The drier second season registered a total ETo about 125 mm higher than the first one, which together with the lower precipitation resulted in a higher (more negative) water deficit for all sites. For all sites in both seasons, January reported the highest water deficit, mainly due to the high ETo and low rainfall, which was compensated by the irrigation applied. In January 2014, the total water (irrigation + rainfall) at WH represented a 32% of the ETo for CON and a 21% for RI (data not shown). For GB5, these percentages corresponded to 11% and 10% for CON and RI, respectively. For GB10, the total water in CON represented a 14% of the ETo, which is higher than that in GB5 due to the higher amount of irrigation applied to GB10 in 2013-2014, while that for RI was 11%. During the second drier season, these percentages for January decreased at WH and GB5, while in GB10 these remained almost unaltered. Thus, the percentage of the ETo that the total water represented corresponded to 21% for CON and 17% for RI at WH, 10% for CON and 8% for RI at GB5, and 14% for CON and 10% for RI at GB10. Due to the high amount of rainfall by the end of the first season, the total water applied for the season represented about 60% of the ETo for all sites, being only slightly lower for the RI treatment. In the second season, the differences between CON and RI for water balance and the percentage that total water applied as a proportion of ETo were more pronounced as higher proportion of the water received by the vines was as irrigation. Thus, the percentage that the total water of the season represents for CON and RI in WH was 33% and 26%, respectively. At GB5, these were 22% for CON and 20% for RI, while in GB10 these reported 26% for CON and 22% for RI. Thus, in addition to differences in water availability imposed through irrigation treatments (CON, RI), there were differences between sites and in the seasonal distribution in each season.

## 2.5 Conclusions

This analysis of the mesoclimate of the Waipara region, together with some detailed microclimate measurements has shown distinct differences both across the Waipara area, as well as the variations across the sites selected for the different evaluations. Differences between seasons were also found, whose potential effect on physiological parameters, and grape and wine composition will be evaluated in the following chapters.

The large differences between two plots located in the same property, and also similarities between two plots separated by more than 5 km, underlined the importance of evaluating the climatic conditions of each plot in particular. These results contribute to the understanding of the spatial variability across the valley, which may have a big impact in terms of irrigation and viticultural practices by increasing the water use efficiency, and therefore, reducing the operational costs for the winegrowers of the region.

The results of water balance calculations showed that soil characteristics were an important driver of irrigation amount and frequency for Pinot noir in Waipara. The total amount of water applied followed the order WH>GB10>GB5 in both seasons, although the differences were rather small in 2013-2014 because of large rainfall events at the end of the season. Thus, as anticipated the available water-holding capacity of the soils (influenced by texture and rooting depth in this study) was influential in determining the irrigation water applied at each site.

Overall, these data suggest that the sites selected for this study demonstrate important differences in the environments experienced the vines. As indicated above, these sites were selected in part because differences in vine material (rootstock), vine age, vine spacing, and trellis systems were relatively minor. Therefore, in addition to providing information on the effect of restricting irrigation, they provide a useful basis for exploring the broader influences of environment, and in particular water availability, on grape and wine composition.

# Chapter 3

# The effect of reduced irrigation on grapevine physiology at three Pinot noir vineyards with contrasting soils

## 3.1 Introduction

Water stress induces changes in vine water status, but the intensity of these varies across varieties, regions, climatic and soil conditions, among other factors. It is forecasted that water deficit may become a limiting factor in wine production, which already occurring in some cool climate regions (Chaves *et al.* 2010). Grapevines have developed some drought adaptive responses, but those are cultivar-dependent, as well as site-related. For example, Pinot noir has shown to be very sensitive to water stress, but its adaptive strategy to deal with soil water scarcity varies depending on the intensity of water stress and climatic conditions (Lovisolo *et al.* 2010). So, knowing how Pinot noir behaves under a water deficit scenario might contribute to anticipating some viticultural issues that the wine industry will face in the near future.

There are several methods to evaluate plant water status. Some of them are designed to detect variations in plant water status in the short term, while others aim to report the accumulated effect of water availability over the season (Choné *et al.* 2001, Tomás *et al.* 2014, Tomás *et al.* 2012). Stem and leaf water potential are commonly used by viticulturists to schedule irrigation, but others require specialised equipment and are almost exclusively used in viticultural science. Among them, stomatal conductance (Bondada and Shutthanandan 2012, Flexas *et al.* 2002), leaf carbon isotope ratio (Santesteban *et al.* 2015), leaf area (Fernandes De Oliveira *et al.* 2013b, Intrigliolo and Castel 2009), leaf proline content (Kavi Kishor and Sreenivasulu 2014), and pruning weight (Edwards and Clingeleffer 2013) have been frequently included in vine water stress studies, whereas chlorophyll levels, leaf osmotic potential, and root carbohydrates concentration have been less considered.

The objective of this research was to evaluate the physiological effects of reducing irrigation about 50% in three commercial Pinot noir vineyards in Waipara over two seasons.

## 3.2 Materials and methods

### 3.2.1 Experimental design

The experimental design, reduced irrigation treatments, and edapho-climatic conditions of each site and season have been described in Chapter 2. Please refer there for more details.

### 3.2.2 Phenology

The system for identifying grapevine growth stages of Eichhorn and Lorenz (1977) modified by Coombe (1995) was used to describe the main phenological stages through the seasons. The date in which budburst, full bloom, fruit-set, veraison, and harvest occurred were recorded and used to determine differences between sites and treatments for this parameter.

### 3.2.3 Leaf area

At veraison, primary and lateral leaf area per plant was determined using the non-destructive method proposed by Lopes and Pinto (2005). As the method has not been used in the past for Pinot noir, this was validated prior to the field evaluation in both seasons, as described below.

#### Validation

The method was validated by randomly collecting 20 entire shoots per site about one week prior to the field evaluation in both seasons. All leaves from each shoot were removed and counted. Then, individual leaf area was determined by a leaf area meter (LI-3100; LICOR, Lincoln, NE), and recorded with respect to primary and lateral position.

The observed primary leaf area per shoot was determined by adding the individual leaf area of all the primary leaves of the shoot. The same protocol was followed for the observed lateral leaf area. To determine the estimated leaf area per shoot (both primary and lateral), five variables were calculated as described in Lopes and Pinto (2005): area of the largest leaf, area of the smallest leaf, mean leaf area [(area of the largest leaf + area of the smallest leaf) / 2)], number of leaves, and the mean leaf area per shoot [(mean leaf area * number of leaves)]. For primary leaf area, leaves from the basal node and those whose leaf area at veraison represented less than the 30% of the original expanded leaf (mainly due to mechanical damage) were excluded, as well as young unexpanded leaves with a primary vein length <3 cm. Under the conditions of this research, the latter showed a leaf area about 15 $cm^2$ or lower. For lateral leaf area, a lateral was measured if it had at least three expanded leaves, each with more than 3 cm of primary vein length. Leaves damaged or showing an abnormal shape were also not considered.

A linear regression analysis was performed to determine the relationship between observed and estimated leaf area (both primary and lateral), in order to calibrate the method under the particular conditions of this study. Microsoft® Excel® 2013 was used to obtain the regression equation and coefficient of determination ($R^2$) for each site (Appendix A). Each equation was used later to calculate the estimated leaf area per shoot from the field data.

**Field evaluation**

At veraison in both seasons, the number of shoots per plant was counted from each plant of the trial. Then, the number of leaves per shoot was counted from two shoots per plant (one per side). The smallest and largest leaves were collected from those shoots and transported to the laboratory to determine their individual area using a leaf area meter (LI-3100; LICOR, Lincoln, NE). These data were used to calculate the leaf area per shoot, which was multiplied by the number of shoots per plant, to obtain the estimated leaf area per plant. The same procedure was used to determine lateral leaf area.

### 3.2.4 Point Quadrat

A Point Quadrat (PQ) assessment was done at the same time as leaf area following the method described in Smart and Robinson (1991). Insertions were made at 10 cm intervals on each vine (11 insertions per plant), and the sequential contacts of leaves, clusters and canopy gaps in the fruit zone were recorded and used to calculate the percent gaps (PG), leaf layer number (LLN), percent interior leaves (PIL), and percent interior clusters (PIC).

### 3.2.5 Stem water potential

Stem water potential ($\psi_s$) (MPa) was determined from one healthy and fully expanded leaf per replicate, selected from the middle part of the shaded side of the canopy, as described in Choné *et al.* (2001). Leaves were covered with both a sandwich-sized zip-lock-type bag and aluminium foil at least two hours before the evaluation. Measurements were performed near noon (11:30 - 13:30), using a pressure chamber (Model 3000; Soil Moisture Equipment Corporation, Santa Barbara, CA). During the measurements, the precautions suggested by Turner (1988) were considered. Irrigation was stopped at least 48 hours before the evaluation, to standardise the conditions of all the sites. For any given date, all sites were evaluated during the same day.

### 3.2.6 Stomatal conductance

Stomatal conductance ($g_s$) (mmol $m^{-2}s^{-1}$) was measured on three healthy and expanded leaves per replicate, selected from the mid part of the sun-exposed side of the canopy. It was evaluated near noon (11:30-13:30) on a sunny day, using a leaf porometer (Model SC-1; Decagon Devices, Inc.,

Pullman, WA). This was performed at veraison and pre-harvest in both seasons. For any given date, all sites were evaluated during the same day. As well as for $\psi_s$, irrigation was stopped at least 48 hours before the evaluation, to standardise the conditions of all the sites.

### 3.2.7 Leaf proline content

Proline content was determined as described initially in Magné and Larher (1992) and later modified by Hofmann *et al.* (2003). One leaf per replicate from the middle part of the canopy were collected into sandwich-sized zip-lock-type bags, transported to the laboratory at a cool temperature, frozen, and stored at -20°C until analysed. These were collected at 5 different dates during the season, with all leaves for any given date being collected during the same day for all sites. Leaves were ground in liquid nitrogen, freeze-dried and 30 mg weighed into 1.7 mL clear microtubes. Proline was extracted in 1.2 mL of 3% (w/v) sulphosalicylic acid (Merck Schuchardt OHG, Hohenbrunn, Germany) under vortex shaking for 20 s and the mixture then centrifuged at 12,000 *g* for 7 min. 700µL of supernatant were removed and after renewed centrifugation, 500µL collected and the volume adjusted to 1 mL with deionized water into a 10 mL screw cap test tube. Then, 2.0 mL of ninhydrin reagent was added and the tube vortexed for 20 s. Ninhydrin reagent was prepared by warming 1% (w/v) ninhydrin (Sigma-Aldrich, St. Louis, MO) in 60% (v/v) glacial acetic acid (HPLC grade, Fisher Scientific UK Limited, UK) under constant stirring until dissolved. Sample tubes were incubated in a water bath for 1 h at 98 °C and the reaction was stopped in an ice-water bath. The reaction mixture was extracted with 2 mL of toluene (92.14 g/mol, Analar®, VWR International Ltd., England) and vortex shaking for 20 s. The phases were allowed to separate for at least 10 minutes at room temperature and 1 mL of the toluene (top phase) was carefully collected and examined spectrophotometrically in a 10 mm pathlength quartz cuvette (Starna Pty Ltd, NSW, Australia) at 520 nm, using a Shimadzu UV-1800 Spectrophotometer (Shimadzu Corporation, Kyoto, Japan). The concentration of free proline was calculated from a standard curve of known proline concentration (0 – 10 µg $mL^{-1}$) by diluting a stock solution of 100 µg/mL of proline (99+%, Sigma-Aldrich Chemical Company, WI) in 3% (w/v) sulphosalicylic acid to complete 1.2 mL.

The performance of this analytical procedure was evaluated using spiked samples. These followed exactly the same protocol as the leaf samples alongside each batch of samples. The percent recovery varied from 90.6 to 92.5% (data not shown).

### 3.2.8 Leaf osmotic potential

Osmolality was measured from one leaf per replicate at different times through the season. Leaves from the middle part of the canopy were collected into sandwich-sized zip-lock-type bags, transported to the laboratory at a cool temperature, frozen, and stored at -20°C until analysed.

Leaves broken into small pieces, thawed at room temperature (21°C), placed into 1.7mL micro tubes, submerged into liquid nitrogen, and then, centrifuged at 13,400 *g* for three minutes. Osmolality (mmol $kg^{-1}$) was analysed on 10 µL of sap using a vapour pressure osmometer (Wescor VAPRO® 5520; Logan, UT). Osmolality was converted to osmotic potential ($\psi_\pi$) (MPa) using the conversion factor -0.002438, based on the Van't Hoff equation, as described in Romero *et al.* (2012).

### 3.2.9 Estimated leaf chlorophyll content

Leaf chlorophyll content was estimated with a chlorophyll meter (SPAD-502; Minolta Co., Ltd, Tokyo, Japan) from veraison to pre-harvest. The average of five measurements from five different leaves per plant from above the cluster zone ($4^{th}$ to $6^{th}$ leaves) was used for comparisons. The results were reported in SPAD units.

### 3.2.10 Carbon isotope ratio in leaf dry matter

Two leaves per replicate, located above the cluster zone, were collected at harvest, transported to the laboratory at a cool temperature, ground in liquid nitrogen and freeze-dried. Carbon isotope composition ($\delta^{13}C$) from 4 mg of freeze-dried leaf material was analysed by EA-IRMS (Elemental Analyser Isotope Ratio Mass Spectrometry), using a Sercon GSL elemental analyser (Crewe, UK), and a Sercon 20-22 IRMS (Isotope Ratio Mass Spectrometer). Samples were analysed with a duplication rate of one in eight. $\delta^{13}C$ was calculated as proposed by Farquhar and Richards (1984). All values were referenced to Vienna-Pee Dee Belemnite standard (V-PDB).

### 3.2.11 Root carbohydrates

#### Sample collection and preparation

Root samples with a diameter between 5 and 10 mm were collected from near the base of the vines during midwinter (June) in 2015 only. The three middle plants of each replicate were selected for this. The samples were washed with distilled water, cut into smaller pieces to facilitate freeze-drying, weighed, and then frozen at -20°C on the day of collection. One week later, the samples were freeze-dried, weighed, ground using a coffee grinder with a stainless steel blade system (Breville BCG200, Australia), and finally ground in liquid nitrogen, as suggested by Rose *et al.* (1991). Root water content (%) was calculated from the difference between fresh and dry weights.

#### Soluble sugar analysis

The methods of Allen *et al.* (1974) and Rose *et al.* (1991), including the modifications suggested by Bennett (2002), were used to determine soluble sugar concentration in grapevine root samples. Soluble sugars were extracted from 100 mg ground samples using 10 mL of 80% ethanol in a hot water bath at 85°C for 10 minutes. The tubes were centrifuged at 1,260 *g* for 8 minutes and the

supernatant transferred to a new tube and stored in the refrigerator until required. The pellet was resuspended in 5 mL of 80% ethanol, heated at 85°C for 10 minutes and centrifuged as same as the first extraction. The supernatant was pooled with the first extraction and stored in the refrigerator, covered with aluminium foil to prevent exposure to the bright light, until analysed. On the same day, 1 mL aliquots of soluble sugar extracts were mixed with 10 mL of anthrone reagent and heated at 85°C for 15 minutes to allow colour reaction. The anthrone reagent was prepared dissolving 1.5 g of anthrone (A-1631; Sigma Chemical Co., St. Louis), 1.0 g of thiourea (VWR International BVBA, Belgium), in 700 mL $H_2SO_4$ (95-97%; Scharlab S. L., Spain): 300 mL deionised water, using an ultrasonic water bath. The absorbance was measured at 625nm using a Shimadzu UV-1800 Spectrophotometer (Shimadzu Corporation, Kyoto, Japan). D(+) glucose (AnalaR®; BDH Laboratory Supplies, England) was used as standard in the range of 0 to 0.8 mg/mL.

After absorbances were corrected by the equation obtained from the standards, soluble sugar concentration was calculated using the equation described in Allen *et al.* (1974), as follows:

$$\text{Soluble carbohydrates (\%)} = \frac{\text{C (mg)} * \text{extract volume (mL)}}{10 * \text{aliquot (mL)} * \text{sample wt (g)}}$$

Where: C = mg glucose obtained from the standard curve

### Starch enzymatic digestion

After the second soluble sugar extraction, the remaining solid tissue was dried in an oven at 85°C for 1.5 hours to evaporate ethanol and water. Once dried, the pellet was resuspended in 5 mL of deionised water and tightly capped. Starch was gelatinised by heating the tubes in a hot water bath at 85°C for one hour. The reaction was then stopped in an ice water bath for 15 minutes. 1 mL of starch digestion solution containing 400 enzyme units/mL α-amylase (A6255 – 25MG; Sigma-Aldrich Co., USA), 2 enzyme units/mL amyloglucosidase (A1602; Sigma-Aldrich Co., USA), adjusted to pH 5.1 using sodium acetate buffer, was added to the tubes and incubated at 50°C for 48 hours in the dark. After incubation, the samples were centrifuged at 1,260 *g* for 5 minutes and the supernatant transferred to a new tube for analysis. Supernatant aliquots of 100 µL and glucose standards in the range of 0 to 2.5 mg/mL were mixed with 5 mL of o-toluidine reagent (1.0 g thiourea (VWR International BVBA, Belgium), 940 mL glacial acetic acid (Univar, Auckland, NZ), and 60 mL o-toluidine (99+%, 185426-100G; Sigma-Aldrich, USA), capped and heated for 20 minutes in the dark at 85°C in a hot water bath. The absorbance at 625nm was measured using a Shimadzu UV-1800 Spectrophotometer (Shimadzu Corporation, Kyoto, Japan).

After correcting absorbances by the equation obtained from the standards, starch concentration (mg of starch/mg of sample) was calculated using the equation described in Rose *et al.* (1991), as follows:

$$\text{mg of starch/mg of sample} = y_g d_f v h_f / dw$$

where $y_g$ is the glucose concentration (mg/mL), $d_f$ is the dilution factor (if needed), v is the original volume of starch extract (5mL deionised water + 1 mL starch digestion solution = 6mL), $h_f$ is the starch hydrolysis factor 0.9 (Volenec, 1986), and dw is the original dry weight of the sample (mg).

### 3.2.12 Pruning weight

At the end of the second season (pruning weights in the first season were not able to be collected due to vineyard manager's oversight), pruning weight was determined to quantify the cumulative effect of the treatment on this parameter after two seasons of study. Each plant was individually pruned and the weight of all removed parts determined *in situ* using a digital scale. Afterwards, pruning weight was calculated per metre of row.

### 3.2.13 Statistical analyses

A series of statistical analyses were performed depending on the set of data. Firstly, a one-way analysis of variance (ANOVA) and, equivalently, the least significant difference (LSD) test were used to determine statistical differences between CON and RI for each site and season at the 5% level ($p < 0.05$). Then, to evaluate the average treatment difference across sites, the two treatment means for each site were input into a randomised complete block design ANOVA with blocking factor "site" and treatment factor "treatment" with LSD test at 5% for leaf area, Point Quadrat, stem water potential, stomatal conductance, leaf $\delta^{13}C$, root carbohydrates, and pruning weight. Data obtained at several points of the season, such as leaf osmotic potential, estimated leaf chlorophyll content, and proline were analysed in the same manner, but in this case and in order to evaluate cumulative effects, the mean value for each variable derived using the area under the curve (AUC) was calculated from all values of the season and used in the above ANOVAs. Finally, and only for sets of data including the two seasons, the seasonal effect was obtained by calculating the mean difference between the seasons 2013-2014 and 2014-2015 for the same treatment and site (e.g. difference between CON WH 2013-2014 and CON WH 2014-2015), using the above randomised complete block design ANOVA with blocking factor "site" and treatment factor "treatment". All the analyses were performed using Genstat 16 (GenStat for Windows, VSN International Limited, UK).

Residual plots, including plots of residuals against fitted-value and histogram of residuals were obtained for each set of data to evaluate whether data need any square root or logarithm transformation. Based on this, no transformations were carried out.

All means, LSD 5%, and p values for all two-way ANOVAs of this chapter can be found in Appendix B.

A linear regression analysis was performed to investigate the relationship between $\psi_s$ and $g_s$ at veraison and pre-harvest. Microsoft® Excel® 2013 was used to obtain the regression equation and coefficient of determination ($R^2$) and Genstat 16 (GenStat for Windows, VSN International Limited, UK) was used to calculate the significance at $p < 0.05$.

No statistical analyses were carried out for phenology data.

## 3.3 Results

### 3.3.1 Phenology

Reduced irrigation did not appear to alter the occurrence of the main phenological stages at any site over the two seasons, and few differences between sites and seasons were observed (Figure 17). Phenological development followed a particular dynamic at each site, with the period between fruit-set and harvest showing the main differences across sites in both seasons.

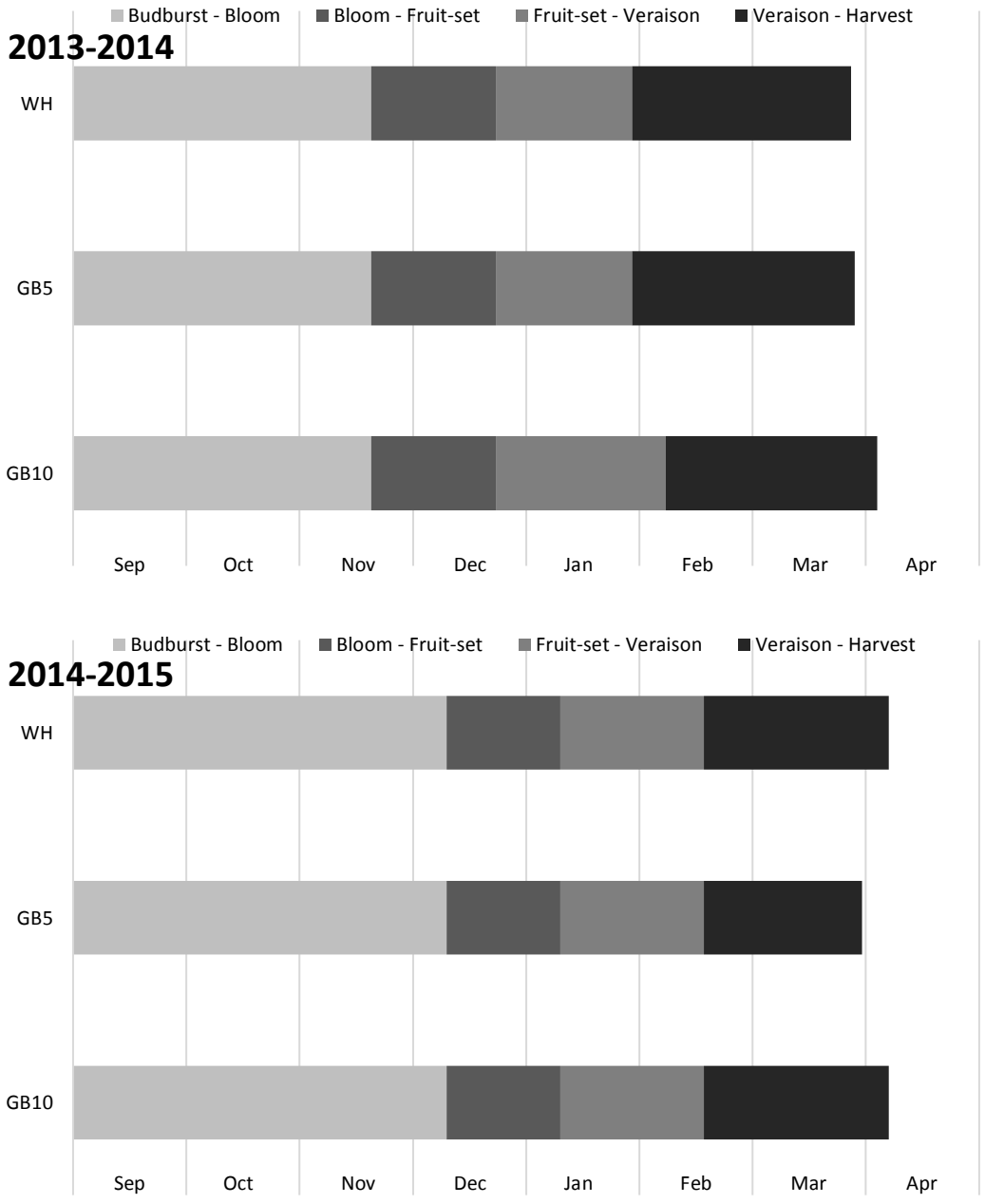


**Figure 17 Occurrence of main phenological stages during the 2013-2014 and 2014-2015 seasons. No differences between treatments were detected in either season or any site**

Harvest dates (based on reaching a target Brix) were very similar at all sites in either season (late March – early April). Season 2013-2014 had an average duration of 209 days, whereas 2014-2015 was longer with 214 days. GB10 was the last site harvested in both seasons.

The period between budburst and bloom lasted for 80 days in 2013-2014, but was about 20 days longer in 2014-2015. The number of days between bloom and fruit-set remained the same either in 2013-2014 or 2014-2015, as same as the period between fruit-set and veraison, except for GB10 being a bit longer than the others in 2013-2014. Although 2014-2015 growing season was few days longer than 2013-2014, the ripening period (between veraison and harvest) was about 10 days shorter.

### 3.3.2 Leaf area

#### Primary leaf area

Primary leaf area was reduced by RI at GB5 in either season, while this was affected at GB10 in the second season only. In contrast, primary leaf area was not affected by RI in either season at WH. (Table 7). RI at GB5 showed a 27% reduction of primary leaf area in 2013-2014 compared to CON, whereas this was 60% in the next season. A similar trend was found at GB10, with the plants under RI showing a 57% smaller leaf area values than CON in 2014-2015.

**Table 7 Primary leaf area ($m^2$/plant) during seasons 2013-2014 and 2014-2015. Each value is the average of four replicates.**

| Season 2013-2014 | | | |
|---|---|---|---|
| Treatment | Site | | |
| | WH | GB5 | GB10 |
| CON | 4.72 | 4.88 | 1.81 |
| RI | 3.93 | 3.57 | 2.98 |
| LSD 5% | 1.15 | 1.06 | 0.68 |
| p value | 0.142 | **0.024** | 0.241 |
| Season 2014-2015 | | | |
| Treatment | Site | | |
| | WH | GB5 | GB10 |
| CON | 4.67 | 4.42 | 3.08 |
| RI | 3.23 | 1.76 | 1.31 |
| LSD 5% | 1.79 | 1.01 | 1.24 |
| p value | 0.095 | **<.001** | **0.013** |

Figure 18 illustrates the differences between CON and RI at GB5 during the second season. The reduction of primary leaf area was noticeable about three weeks before harvest, when the pictures were taken.

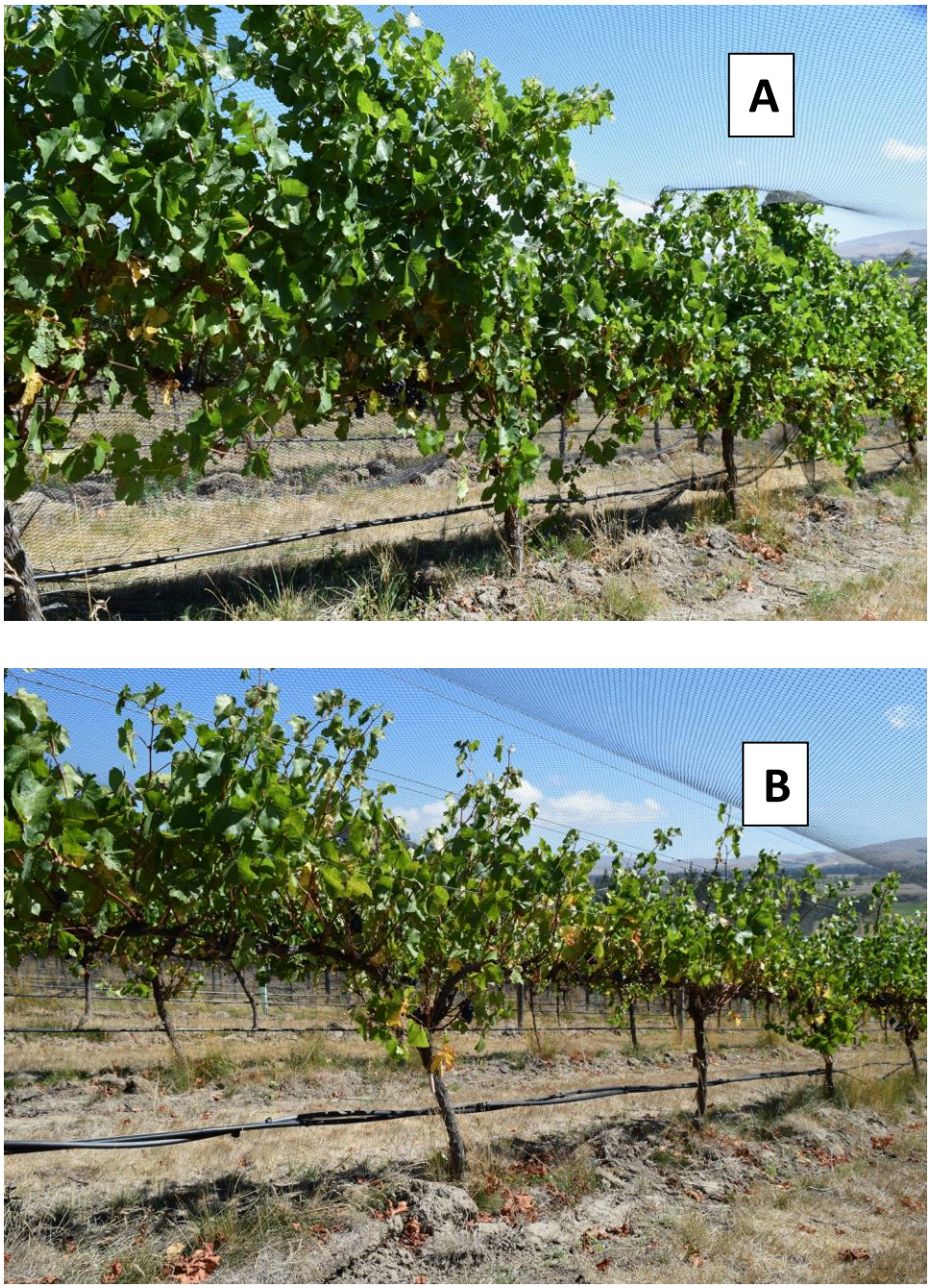


**Figure 18 Differences in leaf area between CON (A) and RI (B). Pictures were taken at GB5 on the 10th of March 2015 during the second season**

The results in Table 8 indicate that there were differences across sites in 2013-2014, while the treatment did not have an overall effect. The opposite tendency was found in 2014-2015.

The combined analysis including all the data from all sites, treatments, and seasons indicated an effect of all factors.

**Table 8 Results of two-way ANOVA on the effects of "site" (WH, GB5 and GB10), "treatment" (CON and RI), and "season" on primary leaf area. Numbers correspond to p values.**

| Factor | Season | |
|---|---|---|
| | 2013-2014 | 2014-2015 |
| Site | **0.023** | 0.113 |
| Treatment | 0.095 | **0.033** |
| | Combined analysis | |
| Site | **0.046** | |
| Treatment | **0.043** | |
| Season | **0.042** | |

## Lateral leaf area

Overall, RI did not affect lateral leaf area, except at GB10 in 2014-2015 (Table 9).

**Table 9 Lateral leaf area ($m^2$/plant) seasons 2013-2014 and 2014-2015. Each value is the average of four replicates.**

| Season 2013-2014 | | | |
|---|---|---|---|
| Treatment | Site | | |
| | WH | GB5 | GB10 |
| CON | 0.47 | 0.36 | 0.41 |
| RI | 0.50 | 0.34 | 0.28 |
| LSD 5% | 0.23 | 0.19 | 0.30 |
| p value | 0.759 | 0.836 | 0.333 |
| Season 2014-2015 | | | |
| Treatment | Site | | |
| | WH | GB5 | GB10 |
| CON | 0.45 | 0.43 | 0.48 |
| RI | 0.23 | 0.17 | 0.10 |
| LSD 5% | 0.36 | 0.62 | 0.30 |
| p value | 0.176 | 0.348 | **0.021** |

Table 10 shows no effect of either site or treatment in 2013-2014, whereas only treatment showed differences in 2014-2015. The combined analysis revealed differences between seasons only.

**Table 10 Results of two-way ANOVA on the effects of "site" (WH, GB5 and GB10), "treatment" (CON and RI), and "season" on lateral leaf area. Numbers correspond to p values.**

| Factor | Season | |
|---|---|---|
| | 2013-2014 | 2014-2015 |
| Site | 0.212 | 0.722 |
| Treatment | 0.503 | **0.027** |
| | Combined analysis | |
| Site | 0.388 | |
| Treatment | 0.075 | |
| Season | **<.001** | |

### 3.3.3 Point Quadrat

#### Percent of gaps (PG)

In general, no differences were observed between treatments for PG. Only at WH in 2014-2015 did RI increase PG (Table 11).

**Table 11 Percent of gaps (PG) for seasons 2013-2014 and 2014-2015. Each value is the average of four replicates.**

| Season 2013-2014 | | | |
|---|---|---|---|
| Treatment | Site | | |
| | WH | GB5 | GB10 |
| CON | 5.45 | 4.55 | 3.64 |
| RI | 3.18 | 6.82 | 6.37 |
| LSD 5% | 6.64 | 12.96 | 7.59 |
| p value | 0.435 | 0.683 | 0.412 |
| Season 2014-2015 | | | |
| Treatment | Site | | |
| | WH | GB5 | GB10 |
| CON | 3.79 | 2.27 | 4.55 |
| RI | 10.61 | 21.97 | 15.15 |
| LSD 5% | 6.68 | 29.15 | 14.04 |
| p value | **0.047** | 0.149 | 0.114 |

No differences in PG were found between sites and treatments in either season. The same result was seen in the combined analysis (Table 12).

**Table 12 Results of two-way ANOVA on the effects of "site" (WH, GB5 and GB10), "treatment" (CON and RI), and "season" on percent of gaps (PG). Numbers correspond to p values.**

| Factor | Season | |
|---|---|---|
| | 2013-2014 | 2014-2015 |
| Site | 0.805 | 0.643 |
| Treatment | 0.626 | 0.084 |
| | Combined analysis | |
| Site | 0.657 | |
| Treatment | 0.118 | |
| Season | 0.062 | |

## Leaf layer number (LLN)

No differences were observed between CON and RI with regard to LLN during the 2013-2014 season, contrasted with 2014-2015 where RI reduced LLN at all sites (Table 13).

**Table 13 Leaf layer number (LLN) for seasons 2013-2014 and 2014-2015. Each value is the average of four replicates.**

| Season 2013-2014 | | | |
|---|---|---|---|
| Treatment | Site | | |
| | WH | GB5 | GB10 |
| CON | 1.80 | 1.87 | 2.36 |
| RI | 2.05 | 1.41 | 1.89 |
| LSD 5% | 0.50 | 1.08 | 0.67 |
| p value | 0.272 | 0.339 | 0.133 |
| Season 2014-2015 | | | |
| Treatment | Site | | |
| | WH | GB5 | GB10 |
| CON | 2.14 | 3.04 | 2.94 |
| RI | 1.59 | 1.27 | 1.38 |
| LSD 5% | 0.30 | 0.94 | 1.10 |
| p value | **0.004** | **0.004** | **0.013** |

No differences were found in LLN across sites and treatments in either season. Differences between seasons were reported only from the combined analysis (Table 14).

**Table 14 Results of two-way ANOVA on the effects of "site" (WH, GB5 and GB10), "treatment" (CON and RI), and "season" on leaf layer number (LLN). Numbers correspond to p values.**

| Factor | Season | |
|---|---|---|
| | 2013-2014 | 2014-2015 |
| Site | 0.414 | 0.788 |
| Treatment | 0.436 | 0.076 |
| | Combined analysis | |
| Site | 0.775 | |
| Treatment | 0.131 | |
| Season | **0.019** | |

## Percent interior leaves (PIL)

Differences were found for PIC at all sites during the second season only, where CON had 60% to 70% more interior leaves than RI at GB5 and GB10, with this difference being about 30% at WH (Table 15).

**Table 15 Percent interior leaves (PIL) for seasons 2013-2014 and 2014-2015. Each value is the average of four replicates.**

| Season 2013-2014 | | | |
|---|---|---|---|
| Treatment | Site | | |
| | WH | GB5 | GB10 |
| CON | 23.7 | 23.5 | 35.7 |
| RI | 23.0 | 17.6 | 27.8 |
| LSD 5% | 7.90 | 14.60 | 10.16 |
| p value | 0.830 | 0.367 | 0.106 |
| Season 2014-2015 | | | |
| Treatment | Site | | |
| | WH | GB5 | GB10 |
| CON | 22.5 | 39.4 | 40.4 |
| RI | 15.7 | 10.8 | 12.4 |
| LSD 5% | 6.52 | 17.24 | 14.99 |
| p value | **0.045** | **0.007** | **0.004** |

Table 16 shows that the “site” and “treatment” did not have an effect on PIL, as same as the combined analysis, where no differences between seasons were found.

**Table 16 Results of two-way ANOVA on the effects of “site” (WH, GB5 and GB10), “treatment” (CON and RI), and “season” on percent interior leaves (PIL). Numbers correspond to p values.**

| Factor | Season | |
|---|---|---|
| | 2013-2014 | 2014-2015 |
| Site | 0.091 | 0.719 |
| Treatment | 0.152 | 0.099 |
| | Combined analysis | |
| Site | 0.481 | |
| Treatment | 0.107 | |
| Season | 0.088 | |

## Percent interior clusters (PIC)

RI had an effect on PIC at WH in 2013-2014. During the second season, differences between CON and RI were found at GB5 only (Table 17).

**Table 17 Percent interior clusters (PIC) for seasons 2013-2014 and 2014-2015. Each value is the average of four replicates.**

| Season 2013-2014 | | | |
|---|---|---|---|
| Treatment | Site | | |
| | WH | GB5 | GB10 |
| CON | 30.3 | 31.6 | 45.7 |
| RI | 51.3 | 25.2 | 40.4 |
| LSD 5% | 16.48 | 28.49 | 21.83 |
| p value | **0.021** | 0.606 | 0.576 |
| Season 2014-2015 | | | |
| Treatment | Site | | |
| | WH | GB5 | GB10 |
| CON | 33.3 | 65.0 | 42.7 |
| RI | 8.3 | 11.3 | 14.6 |
| LSD 5% | 35.32 | 41.98 | 56.75 |
| p value | 0.134 | **0.020** | 0.271 |

No differences were described across sites and treatments in either season. Differences between seasons were only present in the combined analysis (Table 18).

**Table 18 Results of two-way ANOVA on the effects of "site" (WH, GB5 and GB10), "treatment" (CON and RI), and "season" on percent interior clusters (PIC). Numbers correspond to p values.**

| Factor | Season | |
|---|---|---|
| | 2014 | 2015 |
| Site | 0.492 | 0.452 |
| Treatment | 0.759 | 0.059 |
| | Combined analysis | |
| Site | 0.886 | |
| Treatment | 0.183 | |
| Season | **0.040** | |

### 3.3.4 Stem water potential ($\psi_s$)

The results in Table 19 show the dynamic of $\psi_s$ at three different phenological stages over the two seasons. No differences between CON and RI were found overall, with WH at pre-harvest in 2013-2014 being the only exception.

**Table 19 Stem water potential (MPa) at fruit-set, veraison, and pre-harvest during the 2013-2014 and 2014-2015 seasons. Each value is the average of four replicates.**

| Season 2013-2014 | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Fruit-set | | | Veraison | | | Pre-harvest | | |
| Treatment | Site | | | | | | | | |
| | WH | GB5 | GB10 | WH | GB5 | GB10 | WH | GB5 | GB10 |
| CON | -0.2 | -0.3 | -0.3 | -0.6 | -0.7 | -1.0 | -0.4 | -0.6 | -0.6 |
| RI | -0.2 | -0.2 | -0.3 | -0.8 | -0.8 | -1.0 | -0.4 | -0.6 | -0.7 |
| LSD 5% | 0.10 | 0.13 | 0.10 | 0.29 | 0.55 | 0.46 | 0.05 | 0.30 | 0.35 |
| p value | 0.766 | 0.207 | 0.780 | 0.089 | 0.709 | 0.848 | **0.017** | 0.921 | 0.564 |
| Season 2015-2014 | | | | | | | | | |
| | Fruit-set | | | Veraison | | | Pre-harvest | | |
| Treatment | Site | | | | | | | | |
| | WH | GB5 | GB10 | WH | GB5 | GB10 | WH | GB5 | GB10 |
| CON | -0.3 | -0.3 | -0.3 | -0.7 | -0.8 | -0.9 | -0.9 | -0.7 | -0.7 |
| RI | -0.3 | -0.2 | -0.4 | -0.8 | -1.1 | -1.0 | -0.9 | -1.1 | -0.9 |
| LSD 5% | 0.15 | 0.16 | 0.12 | 0.10 | 0.46 | 0.41 | 0.15 | 0.36 | 0.36 |
| p value | 0.439 | 0.483 | 0.176 | 0.194 | 0.161 | 0.573 | 0.254 | 0.060 | 0.390 |

Confirming the results previously presented, none of the factors had an effect on $\psi_s$ over the two seasons at fruit-set, veraison, or pre-harvest (Table 20).

**Table 20 Results of two-way ANOVA on the effects of "site" (WH, GB5 and GB10), "treatment" (CON and RI), and "season" on stem water potential at fruit-set, veraison, and pre-harvest. Numbers correspond to p values.**

| | Fruit-set | | Veraison | | Pre-harvest | |
|---|---|---|---|---|---|---|
| Factor | Season | | Season | | Season | |
| | 2013-2014 | 2014-2015 | 2013-2014 | 2014-2015 | 2013-2014 | 2014-2015 |
| Site | 0.176 | 0.568 | 0.082 | 0.237 | 0.087 | 0.536 |
| Treatment | 0.251 | 0.860 | 0.182 | 0.172 | 0.800 | 0.147 |
| | Combined analysis | | Combined analysis | | Combined analysis | |
| Site | 0.403 | | 0.065 | | 0.552 | |
| Treatment | 0.529 | | 0.064 | | 0.185 | |
| Season | 0.560 | | 0.795 | | 0.170 | |

### 3.3.5 Stomatal conductance ($g_s$)

In 2013-2014, RI reduced $g_s$ at GB10 at pre-harvest only, while this was affected by the treatment at all sites either at veraison or pre-harvest during 2014-2015 (Table 21).

**Table 21 Stomatal conductance (mmol/m$^2$s) at veraison and pre-harvest during the 2013-2014 and 2014-2015 seasons. Each value is the average of four replicates.**

| Season 2013-2014 | | | | | | |
|---|---|---|---|---|---|---|
| | Veraison | | | Pre-harvest | | |
| Treatment | Site | | | | | |
| | WH | GB5 | GB10 | WH | GB5 | GB10 |
| CON | 626.6 | 654.3 | 479.5 | 598.0 | 528.3 | 327.0 |
| RI | 620.8 | 475.4 | 399.3 | 499.0 | 449.1 | 293.1 |
| LSD 5% | 70.6 | 268.0 | 215.22 | 108.6 | 156.2 | 27.09 |
| p value | 0.846 | 0.153 | 0.397 | 0.066 | 0.261 | **0.022** |
| Season 2014-2015 | | | | | | |
| | Veraison | | | Pre-harvest | | |
| Treatment | Site | | | | | |
| | WH | GB5 | GB10 | WH | GB5 | GB10 |
| CON | 440.7 | 516.5 | 490.2 | 378.4 | 291.8 | 376.5 |
| RI | 314.6 | 267.5 | 306.3 | 295.8 | 151.7 | 232.0 |
| LSD 5% | 67.7 | 192.9 | 52.4 | 50.5 | 45.4 | 29.6 |
| p value | **0.004** | **0.020** | **<.001** | **0.007** | **<.001** | **<.001** |

The results have been reported separately for veraison and pre-harvest aiming to identify a pattern for the differences in stomatal conductance. Thus, either at veraison or pre-harvest, RI had an effect on reducing $g_s$ under the drier conditions of the second season, with no differences across sites. The

combined analysis showed only seasonal differences at veraison, whereas the differences between sites and treatments were observed only at pre-harvest (Table 22). The latter stage seems to be the more sensitive for finding differences between sites and also to see the overall effect of RI when combining all the data.

**Table 22 Results of two-way ANOVA on the effects of "site" (WH, GB5 and GB10), "treatment" (CON and RI), and "season" on stomatal conductance at veraison and pre-harvest. Numbers correspond to p values.**

| | Veraison | | Pre-harvest | |
|---|---|---|---|---|
| Factor | Season | | Season | |
| | 2013-2014 | 2014-2015 | 2013-2014 | 2014-2015 |
| Site | 0.175 | 0.894 | **0.018** | 0.078 |
| Treatment | 0.220 | **0.034** | 0.068 | **0.026** |
| | Combined analysis | | Combined analysis | |
| Site | 0.434 | | **0.007** | |
| Treatment | 0.085 | | **0.005** | |
| Season | **0.022** | | 0.300 | |

### 3.3.6 Correlation of stomatal conductance ($g_s$) and stem water potential ($\psi_s$) at veraison and harvest.

In the first season, a significant correlation was found between $g_s$ and $\psi_s$ in WH at pre-harvest (A) and GB10 at veraison (E). In 2014-2015, significant correlations were found only at GB5 either at veraison or pre-harvest (D) (Figure 19). Generally speaking, the range in values at GB5 and GB10 were greater than at WH in either season.

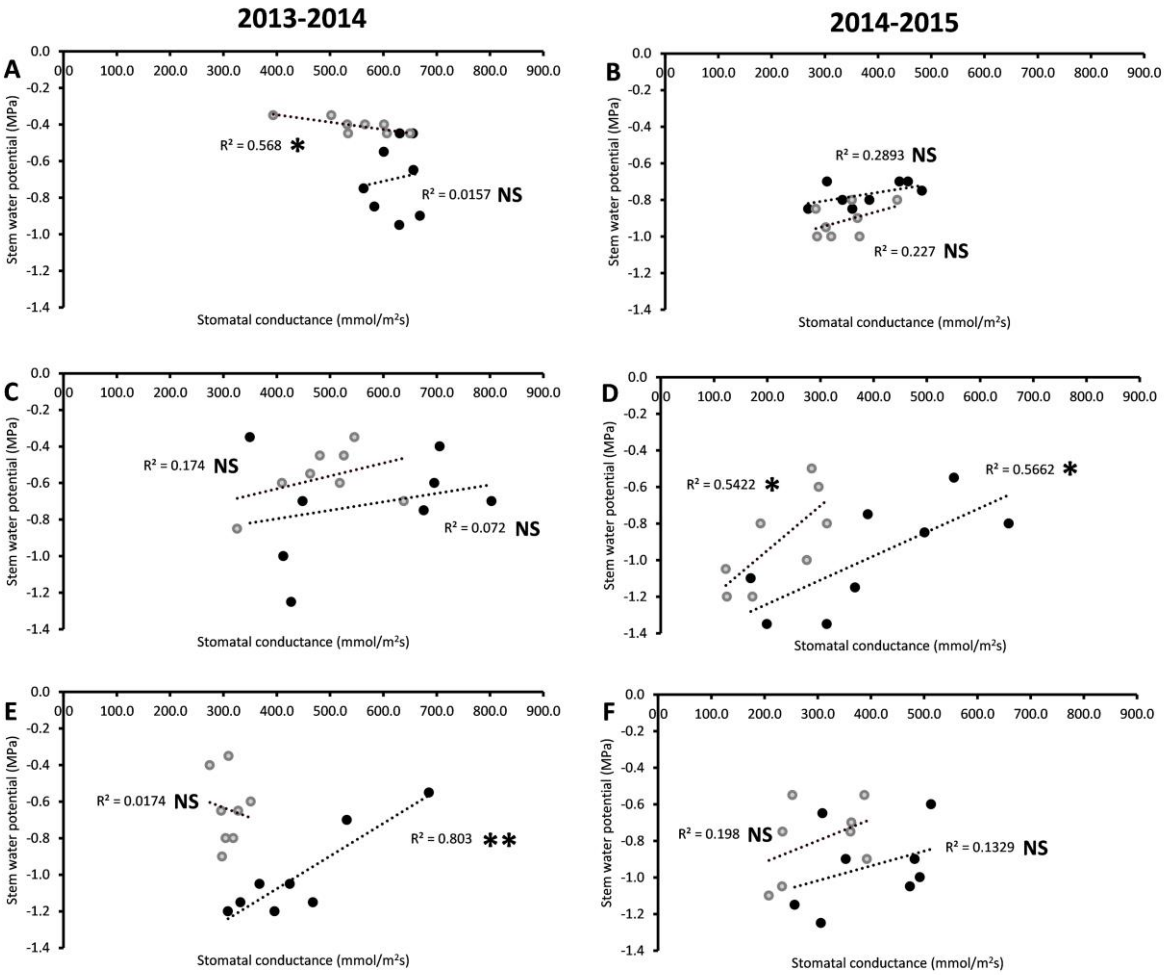


**Figure 19 Correlation between stomatal conductance (mmol/m²s) and stem water potential (MPa) at veraison (black dots) and pre-harvest (grey dots) during the 2013-2014 and 2014-2015 seasons at all sites. Each value is the average of four replicates. Charts correspond to: WH (A, B), GB5 (C, D), GB10 (D, F). Significances are: * p < 0.050; ** p < 0.010; NS non-significant results (p ≥ 0.050).**

### 3.3.7 Leaf proline content

Although similar values for leaf proline content between CON and RI are reported from fruit-set (first date in all charts) to harvest (last date in all charts) at all sites in either season, WH showed a different pattern than the others (Figure 20). The latter site also tended to accumulate more leaf proline at harvest in either season.

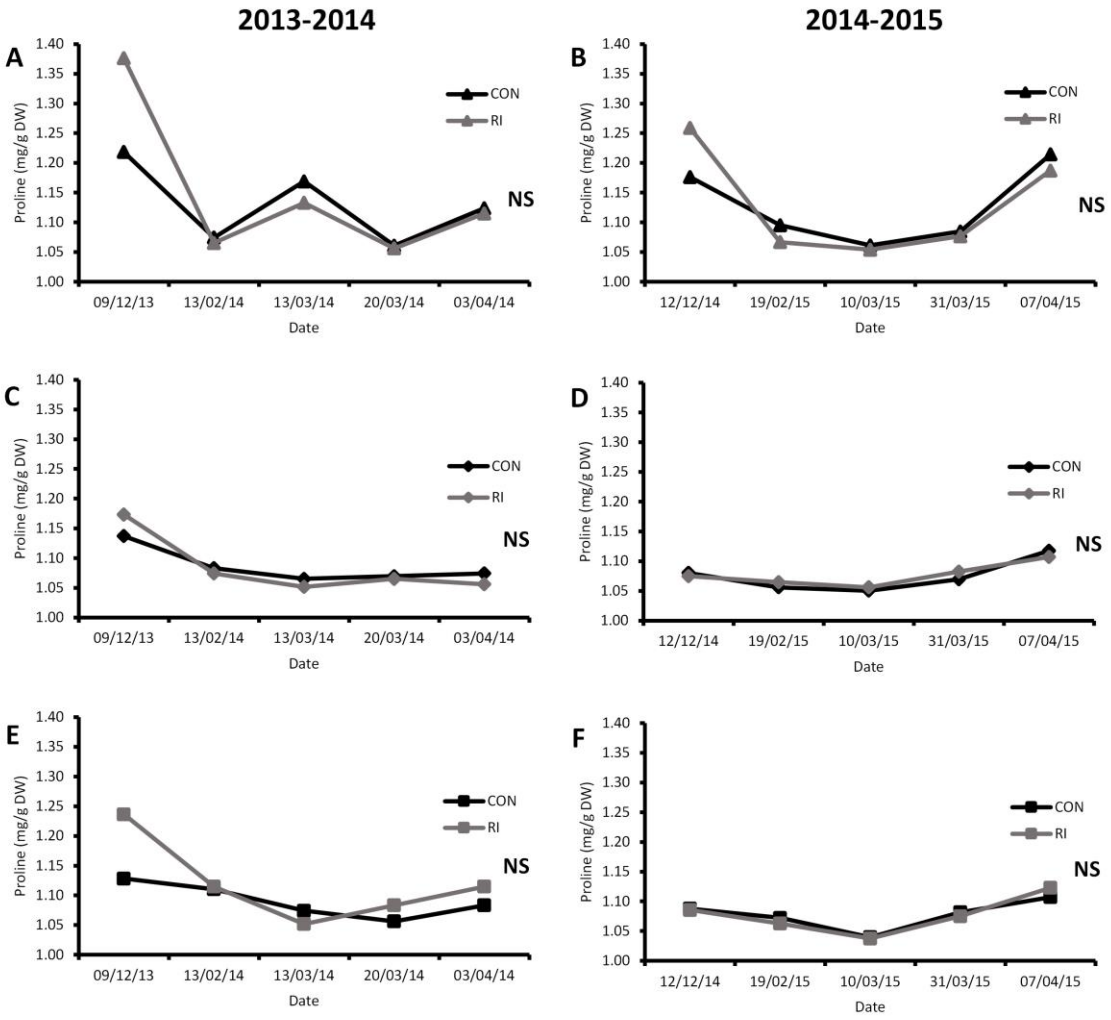


**Figure 20 Evolution of leaf proline content (mg/g DW) during the 2013-2014 and 2014-2015 seasons at all sites. Charts correspond to: WH (A, B), GB5 (C, D), GB10 (D, F). Differences between CON and RI were calculated based on mean area under the curve from all measurements over the season. NS means non-significant results (p ≥ 0.050).**

No differences for proline accumulation were found between CON and RI at all sites in either season (Figure 20). Calculations were carried out based on mean area under the curve. Means, LSD 5%, and p-values can be found in Appendix B.

From the general analysis, only “site” showed differences in 2014-2015, as well as in the combined analysis. No differences between seasons were detected (Table 23).

**Table 23 Results of two-way ANOVA on the effects of “site” (WH, GB5 and GB10), “treatment” (CON and RI), and “season” on leaf proline content. Numbers correspond to p values.**

| Factor | Season | |
|---|---|---|
| | 2013-2014 | 2014-2015 |
| Site | 0.073 | **0.013** |
| Treatment | 0.416 | 0.832 |
| | Combined analysis | |
| Site | **0.012** | |
| Treatment | 0.340 | |
| Season | 0.490 | |

### 3.3.8 Leaf osmotic potential ($\psi_\pi$)

Figure 21 illustrates the evolution of $\psi_\pi$ at all sites over the two seasons. At fruit-set in 2013-2014 (A, C, and D), $\psi_\pi$ in all treatments and sites showed values of about -1.1 MPa, which decreased through the ripening period, and then increased at harvest. $\psi_\pi$ showed a similar trend in 2014-2015 (B, D, and F), but the ranges during the ripening period were less pronounced than in the previous season.

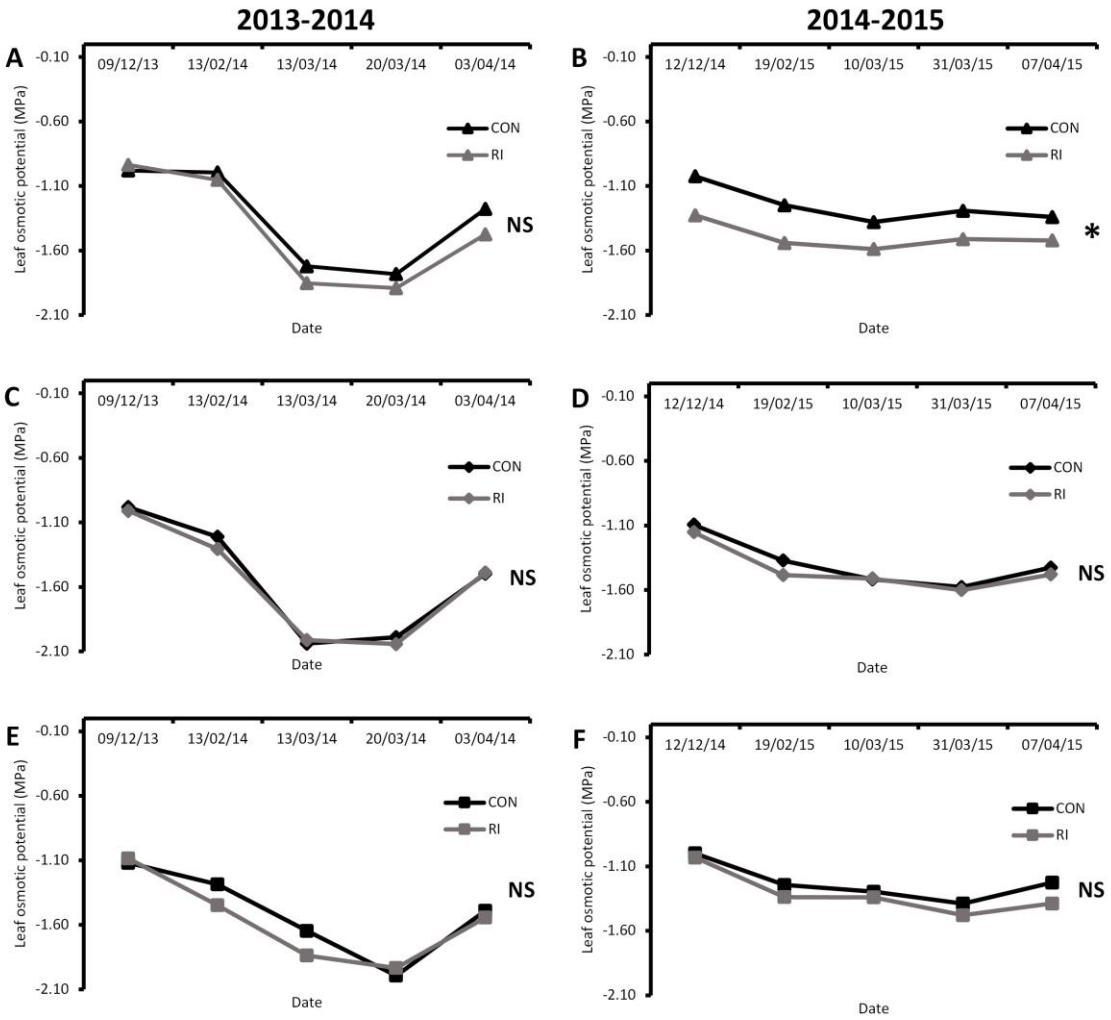


**Figure 21 Evolution of leaf osmotic potential (MPa) over the two seasons at all sites. For any given date, each value represents the average of four replicates. Charts correspond to: WH (A, B), GB5 (C, D), GB10 (D, F). Differences between CON and RI were calculated based on mean area under the curve from all measurements over the season. Significances are: * p < 0.050; NS non-significant results (p ≥ 0.050).**

Evolution of $\psi_\pi$ showed differences only at WH in 2014-2015, whereas at the other sites in either season there were no differences between CON and RI (Figure 21). Means, LSD 5%, and p-values are presented in Appendix B.

Differences among sites were found in the first season only. The other results indicated that the treatment did not alter $\psi_\pi$ in either season. Also, there were no differences between seasons (Table 24).

**Table 24 Results of two-way ANOVA on the effects of "site" (WH, GB5 and GB10), "treatment" (CON and RI), and "season" on leaf osmotic potential. Numbers correspond to p values.**

| Factor | Season | |
|---|---|---|
| | 2013-2014 | 2014-2015 |
| Site | **0.026** | 0.309 |
| Treatment | 0.066 | 0.176 |
| | Combined analysis | |
| Site | 0.208 | |
| Treatment | 0.129 | |
| Season | 0.349 | |

### 3.3.9 Estimated leaf chlorophyll content.

The evolution of estimated leaf chlorophyll content from veraison (first date in all charts) to harvest (last date in all charts) is shown in Figure 22. Overall, at all sites in either season, leaves from above the cluster zone in vines under RI turned yellow earlier than those in CON. This was more pronounced in the second season, with GB5 (D) and GB10 (F) showing large differences from veraison onwards.

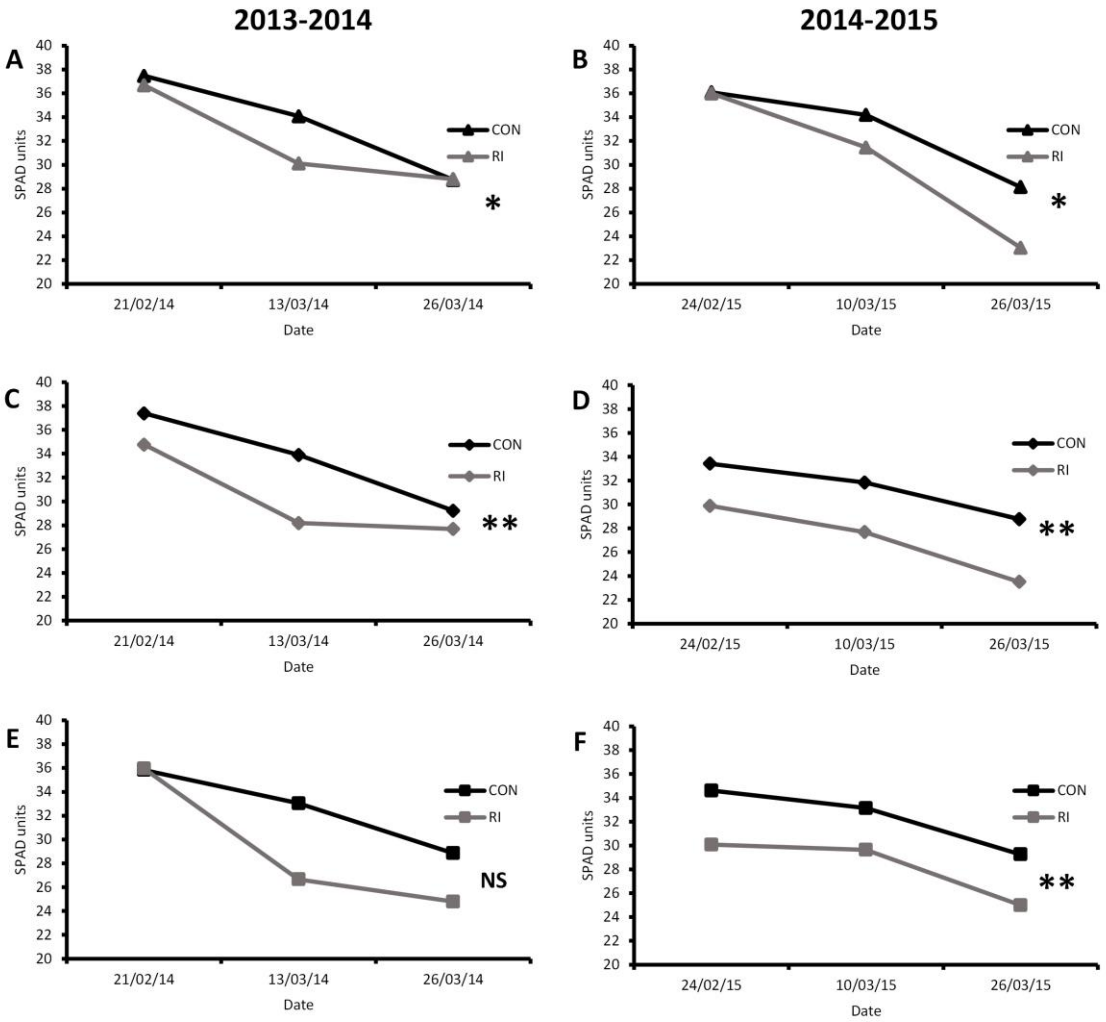


**Figure 22 Evolution of estimated chlorophyll content (SPAD units) through the 2013-2014 and 2014-2015 seasons at all sites. Charts correspond to: WH (A, B), GB5 (C, D), GB10 (D, F). Differences between CON and RI were calculated based on mean area under the curve from all measurements over the season. Significances are: * $p < 0.050$; ** $p < 0.010$; NS non-significant results ($p \geq 0.050$).**

Differences in leaf greenness between CON and RI were found at all sites in either season, except at GB10 in 2013-2014 (Figure 22).

The results of the two-way ANOVAs showed an effect of the treatment in both seasons, with no site effect in 2013-2014 or 2014-2015. The combined analysis also highlighted the effect of the treatment on reducing the estimated chlorophyll content, with no differences between seasons (Table 25).

**Table 25 Results of two-way ANOVA on the effects of "site" (WH, GB5 and GB10), "treatment" (CON and RI), and "season" on estimated leaf chlorophyll content. Numbers correspond to p values.**

| Factor | Season | |
|---|---|---|
| | 2013-2014 | 2014-2015 |
| Site | 0.246 | 0.105 |
| Treatment | **0.032** | **0.018** |
| | Combined analysis | |
| Site | 0.206 | |
| Treatment | **0.024** | |
| Season | 0.435 | |

## 3.3.10 Carbon isotope ratio ($\delta^{13}C$) in leaf dry matter

Results in Table 26 indicate that, in general, RI did not modify leaf $\delta^{13}C$ in water-stressed vines with respect to those normally irrigated over the two seasons. Only differences at GB10 in 2013-2014 were found.

**Table 26 Carbon isotope ratio in leaf dry matter during the 2013-2014 and 2014-2015 seasons. Each value is the average of four replicates.**

| Season 2013-2014 | | | |
|---|---|---|---|
| Treatment | Site | | |
| | WH | GB5 | GB10 |
| CON | -29.14 | -29.09 | -28.41 |
| RI | -29.34 | -28.41 | -29.24 |
| LSD 5% | 1.10 | 1.34 | 0.73 |
| p value | 0.679 | 0.263 | **0.032** |
| Season 2014-2015 | | | |
| Treatment | Site | | |
| | WH | GB5 | GB10 |
| CON | -29.01 | -28.79 | -28.32 |
| RI | -29.14 | -29.02 | -27.72 |
| LSD 5% | 1.13 | 2.30 | 2.00 |
| p value | 0.787 | 0.813 | 0.491 |

When all the data were analysed to obtain the variations between sites, treatments, and seasons, these also indicated that leaf $\delta^{13}C$ was not influenced by either the characteristics of the site or the reduced irrigation treatment, nor altered by the different climatic conditions of each season (Table 27).

**Table 27 Results of two-way ANOVA on the effects of "site" (WH, GB5 and GB10), "treatment" (CON and RI), and "season" on leaf carbon isotope ratio. Numbers correspond to p values.**

| Factor | Season | |
|---|---|---|
| | 2013-2014 | 2014-2015 |
| Site | 0.676 | 0.139 |
| Treatment | 0.817 | 0.791 |
| | Combined analysis | |
| Site | 0.076 | |
| Treatment | 0.897 | |
| Season | 0.801 | |

## 3.3.11 Root carbohydrates

### Root water content

RI did not affect root water content (%) at any site when evaluated in the second season (Table 28).

**Table 28 Root water content (%) in the 2014-2015 season. Each value is the average of four replicates.**

| Treatment | Site | | |
|---|---|---|---|
| | WH | GB5 | GB10 |
| CON | 46.5 | 46.8 | 48.0 |
| RI | 46.0 | 45.5 | 46.8 |
| LSD 5% | 1.2 | 2.0 | 1.8 |
| p value | 0.356 | 0.171 | 0.147 |

Variations site by site, as well as an overall effect of the treatment were not observed (Table 29).

**Table 29 Results of two-way ANOVA on the effects of "site" (WH, GB5 and GB10), "treatment" (CON and RI) on root water content (%). Numbers correspond to p values.**

| Factor | Season |
|---|---|
| | 2014-2015 |
| Site | 0.090 |
| Treatment | 0.057 |

## Root soluble carbohydrates

Root soluble carbohydrates followed a similar trend as root water content, with no differences between CON and RI at all sites (Table 30).

**Table 30 Root soluble carbohydrates (%) in the 2014-2015 season. Each value is the average of four replicates.**

| Treatment | Site | | |
|---|---|---|---|
| | WH | GB5 | GB10 |
| CON | 2.5 | 2.8 | 2.9 |
| RI | 2.4 | 3.0 | 3.0 |
| LSD 5% | 0.2 | 0.5 | 0.3 |
| p value | 0.423 | 0.594 | 0.532 |

Although differences between CON and RI were not found within each site, variations across sites were reported (Table 31).

**Table 31 Results of two-way ANOVA on the effects of "site" (WH, GB5 and GB10), "treatment" (CON and RI) on root soluble carbohydrates (%). Numbers correspond to p values.**

| Factor | Season |
|---|---|
| | 2014-2015 |
| Site | **0.032** |
| Treatment | 0.501 |

## Root starch

No differences for root starch (%) between treatments were found at all sites (Table 32)

**Table 32 Root starch content (%) in the 2014-2015 season. Each value is the average of four replicates.**

| Treatment | Site | | |
|---|---|---|---|
| | WH | GB5 | GB10 |
| CON | 12.0 | 9.2 | 9.8 |
| RI | 11.9 | 8.8 | 10.1 |
| LSD 5% | 1.2 | 1.9 | 1.7 |
| p value | 0.869 | 0.667 | 0.702 |

As found for root soluble carbohydrates, only differences across sites were detected for root starch content (Table 33).

**Table 33 Results of two-way ANOVA on the effects of "site" (WH, GB5 and GB10), "treatment" (CON and RI) on root starch content (%). Numbers correspond to p values.**

| Factor | Season |
|---|---|
| | 2014-2015 |
| Site | **0.011** |
| Treatment | 0.809 |

### 3.3.12 Pruning weight

No differences in pruning weight were found at the end of the second season at all sites (Table 34).

**Table 34 Pruning weight (kg/metre of row) in the 2014-2015 season. Each value is the average of four replicates.**

| Treatment | Site | | |
|---|---|---|---|
| | WH | GB5 | GB10 |
| CON | 1.03 | 0.53 | 0.44 |
| RI | 0.98 | 0.36 | 0.28 |
| LSD 5% | 0.15 | 0.22 | 0.23 |
| p value | 0.430 | 0.116 | 0.146 |

Variations across sites for pruning weight, however, were observed (Table 35).

**Table 35 Results of two-way ANOVA on the effects of "site" (WH, GB5 and GB10), "treatment" (CON and RI) on pruning weight. Numbers correspond to p values.**

| Factor | Season |
|---|---|
| | 2014-2015 |
| Site | **0.008** |
| Treatment | 0.075 |

## 3.4 Discussion

### Phenology

Although RI did not alter the date of occurrence of the main phenological stages in either season, differences between seasons were observed. These differences can be attributed in part to the frost event registered early in the second season (4th and 5th of October, 2014), in which most of the vineyards in the Waipara area were damaged, including those in where this study was carried out. This extended the period between budbreak and bloom by about 20 days in the second season. Despite bloom in 2014-2015 season happening later than in 2013-2014, the period between bloom and fruit-set was very similar at about 30 days. Fruit-set to veraison was shorter in 2013-2014 at WH and GB5, whereas at GB10 it was longer. The main differences between seasons were found in the period between veraison and harvest, which was shorter in 2014-2015. The growing season was a few days longer in 2014-2015 at all the sites. These findings are in agreement with Zsófi *et al.* (2009), who found spatial and seasonal variations in the duration of the main phenological stages in the Eger wine region in Hungary. Their results also coincide with these in which the length of the ripening period showed the main seasonal differences.

Few reports in the literature on field frost damage and its effect on grapevine phenology have been found. Jones *et al.* (2010) reported Pinot noir vines affected by a frost blooming nine days later than those unaffected, which is different than this study. The same authors described no differences on date of veraison between frosted and non-damaged vines.

Temperature has been described as the main climatic parameter influencing the major phenological stages in grapevines (Falcão *et al.* 2010, Jones 2003, Jones and Davis 2000). The research conducted by Falcão *et al.* (2010) found differences in the date of the main phenological stages related to the vineyard altitude, where a more detailed analysis revealed that the longer duration of these at higher altitude was due to the lower mean temperatures registered there. Van Leeuwen *et al.* (2004) studied the differences between three different varieties in Saint-Emilion (France) over five seasons, finding significant seasonal differences in phenology due to climatic differences between seasons, with the date of veraison being the most influenced by the seasonal temperatures. In this research, the similarities in mean temperature and other climatic parameters between GB10 and WH may suggest that there were other parameters like wind speed, site aspect, soil characteristics, etc. influencing the variation between sites in Waipara.

### Leaf area

The method of Lopes and Pinto (2005) was chosen for its simplicity and suitability for this study, even though this has not been used on Pinot noir. References using this method in New Zealand were also not found. Nevertheless, several authors have used this method in field experiments to estimate leaf

area in different wine regions and varieties (Botelho *et al.* 2012, Cruz *et al.* 2012, Fernandes De Oliveira *et al.* 2013a, Fernandes De Oliveira *et al.* 2013b, Lopes *et al.* 2008, Lopes *et al.* 2011).

Lopes and Pinto (2000) originally proposed this as an empirical non-destructive model to estimate leaf area in which the single leaf area was estimated by a model using the length of the two main lateral leaf veins. This incorporated another source of error to the model as the individual leaf area was just estimated instead of the actual as measured by a leaf area meter. As such, any error in that estimation was carried out to the estimation of the leaf area per shoot, and therefore, to the leaf area per plant. For example, Beslic *et al.* (2010) validated the method for cv. Blaufränkisch estimating the individual leaf area using the length of the two inferior leaf veins for calculations. Coefficients of determination ($R^2$) of 0.87 and 0.94 between observed and predicted leaf area were obtained for main and lateral leaf area, respectively. Some authors have validated and improved the accuracy of the method for different varieties and wine regions in Europe, using a leaf area meter. Thus, Lopes and Pinto (2005) validated the method using shoots from four different varieties in Portugal obtaining high $R^2$ between 0.95 and 0.98 for primary leaf area, and between 0.97 and 0.98 for lateral leaf area. Similar results were obtained when the method was validated for Pinot noir under the conditions of this research. Appendix A shows the $R^2$ for primary leaf area ranging between 0.91 and 0.98 in 2013-2014, and between 0.94 and 0.98 in 2014-2015, while for lateral leaf area these were between 0.95 and 0.99 in the first season, and between 0.99 and 1.0 in the second season. In Spain, Sanchez-de-Miguel *et al.* (2011) validated the method for six different varieties, but in that case $R^2$ reported were lower than those reported by Lopes and Pinto (2005) and this study. Based on the high $R^2$ obtained here, a modification to the method of Lopes and Pinto (2005) was proposed. Thus, to improve the accuracy of the method through the reduction of the error when individual leaf area is determined, the smallest and largest leaves of a shoot were collected after counting them to estimate leaf area per shoot. As these leaves were measured using a leaf area meter, the individual leaf area correspond to the real leaf area instead of the estimated one, and therefore, the variations in estimating individual leaf area were eliminated.

Differences in primary leaf area were reported at GB5 and GB10, while the RI treatment did not affect this either in 2013-2014 or 2014-2015 at WH. Differences were found in the two sites having soils with higher profile available water, while no differences were observed for the soil with lower capacity of holding water. WH had the lower water deficit (less negative water balance) across all sites during the two seasons, mainly due to the higher irrigation frequency, and therefore, higher volumes of water received by the vines through the season. This combination of factors showed to have an influence in determining the magnitude of the partial loss of primary leaf area under water stress conditions. Water stress has been described as promoting a partial loss of canopy leaf area under field conditions (Chaves *et al.* 2007, Tomás *et al.* 2014 and literature therein), which is

proposed as a drought adaptation mechanism (Intrigliolo and Castel 2006). Fernandes De Oliveira *et al.* (2013b) evaluated the effect of different deficit irrigation strategies applied between fruit-set and harvest on a series of physiological parameters. Their findings indicated that deficit irrigation did not affect primary leaf area when measured pre-veraison. However, the evaluation pre-harvest showed a significant effect of the treatment on reducing primary leaf area from plants receiving 75% less water than control. This and the results reported here confirm that the loss of canopy leaf area due to water stress is more evident from veraison onwards. In that study, lateral leaf area was not altered either at pre-veraison or pre-harvest (Fernandes De Oliveira *et al.* 2013b), which agrees with the results reported here over two seasons. Pedreira dos Santos *et al.* (2007) described a reduction of primary leaf area in vines under deficit irrigation and partial root-zone drying (PRD), similar than here, but in this case, lateral leaf area was also reduced by the treatments, which is not in agreement with the results in this study. Other studies have reported primary leaf abscission as a response to water stress under field conditions. Ginestar *et al.* (1998) found that different intensities of water deficit applied after veraison decreased leaf area between 11% and 65% in Shiraz in Australia, but data for lateral leaf area were not reported. The magnitude of the reduction in primary leaf area is similar to that found here at GB5 in both seasons and GB10 in 2014-2015. Similar results were also obtained by Romero *et al.* (2013) from a three season study, but again, data for lateral leaf area were not included. Palliotti *et al.* (2000) demonstrated that leaves in lateral shoots had high photosynthetic and transpiration rates after veraison, which contributed to sugar accumulation in the fruit and starch accumulation in the wood. Hence, it is suggested that in vine water stress studies, primary and lateral leaf area should be measured and considered individually.

Studies showing results from either one season (Fernandes De Oliveira *et al.* 2013b, Ginestar *et al.* 1998, Pedreira dos Santos *et al.* 2007) or more than one season (Chaves *et al.* 2007, De Souza *et al.* 2005b, Romero *et al.* 2013) have confirmed that the reduction of leaf area in plants under different levels of water stress can be noticed in the same season in which the treatment was applied. This was confirmed here, especially for primary leaf area.

### Point Quadrat

Point Quadrat analysis has not been widely considered in grapevine water stress studies. This research considered all parameters suggested by Smart and Robinson (1991), while LLN seems to be the parameter of choice in most reports (Lopes *et al.* 2011, Pedreira dos Santos *et al.* 2007). This is likely due to the importance of LLN on cluster exposure, and its correlation with PIL and PIC, described by Reynolds and Vanden Heuvel (2009 and literature therein). Also, it was difficult to find a similar field study comparing all these parameters across different locations within a region.

The effect of RI varied depending on the evaluation. In general, PG was higher in plants under RI only at WH in 2014-2015. This difference was likely due to the drier conditions and frost damage registered in the 2014-2015 season, which reduced the number of shoots per metre (data not shown). Smart and Robinson (1991) suggested that an optimum PG should be 20-40%, far from the values obtained here, even in the second season; only GB5 showed relatively high (22%) gaps under RI in 2014-2015. In Waipara, leaf removal research conducted on Pinot noir by Kemp (2010) found less than 1% gaps in 2008, whereas the value was much higher in 2009, and closer to the optimum suggested by Smart and Robinson (1991).

LLN was reduced by RI in 2014-2015 at all sites, highlighting the differences between seasons. These differences can be explained by drier conditions of the second season, as a bigger proportion of the water received by the vines corresponded to irrigation, and therefore, the RI treatment showed to have a greater effect on grapevine physiology than in the previous season. These results match those observed by Pedreira dos Santos *et al.* (2007) who found a similar trend in a single season trial in cv. Moscatel, where LLN was lower in vines under different irrigation regimes compared to a fully irrigated control. In contrast, Lopes *et al.* (2011) did not observe differences for LLN between plants under conventional, partial rootzone drying (PRD), and regulated deficit irrigation (RDI) over two seasons, likely due to the lack of differences in total and lateral leaf area described there. Data obtained by Kemp (2010) indicated that leaf layer number in non-defoliated vines ranged between 1.9 and 3.4 in Waipara, which are in concordance with those reported here. These results also agree with findings of terroir studies, such as Zsófi *et al.* (2009) who classified terroir for cv. Kékfrankos, where LLN showed significant seasonal and spatial variations within the Eger region in Hungary. Most results for LLN in this study and others elsewhere are above the optimum range (1.0 – 1.5 or less) suggested by Smart and Robinson (1991). A strong correlation has been described between LLN and photosynthetically active radiation (PAR) in the fruiting zone, which in this case may have an influence on grape characteristics (Reynolds and Vanden Heuvel 2009 and literature therein). Their potential relationship will be analysed in chapter 5.

RI reduced PIL values only in 2014-2015. This followed the same trend as leaf layer number, confirming the association between these two parameters described in Reynolds and Vanden Heuvel (2009). From the literature reviewed, only Zsófi *et al.* (2009) included this in their terroir analysis. As found for LLN, the authors described significant spatial and seasonal differences. Here, although the results in 2014-2015 are significant at all sites, spatial and seasonal differences were not found from the general analysis. In all cases, data reported here are higher than the optimum recommended by Smart and Robinson (1991).

PIC was altered by RI at WH in 2013-2014 and GB5 in 2014-2015. Kemp (2010) reported PIC ranging from 29 to 69% in 2008, and 13 to 42% in 2009 in Waipara. Despite those data not being from an irrigation trial, the results observed here are similar to those for the same region and variety. The same seasonal and spatial distribution as for PIL was described by Zsófi *et al.* (2009) in Hungary. A majority of the findings in this study are within the optimum PIC range recommended by Smart and Robinson (1991).

### Stem water potential ($\psi_s$)

$\psi_s$ has been widely used to evaluate grapevine water status due to its sensitivity to reveal small changes in whole vine water status under mild water deficit (Choné *et al.* 2001, Van Leeuwen *et al.* 2010). Van Leeuwen *et al.* (2010) highlighted its accuracy when soil water content is heterogeneous, which is the case of most irrigated vineyards. $\psi_s$ has been reported as being a useful indicator for comparing vine water status among sites, and it is also a suitable tool for irrigation management (Van Leeuwen *et al.* 2009)

Differences between CON and RI were found only in WH at pre-harvest in 2013-2014. Although no differences were observed at fruit-set, this corresponded to the stage in which the vines in this study evidenced less water stress in either season. These findings are consistent with those of Romero *et al.* (2013) who did not find differences in $\psi_s$ between budburst and fruit-set over three seasons in a field-grown Monastrell regulated deficit irrigation study under semiarid conditions in South-Eastern (SE) Spain. Intrigliolo and Castel (2009) also reported low levels of stress early in the season, which increased toward harvest, which coincide with those reported here. As described by Romero and colleagues (2013), the low level of stress at that time of the season is due to high soil water content remaining from winter, the vine canopy not being fully expanded, and lower atmospheric demand. These likely explain the lack of differences obtained in this study at fruit-set in both seasons.

The results at veraison were the lowest of the season for most evaluations, although no differences between CON and RI were observed at all sites over the two seasons. Romero *et al.* (2013) found a significant effect of reduce irrigation on $\psi_s$ at veraison in two out of three seasons, which is in contrast to the non-significant results found here. Intrigliolo and Castel (2009) also described the lower values of $\psi_s$ in their study around veraison, regardless the irrigation treatment in cv. Tempranillo. Here, the lowest values of $\psi_s$ are coincident with the period in which the maximum water deficit was registered (January/February), which together with the maximum temperatures of the season were the likely causes.

WH at pre-harvest in 2013-2014 was the only site showing differences between CON and RI among all sites over the two seasons. In general, during the second, drier, season, all sites showed the minimum values of $\psi_s$ at pre-harvest, which contrast to those in 2013-2014 where these reported

either similar or higher values than those at fruit-set. Similar results to 2014-2015 were described by Romero *et al.* (2013), whose pre-harvest results also corresponded to the maximum level of water stress reported in each season. The same trend was described by Intrigliolo and Castel (2009), where the maximum levels of stress were found pre-harvest, confirming that $\psi_s$ tends to decrease as the season advances. This also suggests that the high amount of rainfall registered pre-harvest in 2013-2014 altered the dynamic of $\psi_s$ under the conditions of this study. In contrast, the lower $\psi_s$ under drier conditions in 2014-2015 were expected.

### Stomatal conductance ($g_s$)

$g_s$ of vines under reduced irrigation was lower than those normally irrigated at all sites at veraison and pre-harvest in 2014-2015, while this was affected by RI only in GB10 at pre-harvest in 2013-2014. The combined analysis showed differences between seasons at veraison, which agree with the opposite climatic conditions registered during the two seasons of this study. The lower soil water available in 2014-2015, due to the lower rainfall and irrigation and higher temperatures, likely reached a point were an adaption response of closing stomata was triggered to maintain grapevine water status at a relatively constant level, despite the lower soil water availability.

Stomatal closure has been described as one of the first responses to soil water scarcity in grapevines (Lovisolo *et al.* 2010, Schultz 2003, Tomás *et al.* 2014). This is considered to be a water saving response to maintain leaf hydration despite low soil water content (Intrigliolo and Castel 2006, Schultz 2003). However, high variability between cultivars has been observed for $g_s$, which suggests that the response to soil water scarcity is variety-dependent (Tomás *et al.* 2014). Pinot noir has been shown to strongly reduce $g_s$ under water-stress, either under field or controlled conditions. Bellvert *et al.* (2013) evaluated the variations in $g_s$ between well-watered and stressed Pinot noir vines under field conditions. Stressed plants showed lower values of $g_s$ at solar noon, although those values were much lower than found in this study. Under controlled conditions, Tomás *et al.* (2012) reported a 65% reduction in $g_s$ in water-stressed vines with respect to the control treatment. The results shown here are in concordance with those found in the literature, confirming the association between water-stress and stomatal closure in Pinot noir. Other studies have suggested an association between leaf area and stomatal sensitivity. Thus, Schultz (2003) noted that a reduction in leaf area caused by water stress in cv. Syrah increased stomatal sensitivity. A similar reduction in leaf area was found by Intrigliolo and Castel (2006) in cv. Tempranillo, but stomatal activity was shown to be less sensitive in Schultz's (2003) study. Their observations suggest that a reduction in vegetative growth together with stomatal closure define the grapevine water saving behaviour.

$g_s$ can be used as an integrative parameter to evaluate the degree of drought. It is known that stomatal closure has a direct impact on photosynthesis, but there is a different effect depending on

the level of stress (Cifre *et al.* 2005, Flexas *et al.* 2002, Medrano *et al.* 2002). Flexas *et al.* (2002) and Medrano *et al.* (2002) have proposed three phases of water stress based on $g_s$. Briefly, a phase of "mild water stress" has been defined for values of $g_s$ ranging from 0.5 to 0.15 mol $H_2O$ $m^{-2}s^{-1}$. "Moderate water stress" is comprised from values of $g_s$ between 0.15 and 0.05, and finally, when $g_s$ drops below 0.05 this is classified as "severe water stress". All findings in this study correspond to values considered as mild water stress.

### Relationship between $g_s$ and $\psi_s$ at veraison and pre-harvest

Though $g_s$ and $\psi_s$ showed to be well correlated for certain phenological stages and sites, most of the analyses indicated a poor correlation between these parameters either at veraison or pre-harvest in both seasons. This relationship has been analysed by some authors, who has reported a wide range of results. For example, Bota *et al.* (2015) studied the stomatal behaviour of 23 grapevine cultivars under progressive water stress. Their findings indicate that for cv. Manto negro there is a high correlation between $g_s$ and $\psi_s$ ($R^2$ = 0.91), which reflect its tight control of stomatal aperture. On the other hand, cvs. such as Escursac and Galmeter showed $R^2$ of 0.32 and 0.20, respectively, similar to those found here at GB5 at pre-harvest in 2013-2014, and WH and GB10 in 2014-2015 at veraison and harvest. In cv. Tempranillo, Intrigliolo and Castel (2009)when the authors pooled data from PRD and conventional drip irrigation to evaluate the relationship between $g_s$ and $\psi_s$, the results indicated a high correlation between them ($R^2$ = 0.74), similar to that found in GB10 at veraison in 2013-2014. Romero *et al.* (2010) also reported a high correlation between midmorning $g_s$ and midday $\psi_s$ under efficient deficit-irrigation management in cv. Monastrell under field conditions. The low correlation between these parameters found in this study suggest that when $g_s$ dropped due to water stress, $\psi_s$ did not always follow the same tendency, maintaining plant water status at a relatively constant level, despite the lower soil water available. This is a behaviour described for isohydric varieties, which will be discussed later in the chapter.

It is also notable that the range of values for $g_s$ and $\psi_s$ was grater at the GB5 and GB10 sites compares to WH. This seems reasonable due to the frequency of irrigations at the latter vineyard, where the low water holding capacity of the gravelly soils means vines are irrigated often, leading to fewer opportunities for the water status of the vines to fall.

### Leaf proline content

Leaf proline content remained essentially constant from fruit-set to harvest in either season, with no differences between CON and RI observed at any site. These results may be in agreement with those of Patakas *et al.* (2002) who showed no differences in proline accumulation between control and stressed vines, although their results were expressed as total amino acids. However, these values differ from some published studies, such as Bertamini *et al.* (2006) and Doupis *et al.* (2011), where

leaf proline content was more than double in stressed plants with respect to those that were well-watered. Furthermore, a twofold increase in proline content was reported in Cabernet Sauvignon and Shiraz vines under water stress compared to control (Hochberg *et al.* 2013b).

Proline accumulation is one of the common responses of plants to lower water availability, and the magnitude depends on the severity of stress (Bertamini *et al.* 2006, Hochberg *et al.* 2013b). Despite its contribution to osmotic adjustment being widely reported (Patakas *et al.* 2002), there are multiple physiological functions of proline that have been less studied. For example, the antioxidant capacity of proline has been described by some authors (Doupis *et al.* 2011, Gunes *et al.* 2006, Kavi Kishor and Sreenivasulu 2014, Ozden *et al.* 2009). This is thought to provide protection against drought and salinity damage by increasing cell antioxidant enzyme activity under stress conditions. Its implication on synthesis of hormones and sugars, the flowering process, and seed development has been also suggested (Kavi Kishor and Sreenivasulu 2014). Interestingly, the same authors suggested that in other species proline accumulated during a stress episode is degraded and used as a source of energy once the stress is relieved. The present results from proline accumulation indicate that RI did not stress the vines enough to activate the mechanisms involved in plant protection under water stress described in the literature.

The role of proline in osmotic adjustment will be discussed in the next section.

### Leaf osmotic potential ($\psi_\pi$)

No differences between CON and RI were observed in either season for $\psi_\pi$. Although significant differences between seasons were not found, $\psi_\pi$ in 2013-2014 followed a different dynamic than in 2014-2015. The lowest $\psi_\pi$ values of the two seasons were registered in 2013-2014 before harvest. Romero *et al.* (2012) reported similar results from different irrigation treatments, where no differences between water-stressed and control vines were found either pre or post-veraison. Padgett-Johnson *et al.* (2000) evaluated the performance of cv. Carignane scion under non-irrigated conditions, and interestingly, despite that this was not a water-stress study, the performance of $\psi_\pi$ under non-irrigated conditions followed a similar trend to that found here in the first season, with $\psi_\pi$ decreasing during most of the season, and then increasing just before harvest.

The capacity of grapevines to osmoregulate under abiotic stress has been widely described (Alsina *et al.* 2007, Patakas *et al.* 2002). Plants can deal with adverse conditions by accumulating compatible solutes inside their cells. This prevents excessive water loss and helps tissues to maintain a higher water potential, supporting continued water uptake (Bray 1997, Zhu 2002). These solutes, which have to be relatively small, hydrophilic, not easily metabolised, and not alter cell functions, include sugars (mainly glucose and fructose), sugar alcohols (e.g. mannitol or glycerol), inorganic ions (e.g. $K^+$, $Na^+$, $Cl^-$ and $SO_4^{2-}$) and amino acids (Patakas *et al.* 2002, Zhu 2002). Among amino acids, proline has

been described as being accumulated to a larger extent than others under stress conditions (Hochberg *et al.* 2013b). However, the role of proline in osmotic regulation remains unclear. Patakas *et al.* (2002) concluded that the accumulation of inorganic ions, such as $K^+$, $Na^+$, $Cl^-$ and $SO_4^{2-}$ are more important than amino acids for osmotic adjustment in stressed vines. Recently, Hochberg *et al.* (2013b) confirmed the results of Patakas *et al.* (2002) concluding that compared to inorganic ions, amino acids made a very small contribution to osmotic adjustment. These may partially explain the small differences in $\psi_\pi$ between CON and RI, based on the results for leaf proline content previously reported.

### Estimated leaf chlorophyll content

Leaves above the cluster zone in vines under RI turned yellow earlier than those in CON in both seasons. During the ripening period, leaves in the cluster zone senesce earlier than those at the top of the canopy, a process that was accelerated by RI in this study. During senescence chlorophyll degrades and chloroplasts break down (Hendry *et al.* 1987). Afterwards, carbon, nitrogen (and other nutrients), nucleic acids, polysaccharides, and lipids are remobilized to be used by other sink organs (such as young leaves, clusters, or roots) (Thomas 2013). The process culminates in leaf abscission, whose effect was reflected in the lower primary leaf area in vines under RI reported earlier.

Despite the SPAD meter having been used in viticulture as a non-destructive method to measure leaf chlorophyll content (Steele *et al.* 2008) and for diagnosis of grapevine nutritional status (Brunetto *et al.* 2012, Covarrubias and Rombolà 2013, Porro *et al.* 2001), its use in studies about vine water stress is very limited. A reduction in total chlorophyll content (including chlorophyll *a* and *b*) was reported by Bertamini *et al.* (2006) from vines under water deficit. In that study, water deficit decreased both chlorophyll *a* and *b*, but chlorophyll *a* showed to be more affected. The findings in this research highlighted the potential usefulness of considering leaf greenness in water-stress studies.

### Carbon isotope ratio in leaf dry matter ($\delta^{13}C$)

$\delta^{13}C$ did not show any differences in the trial. De Souza *et al.* (2005b) also reported no differences in $\delta^{13}C$ measured in primary leaves from different irrigation treatments, with similar values being reported over two seasons as in this study. In a one season experiment, De Souza *et al.* (2003) described differences for $\delta^{13}C$ only between fully irrigated and non-irrigated vines, with those under deficit irrigation and partial rootzone drying showing no differences with respect to fully irrigated ones. Based on the small differences between treatments previously cited and others elsewhere, Santesteban *et al.* (2015) discussed the role of leaves as an organ for evaluating $\delta^{13}C$ under field conditions. The authors suggested that because leaves are formed early in the season, before any significant water deficit is experienced, their $\delta^{13}C$ could be less related to water use efficiency than other parts of the vine. On the other hand, Tomás *et al.* (2012) found higher values in leaves from

water-stressed Pinot noir vines when compared to those well irrigated, but in their case, the differences in $\delta^{13}C$ between treatments were greater than any reported here. Furthermore, Costa *et al.* (2012) and Zyakun *et al.* (2013) showed differences between varieties, sites, and seasons for $\delta^{13}C$. The diversity of results obtained here and elsewhere for $\delta^{13}C$ using mature primary leaves suggests that this measurement is highly varietal and site dependent, with different results reported under different levels of water stress. The latter suggests that in this experiment, the lower values in stomatal conductance in plants under RI reported earlier were not enough to alter their photosynthetic activity, as described in Santesteban *et al.* (2015).

### Root carbohydrates

As described in Bota *et al.* (2004), the distribution of carbohydrates and other photosynthetic products to the different parts of the plant have been extensively studied in grapevines: the effect of different cultural practices such as shoot pinching, canopy manipulation and crop load have been tested to understand the carbohydrate dynamic under different conditions. However, the effects of environmental factors, like water stress, have been less studied. Among the literature reviewed, Bota *et al.* (2004), Holzapfel *et al.* (2010 and references therein), and Rogiers *et al.* (2011) described the effect of water stress on this parameter.

RI did not affect root water content measured during midwinter in the second season. Only Bennett (2002) have reported root water content in similar manner, and his values (58%) are higher than those obtained here. This might be due to varietal differences such as using ungrafted Chardonnay instead of grafted Pinot noir, and the differences in location.

Root soluble carbohydrates (%), which include sucrose, glucose, and fructose, were not altered by RI, with only differences between sites being reported. These results are in concordance with those reported in Holzapfel *et al.* (2010 and references therein) who showed that root soluble sugars concentration was unaltered by a reduction of irrigation in cv. Shiraz. In the same review, the authors also described a study (J.P. Smith and B.P. Holzapfel (unpublished), as cited in Holzapfel *et al*., 2010, p. 163) reporting similar results in which root soluble sugars did not show differences when comparing irrigated and non-irrigated vines.

Root starch (%) during dormancy was also unaffected by RI, though there were site by site differences. Starch is the major carbohydrate reserve in roots (De Herralde *et al.* 2010, Holzapfel and Smith 2012, Pellegrino *et al.* 2014). In Pinot noir, Zapata *et al.* (2004) reported that roots contain more than 90% of the starch stored in the vine at the beginning of the season. However, its accumulation seems to be more sensitive to water stress than soluble sugars. Holzapfel *et al.* (2010) showed results in which different irrigation strategies affected root starch accumulation, where Shiraz vines either under regulated deficit irrigation or partial rootzone drying had lower starch

concentration than those well irrigated. Similar results have been described in the same review where non-irrigated vines showed lower root starch concentration than those under irrigation. Rogiers *et al.* (2011) demonstrated that 10 days of water deficit about veraison were enough to reduce root starch concentration in potted Grenache vines, while in Semillon this was not altered.

Sucrose is formed in the leaves directly from photosynthates or from the degradation of starch stored within the chloroplasts, and then distributed to various grapevine organs to provide energy and carbon for structural growth and storage through phloem. Sucrose provides the substrate for starch formation in the plastids (amyloplasts) of roots, trunk, canes, stem, and buds, which is stored for use later on (Holzapfel *et al.* 2010). The distribution of starch within the vine depends on several factors and follows a seasonal pattern. For instance, Zapata *et al.* (2004) described that more than 90% of the starch in Pinot noir is stored in the roots during dormancy, but 40% of that has been used before the first leaf is expanded in the next season. The crop is an important carbohydrate sink that affect the distribution pattern within the vine. Thus, Bota *et al.* (2004) using $^{14}C$-labelled ($^{14}CO_2$) studied the partitioning of photosynthates under water stress conditions. They concluded that the presence or absence of fruit was more important than water stress in changing the distribution of photosynthates within the vine. As sucrose is formed in the leaves, a lower leaf area together with lower photosynthetic rate have been proposed as limiting factors for carbohydrate accumulation (Holzapfel *et al.* 2010, Pellegrino *et al.* 2014). In this study, the lower leaf area and lower stomatal conductance in vines under RI did not have an effect on root carbohydrate reserves, or root water content. Additionally, since the rootstocks are different between WH and the two sites at Greystone vineyard, this did not result in a variation in carbohydrate accumulation.

### Pruning weight

No differences in pruning weight were observed at all sites between CON and RI, but there were variations across sites. Acevedo-Opazo *et al.* (2010) reported that a reduction in irrigation pre and post-veraison decreased pruning weights in cv. Cabernet Sauvignon, but those differences were non-significant over the three seasons of their research. A decrease in pruning weight as a consequence of reduced irrigation has been reported in other varieties and wine regions, such as De Souza *et al.* (2005a), De Souza *et al.* (2005b) and Chaves *et al.* (2007) in Moscatel and Castelão (all of them in Portugal), Edwards and Clingeleffer (2013) in Cabernet Sauvignon in Australia, and De la Hera *et al.* (2007) in Spain in cv. Monastrell. Interestingly, when Padgett-Johnson *et al.* (2003) compared 17 different *Vitis* species under irrigated and non-irrigated conditions, pruning weights showed lower values in all the species (including *Vitis vinifera*) under non-irrigated conditions. The lack of differences between CON and RI at all sites after two seasons of reduced irrigation for root carbohydrates and pruning weight, despite the lower leaf area and stomatal conductance observed during the second season, may suggest that two seasons under this level of stress were not enough

to alter these parameters, being necessary to extend the trial for more seasons to evaluate the cumulative effect of reduced irrigation in the long term.

### Isohydric behaviour of Pinot noir under field conditions

In general, *Vitis vinifera* has been classified as a "drought avoiding" species, with an efficient stomatal control over transpiration (Chaves *et al.* 2010, Poni *et al.* 2014). However, some varieties have shown better stomatal control than others in response to water scarcity. Based on this, these can be classified into two categories: isohydric and anisohydric (Schultz 2003). Briefly, isohydric (drought avoiding or "pessimistic") varieties are those that would modify their growth and physiology to preserve their water status and show a more conservative use of future resources under drought. In contrast, anisohydric ("optimistic") varieties use all the resources available to them in expectation of more arriving (Poni *et al.* 2014, Schultz 2003). As described in Lovisolo *et al.* (2010), isohydric cultivars tend to keep their leaf water potential above a certain threshold, regardless of soil water availability or atmospheric water demand, whereas anisohydric cultivars are those in which leaf water potential drops when the vine faces restricted soil water availability or high atmospheric demand. Usually, grapevines have been classified as isohydric species due to their ability to close their stomata aiming to decrease stomatal conductance in response to low soil water availability to avoid cavitation (Düring 1987, Galmés *et al.* 2007). However, the same variety can behave as iso- or anisohydric depending on the environmental conditions (Lovisolo *et al.* 2010).

Pinot noir has been classified as anisohydric when water stress is applied pre-veraison and as isohydric when it is applied post-veraison (Lovisolo *et al.* 2010). However, there is an unresolved issue in how cultivars can be classified as iso- or anisohydric. In general, the first criterion to classify varieties as being either iso- or anisohydric is how leaf water potential responds to a reduction in soil water availability (Chaves *et al.* 2010). The second criterion to classify cultivars into one of those groups is based on their stomatal sensitivity to respond to soil-water deficit (Schultz 2003). As previously described, isohydric represents a plant behaviour in which leaf water potential is kept steady, regardless of soil water status. This also includes a decrease in stomatal conductance and a decline in the transpiration rate aiming to maintain the vine water status constant. In this study, the lack of differences in $\psi_s$ between CON and RI at all sites in either season, together with the stomatal closure showed by the vines under RI, suggest that Pinot noir behave is an isohydric cultivar.

## 3.5 Conclusions

Pinot noir showed a series of adaptive responses to soil water scarcity over two seasons, which varied across the sites selected for this study. Premature abscission of primary leaves from the cluster zone in plants under RI appeared as the first adaptive strategy, which was registered especially in the second season. The remaining leaves in those plants closed their stomata in order to

maintain normal water status, which was reflected in the lack of differences found for $\psi_s$. However, despite the lower leaf area and stomatal conductance, RI did not increase either leaf proline content or leaf osmotic potential. Leaf $\delta^{13}C$, a known long-term vine water status indicator was also unaltered. This indicates that under the conditions of this study, either in a wet or dry season, primary leaf abscission and stomatal closure act as short-term responses to water stress, and that this response was sufficient to avert significant water deficit stress as evidenced by the levels of proline and leaf osmotic potential.

Stomatal closure also did not impact root carbohydrates concentration, which together with the non-significant variations in $\delta^{13}C$, indirectly suggest that photosynthesis was not negatively affected in leaves after veraison.

The significant differences between sites, even when no differences between CON and RI were observed at each site, highlighted the importance of the site-related factors (such as soil characteristics, and related to it, irrigation frequency and volume) in determining the vine responses under a water-restricted scenario, underlining to the understanding to the importance of terroir for Pinot noir.

Finally, although $\psi_s$ was used instead of leaf water potential to determine vine water status at three different times of the season, the lack of differences in $\psi_s$ between CON and RI through the two seasons at all sites, together with the sensitive response of stomata under water restricted conditions, may suggest an isohydric behaviour pattern of Pinot noir.

# Chapter 4

# The effect of reduced irrigation on grape characteristics at three Pinot noir vineyards with contrasting soils

## 4.1 Introduction

Water stress has been shown to affect berry growth (Ojeda *et al.* 2001), Brix, pH and titratable acidity (Edwards and Clingeleffer 2013), skin phenolic compounds (Ojeda *et al.* 2002), and berry amino acids (De Royer Dupré *et al.* 2014), among other factors. However, most of the studies evaluating the effect of water stress on berry parameters have reported results at harvest, whereas their evolution and variations through the ripening season have been little studied.

As discussed in the previous chapter, Pinot noir did show different adaptive responses to water scarcity aiming to maintain vine water status constant. The climatic differences between sites described earlier may be linked with the variations in plant water status and grape composition. The direct relationship between grape characteristics and wine quality has been extensively studied (De Andrés-de Prado *et al.* 2007, Reynard *et al.* 2011). Berries of Pinot noir, however, have demonstrated to be very responsive to changes in soil water availability, temperatures, solar radiation, viticultural managements etc. (Berdeja *et al.* 2014, Feng *et al.* 2015, Kemp *et al.* 2011, Pastor del Rio and Kennedy 2006), highlighting the importance of evaluating the impact of reducing irrigation. As any change in berry composition may alter wine characteristics, the results of studying this under commercial conditions could have a direct impact on improving water use efficiency, which may be also reflected in specific viticultural managements based on each the site's specific characteristics.

The aim of this study was to quantify the impact of a 50% reduction in the irrigation applied by the viticulture managers' on berry characteristics during two seasons in three commercial Pinot noir vineyards in Waipara.

## 4.2 Materials and methods

Unfortunately, the frost that affected Waipara in October 2014 caused a shortage of grapes in most of the vineyards in the region, including those participating in this project. Therefore, parameters like yield, number of clusters, and typical cluster weights could not be obtained this season. However, there was sufficient crop to collect berry samples regularly from mid-veraison until harvest in 2014-2015. As a result, only berry weight data were used to compare the effect of the treatment over the two seasons.

### 4.2.1 Experimental design

The experimental design, reduced irrigation treatments, and edapho-climatic conditions of each site and season have been described in Chapter 2. Please refer there for more details.

### 4.2.2 Berry samples

Berry samples were collected weekly (weather dependent) from 50% veraison until harvest in both seasons. Fifteen berries per replicate, corresponding to three berries per plant, were randomly sampled from the top, medium, and bottom part of different clusters, to follow the evolution of a series of parameters through the ripening period. A fifteen berry sample was determined before sampling started as the maximum size to obtain weekly samples from 50% veraison until harvest while not dramatically altering crop load at the end of the season, and as well providing enough material to obtain the all data needed from them. This was calculated based on number of clusters and estimated number of berries per cluster before veraison in the first season. Samples were transported in sandwich-sized ziplock-type bags, frozen and stored at -35°C until analysed.

50% veraison was visually determined during regular field evaluations after colour change was detected in the first berries. This was coincident with an increase of Brix, as can be observed in the results section.

#### Skin, seeds, and juice separation

**Average berry weight** was determined by weighing each 15 berry sample and dividing the total weight by 15. Skins, seeds, and grape juice were then separated and stored for different analyses following these procedures. Berries were removed from -35°C and allowed to thaw at room temperature (21°) for about 10 minutes. Each was gently squeezed to separate the skin from the rest of the berry. Skins were weighed and stored at -35°C until required. Grape juice was extracted by manually squeezing the berry pulp using a stainless steel strainer and immediately frozen at -35°C to avoid oxidation. Seeds were quickly counted, weighed to obtain **seed fresh weight**, and then submerged into liquid nitrogen to stop oxidation and stored at -35°C overnight. Afterwards, seeds

were freeze-dried for 48 hours, weighed, and **seed dry weight**, and therefore, **seed water content (%)** were determined. Freeze-dried seed samples were stored at room temperature until they were needed for evaluations.

### 4.2.3 °Brix, pH and titratable acidity from veraison to harvest

Brix were determined using an Atago Pocket refractometer (PAL – 1; Atago Inc., USA). Grape juice pH was measured using a Suntex pH/mV/temperature meter (SP-701; Suntex, Taiwan) with a Eutech Instruments probe (EC 620133; Eutech Instruments Pte Ltd, Singapore). Before analyses, the pH meter was calibrated using two standard buffer solutions of pH 4.0 and 7.0. Titratable acidity (TA) was determined by titration to pH 8.2 using 0.1 M NaOH (LabServ, 97% min; Biolab (Australia) Ltd.). TA was measured on 1 mL of juice for the samples at mid-veraison, and 1.5 mL for all the rest. 0.1 M NaOH was carefully added under constant stirring using micro pipettes and the mL used for titration until pH 8.2 was recorded and used for calculations. The results were reported in g/L as tartaric acid ($H_2T$), and calculated as described in Iland *et al.* (2004):

$$\text{Titratable acidity } (g/L \text{ as } H_2T) = 75 * \text{molarity of NaOH} * \frac{\text{Titre value (mL)}}{\text{Volume of juice (mL)}}$$

### 4.2.4 Methy cellulose precipitation (MCP) from seeds and skins

#### Sample preparation

Skins were thawed at room temperature and 8 mL of deionised water added to each sample (15 skins) to facilitate homogenisation with a Polytron PT 3100 homogeniser (Kinematica AG, Switzerland) for 5 minutes at 23,000 rpm.

Freeze-dried seeds were ground using an IKA A10 analytical grinder (Spectrum Chemical mfg. Corp., CA) for 30 seconds.

#### Extraction

For skin extractions, 10 ml of aqueous ethanol (50% v/v) were added to approximately 1 g of skin homogenate. For seeds, the same volume of aqueous ethanol + 1 mL of deionised water was added to 6 mg of ground seeds. Then, samples were mixed in a rotary shaker for 60 minutes at 60 rpm, and finally centrifuged at 1960 X *g* for 5 minutes.

#### Methylcellulose precipitable tannin concentration

The 1 mL assay proposed by Mercurio *et al.* (2007) was used to determine the tannin concentration of skin and seed extracts. Methylcellulose solution (0.04% w/v, 1500 cP viscosity at 2%, M-0387,

Sigma-Aldrich, USA) and saturated ammonium solution (Sigma-Aldrich, Auckland) were prepared as described in Mercurio *et al.* (2007). Aliquots (100 µL) were used for either skin or seed extractions. Measurements at 280 nm were carried out on a Shimadzu UV-1800 Spectrophotometer (Shimadzu Corporation, Kyoto, Japan), using 1cm pathlength methacrylate disposable cuvettes. As suggested in Mercurio *et al.* (2010), the reading of the control samples at 280 nm was used to determine total phenolics content. Epicatechin was used as standard alongside each batch of samples.

### 4.2.5 Carbon isotope composition in grape juice

Carbon isotope composition ($\delta^{13}C$) of sugars in grape juice was measured as described by Gaudillère *et al.* (2002). Five microlitres of grape juice, obtained from the samples collected at harvest, were pipetted into a tin capsule containing an absorbent (Chromosorb W 30-40 mesh), sealed and loaded into a Sercon (Crewe, UK) GSL elemental analyser. The samples were combusted in the presence of oxygen to convert the carbon in the material to $CO_2$ gas. The resultant $CO_2$ was resolved on a gas chromatograph packed column and passed into a Sercon 20-22 IRMS, where masses 44, 45 and 46 were determined. The samples were analysed with a duplication rate of one in eight. $\delta^{13}C$ was calculated as proposed by Farquhar and Richards (1984). All values were with reference to Vienna-Pee Dee Belemnite standard (V-PDB).

### 4.2.6 YAN (Yeast Assimilable Nitrogen)

Primary amino acid nitrogen (PAAN) and ammonia nitrogen (AN) content were measured using commercial analysis kits (Vintessential Laboratories, Australia), following the manufacturer's instructions. For both PAAN and AN, 1 cm pathlength methacrylate cuvettes were used on a Shimadzu UV-1800 Spectrophotometer (Shimadzu Corporation, Kyoto, Japan), measuring absorbances at 335 and 340nm, respectively. Yeast Assimilable Nitrogen was determined by adding PAAN content to AN content, as indicated by the manufacturer.

### 4.2.7 Grape juice amino acid content

#### Sample preparation

Grape juice samples were diluted 1:10 for 2013-2014 and 1:15 for 2014-2015 with deionised water, passed through a 0.45 µmol/L nylon syringe filter into an HPLC glass vial and capped tightly. These were stored in a fridge for 24 hours until analysed.

#### Equipment

Each sample was analysed on a Hewlett-Packard Agilent 1100 series HPLC system with a 250 x 4.6 mm, 5µm prodigy reverse phase HPLC column (Phenomenex).

### Chromatography

To derivatize the primary amino acids, o-phthaldialdehyde (OPA) was used as a fluorescence derivative reagent for primary amino acid, and 9-fluorenylmethyl chloroformate (FMOC) was a fluorescence derivative for proline. Detection utilised a fluorescence detector with an excitation of 335 nm and emission of 440 nm for primary amino acids. At 26 min, the detector was switched to excitation 260 nm and emission 315 nm to detect proline. Known concentrations of mixed amino acid standards were analysed in parallel to generate calibration curves for quantification of unknown samples. 20 amino acids and 1 non-protein amino acid (taurine) were considered in this study.

The HPLC separation used solvent A (0.01 M $Na_2HPO_4$ with 0.8% THF adjusted to pH 7.5 with $H_3PO_4$) and solvent B (20% solvent A, 40% methanol, 40% acetonitrile). The pump gradient was: 0 min, 0% B; 14 min, 40% B; 22 min, 55% B; from 27 min to 35 min, 100% B; 36 min, 0% B; and equilibrating at 0% B until 40 min with a flow rate of 1.0ml/min. Data were analysed using the Chemstation (Agilent) chromatography data system.

### Abbreviations

For a better understanding of the information presented in the results section, the amino acid names have been abbreviated as follows: aspartic acid (aspartate, ASP), glutamic acid (glutamate, GLU), cysteine (CYS), asparagine (ASN), serine (SER), glutamine (GLN), histidine (HIS), glycine (GLY), threonine (THR), arginine (ARG), alanine (ALA), tyrosine (TYR), valine (VAL), methionine (MET), tryptophan (TRP), phenylalanine (PHE), isoleucine (ILE), lysine (LYS), leucine (LEU), proline (PRO) and taurine (TAU). All the results of amino acid concentration are showed in order of elution.

## 4.2.8 Statistical analyses

A series of statistical analyses were performed depending on the set of data. Firstly, a one-way analysis of variance (ANOVA) and, equivalently, the least significant difference (LSD) test were used to determine statistical differences between CON and RI for each site and season at the 5% level ($p < 0.05$). Then, to evaluate the average treatment difference across sites, the two treatment means for each site were put into a randomised complete block design ANOVA with blocking factor “site” and treatment factor “treatment” with LSD test at 5% for grape juice $\delta^{13}C$, PAAN, AN, YAN, and juice protein amino acids and taurine contents. Data obtained at several points of the season, such as berry weight, seed fresh weight, seed dry weight, seed water content, Brix, pH, TA, seed and skin tannin concentration, and seed and skin total phenolics were analysed in the same manner, but in this case and in order to evaluate cumulative effects, the mean value for each variable derived using the area under the curve (AUC) was calculated from all values of the season and used in the above ANOVAs. Finally, and only for sets of data including the two seasons, the seasonal effect was obtained by calculating the mean difference between the seasons 2013-2014 and 2014-2015 for the

same treatment and site (e.g. difference between CON WH 2013-2014 and CON WH 2014-2015), using the above randomised complete block design ANOVA with blocking factor "site" and treatment factor "treatment". All the analyses were performed using Genstat 18 (GenStat for Windows, VSN International Limited, UK).

Residual plots, including plots of residuals against fitted-value and histogram of residuals were obtained for each set of data to evaluate whether data need any square root or logarithm transformation. Based on this, no transformations were carried out.

All means, LSD 5%, and p values for all two-way ANOVAs of this chapter can be found in Appendix C.

A linear regression analysis was performed to investigate the relationship between grape juice $\delta^{13}C$ at harvest and minimum stem water potential ($\psi_s$). Microsoft® Excel® 2013 was used to obtain the regression equation and coefficient of determination ($R^2$) and Genstat 18 (GenStat for Windows, VSN International Limited, UK) was used to calculate the significance at $p < 0.05$.

Principal component analysis (PCA) was performed on data from the first and second season. For both seasons, five grapevine physiology and eight berry parameters were used to obtain differences between treatments and sites. All analyses were carried out using Genstat 18 (GenStat for Windows, VSN International Limited, UK).

## 4.3 Results

### 4.3.1 Berry parameters

#### Berry weight

Differences in berry weight over the season (AUC) were found at GB5 and GB10 in 2013-2014, whereas in 2014-2015 these were observed at WH and GB5 (Figure 23).

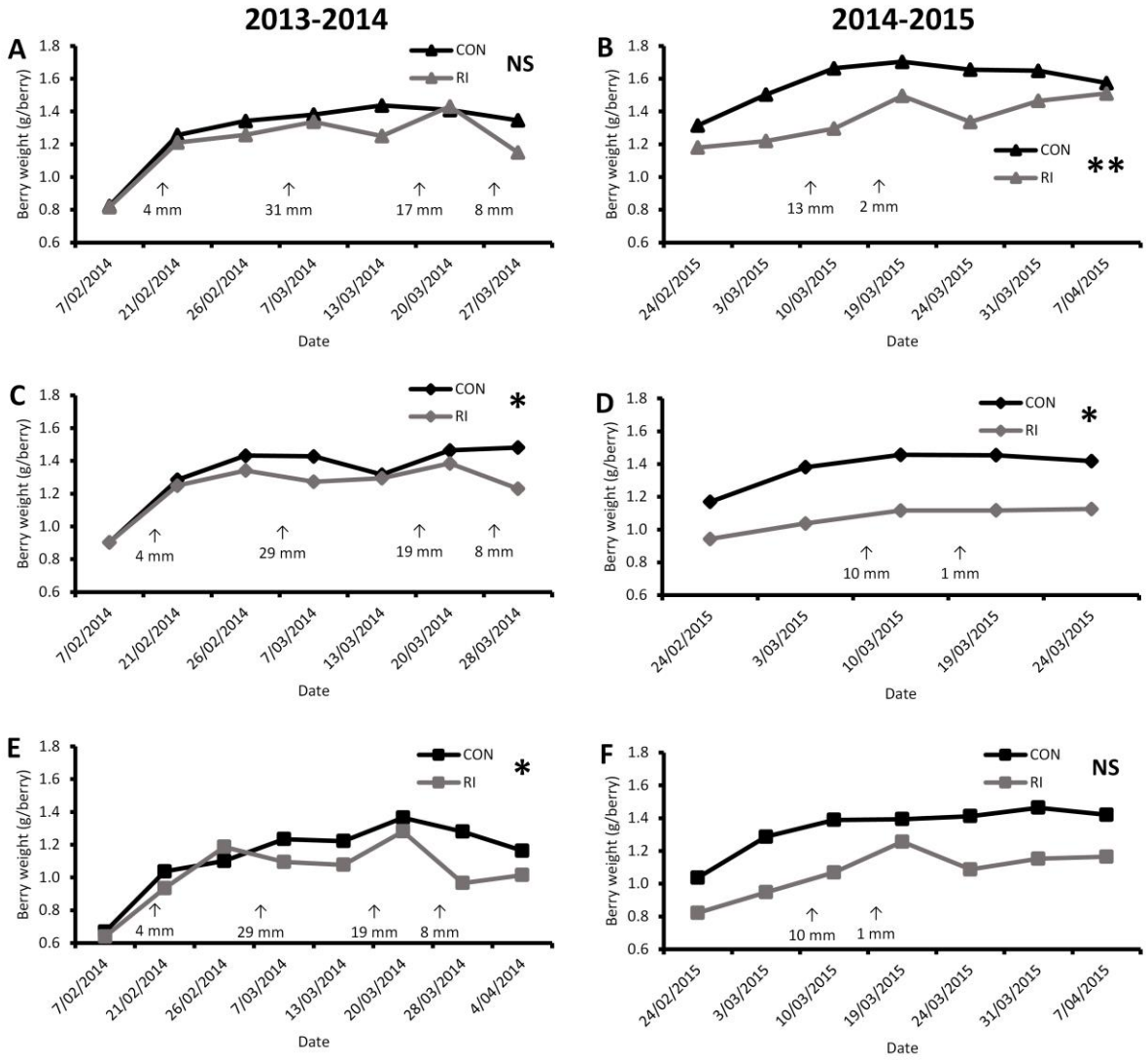


**Figure 23** **Evolution of berry weight (g/berry) over the two seasons at all sites. For any given date, each value represents the average of four replicates. Arrows represent the amount of rainfall registered in certain days of the ripening period. Charts correspond to: WH (A, B), GB5 (C, D), GB10 (E, F). Differences between CON and RI were calculated based on mean area under the curve from all measurements over the season. Significances are: * $p < 0.050$; ** $p < 0.010$; NS non-significant results ($p \geq 0.050$).**

In the first season, berry weights had similar values at veraison (07/02/2014), where the differences between CON and RI started to be noticeable a few weeks later. On the other hand, the effect of RI on berry weight was evident from veraison (24/02/2015) onwards in the second season. Moreover, as can be seen in Figure 23, berries in water-stressed plants were more responsive to rainfall during ripening. Thus, in 2013-2014 berries under RI that started losing weight two or three weeks before harvest recovered part of their weight after a 17mm (WH) and 19mm (GB5 and GB10) rainfall, reaching similar weights as CON. Yet, that water gained during and after the rainfall was rapidly lost during the next two or three weeks, with those berries returning to similar or even lower weight than before the rainfall. The same response was registered in 2014-2015, but was less pronounced because only one big rain event was registered during the ripening period.

The differences between CON and RI in berry weight were also observed in the combined analysis, where the results indicated differences between sites, treatments, and seasons, evidencing the high impact of RI on this parameter (Table 36)

**Table 36 Results of two-way ANOVA on the effects of "site" (WH, GB5 and GB10), "treatment" (CON and RI), and "season" on berry weight. Numbers correspond to p values.**

| Factor | Season | |
|---|---|---|
| | 2013-2014 | 2014-2015 |
| Site | **0.006** | **0.019** |
| Treatment | **0.012** | **0.007** |
| | Combined analysis | |
| Site | **0.010** | |
| Treatment | **0.005** | |
| Season | **0.017** | |

### Seed fresh weight

When the evolution of fresh seed weight was analysed for each site and season, RI did not show an effect on this at any site (Figure 24).

As can be observed in Figure 24, fresh seed weight tended to decrease during the ripening period, reaching its minimum at harvest. Moreover, seed fresh weight was higher at all sites in 2014-2015 in either treatment compared to the previous season.

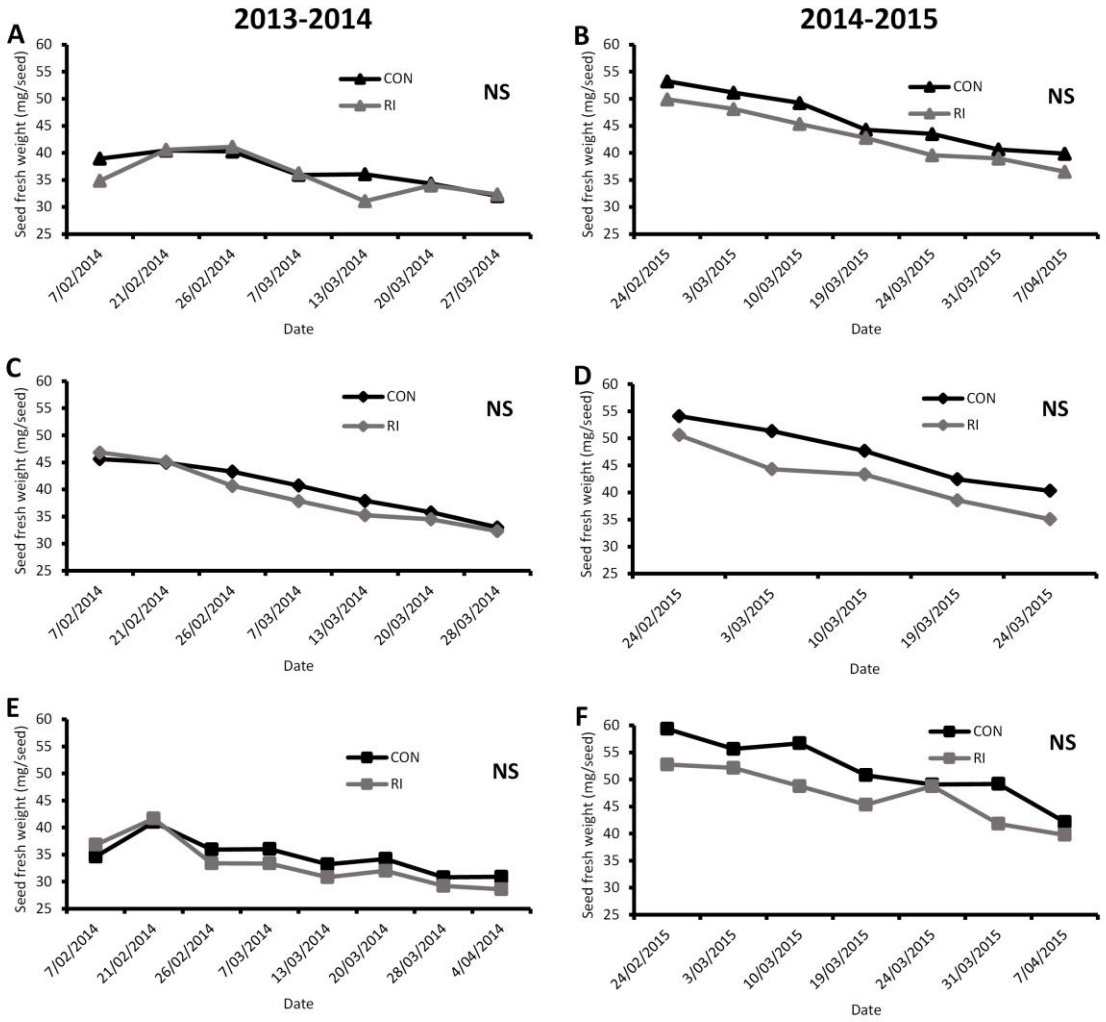


**Figure 24** **Evolution of seed fresh weight (mg/seed) over the two seasons at all sites. For any given date, each value represents the average of four replicates. Charts correspond to: WH (A, B), GB5 (C, D), GB10 (E, F). Differences between CON and RI were calculated based on mean area under the curve from all measurements over the season. NS means non-significant results ($p \geq 0.050$).**

In both seasons there were differences in seed fresh weight across sites, with the treatment also showing an overall effect (Table 37). However, when the results of both seasons were combined, no differences across sites were observed, while the treatment and season showed differences.

**Table 37 Results of two-way ANOVA on the effects of "site" (WH, GB5 and GB10), "treatment" (CON and RI), and "season" on fresh seed weight. Numbers correspond to p values.**

| Factor | Season | |
|---|---|---|
| | 2013-2014 | 2014-2015 |
| Site | **0.003** | **0.038** |
| Treatment | **0.018** | **0.024** |
| | Combined analysis | |
| Site | 0.151 | |
| Treatment | **0.022** | |
| Season | **0.027** | |

### Seed dry weight

Seed dry weight was not altered by RI in either season, except at GB10 in 2013-2014 (Figure 25). Although in most of the cases no differences were found, it is worth noting that seed dry weight in vines under RI was slightly lower than in CON.

In general, seed dry weight remained stable from veraison onwards in either season. Furthermore, in Figure 25 can be seen that seed dry weight reported higher values in the second season compared to the first one.

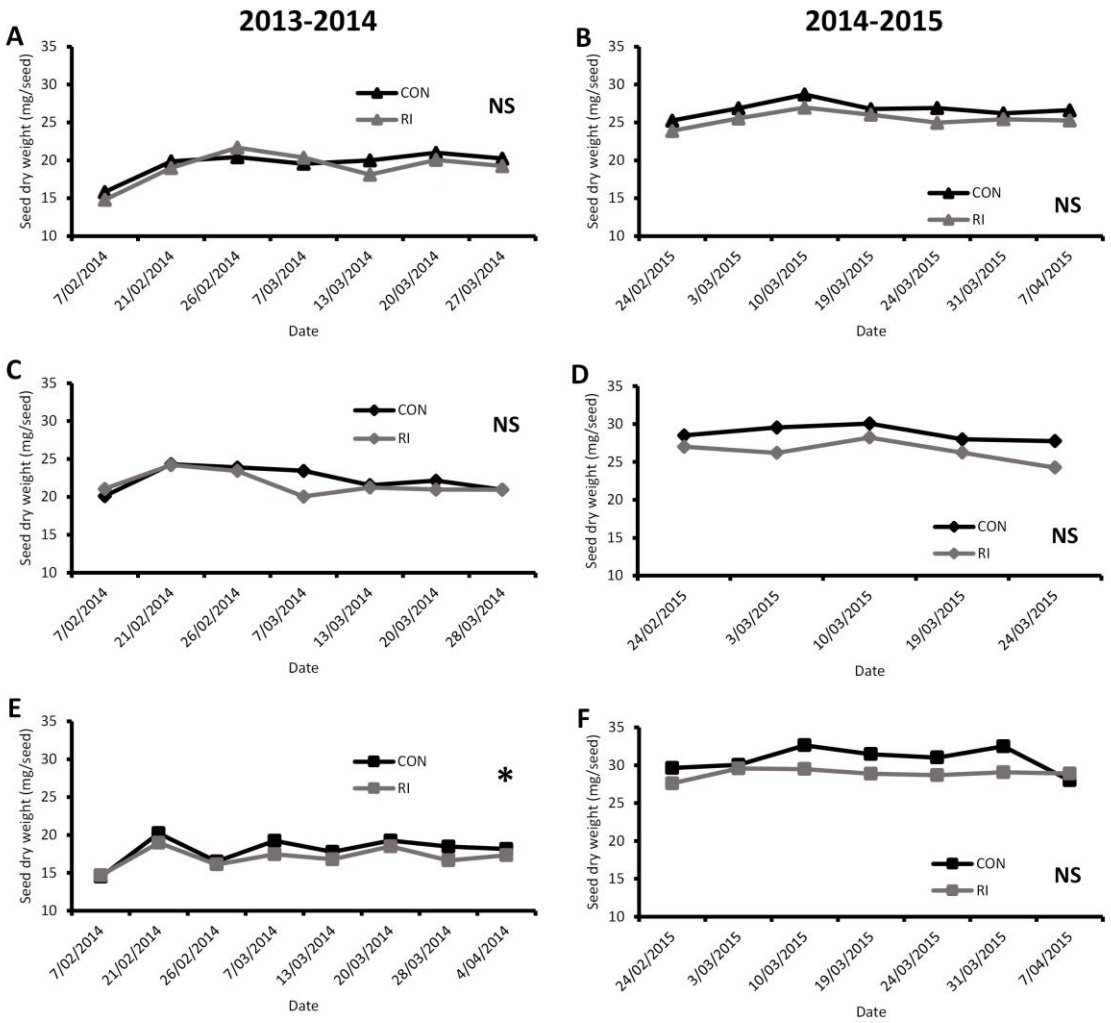


**Figure 25** **Evolution of seed dry weight (mg/seed) over the two seasons at all sites. For any given date, each value represents the average of four replicates. Charts correspond to: WH (A, B), GB5 (C, D), GB10 (E, F). Differences between CON and RI were calculated based on mean area under the curve from all measurements over the season. Significances are: * $p < 0.050$; NS non-significant results ($p \geq 0.050$).**

Although no differences were observed in seed dry weight when data from CON and RI when analysed for each site (Figure 25), the overall analysis indicated that there were variations across sites and seasons, and an overall effect of the treatment was also reported (Table 38).

**Table 38 Results of two-way ANOVA on the effects of "site" (WH, GB5 and GB10), "treatment" (CON and RI), and "season" on seed dry weight (mg/seed). Numbers correspond to p values.**

| Factor | Season | |
|---|---|---|
| | 2013-2014 | 2014-2015 |
| Site | **0.005** | **0.020** |
| Treatment | **0.048** | **0.026** |
| | Combined analysis | |
| Site | **0.037** | |
| Treatment | **0.029** | |
| Season | **0.028** | |

### Seed water content (%)

Seed water content progressively decreased through the ripening season, showing no differences between CON and RI, except at GB5 in the second season (Figure 26). The results also indicate that higher values were reported in the first season (A, C, and E) compared to 2014-2015.

Overall, in either season and in most of the sampling dates, seed water content was marginally lower in water-stressed vines, despite no differences being detected.

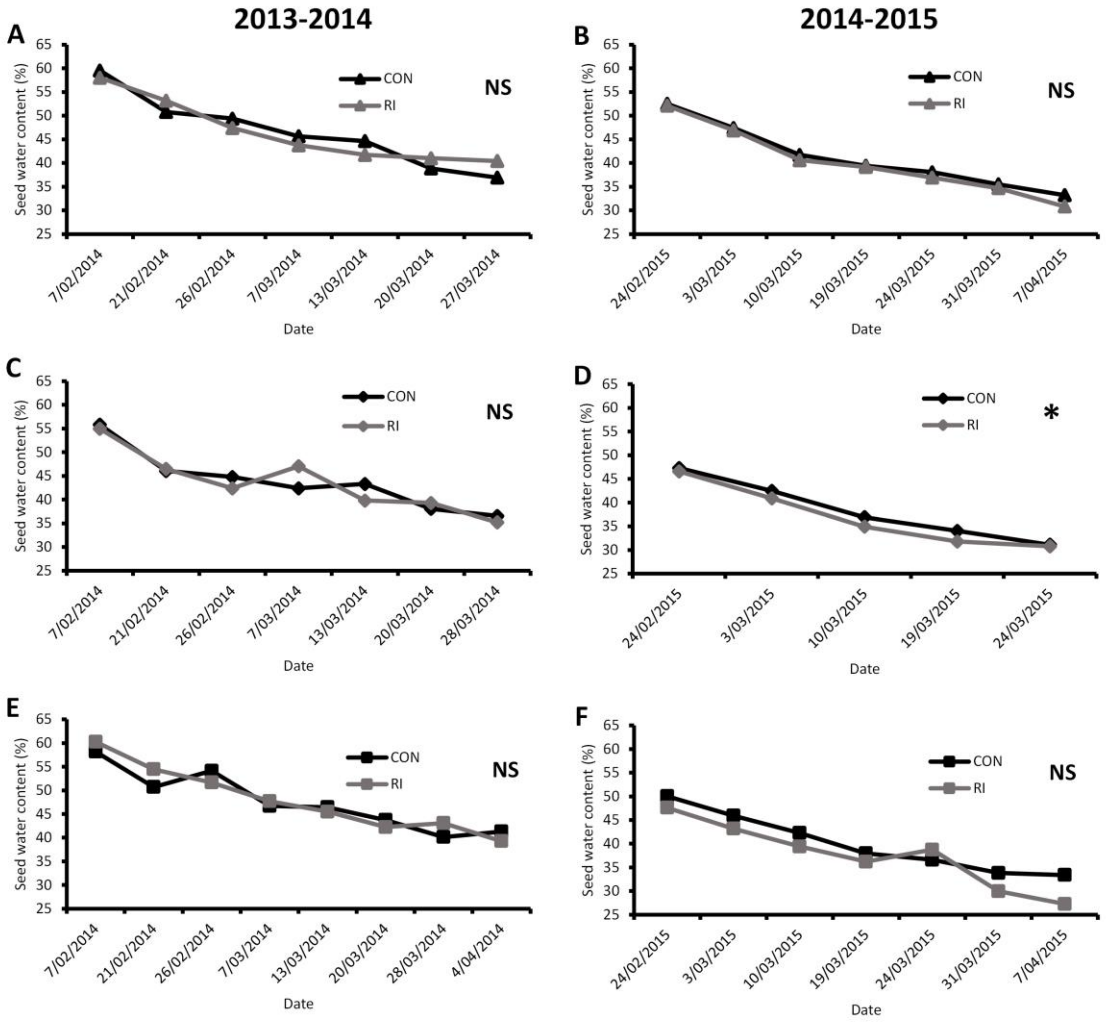


**Figure 26** **Evolution of seed water content (%) over the two seasons at all sites. For any given date, each value represents the average of four replicates. Charts correspond to: WH (A, B), GB5 (C, D), GB10 (E, F). Differences between CON and RI were calculated based on mean area under the curve from all measurements over the season. Significances are: * $p < 0.050$; NS non-significant results ($p \geq 0.050$).**

Similar to the other seed parameters reported earlier, differences across sites in seed water content were found in both seasons. The variations site by site and the effect of the treatment showed also differences in the combined analysis. However, no variations between seasons were observed (Table 39).

**Table 39 Results of two-way ANOVA on the effects of "site" (WH, GB5 and GB10), "treatment" (CON and RI), and "season" on seed water content (%). Numbers correspond to p values.**

| Factor | Season | |
|---|---|---|
| | 2013-2014 | 2014-2015 |
| Site | **0.006** | **0.047** |
| Treatment | 0.884 | 0.057 |
| | Combined analysis | |
| Site | **0.004** | |
| Treatment | **0.021** | |
| Season | 0.108 | |

### 4.3.2 Brix, pH and TA through the ripening period

#### Brix

Differences in evolution of Brix were reported only at WH in both seasons (Figure 27).

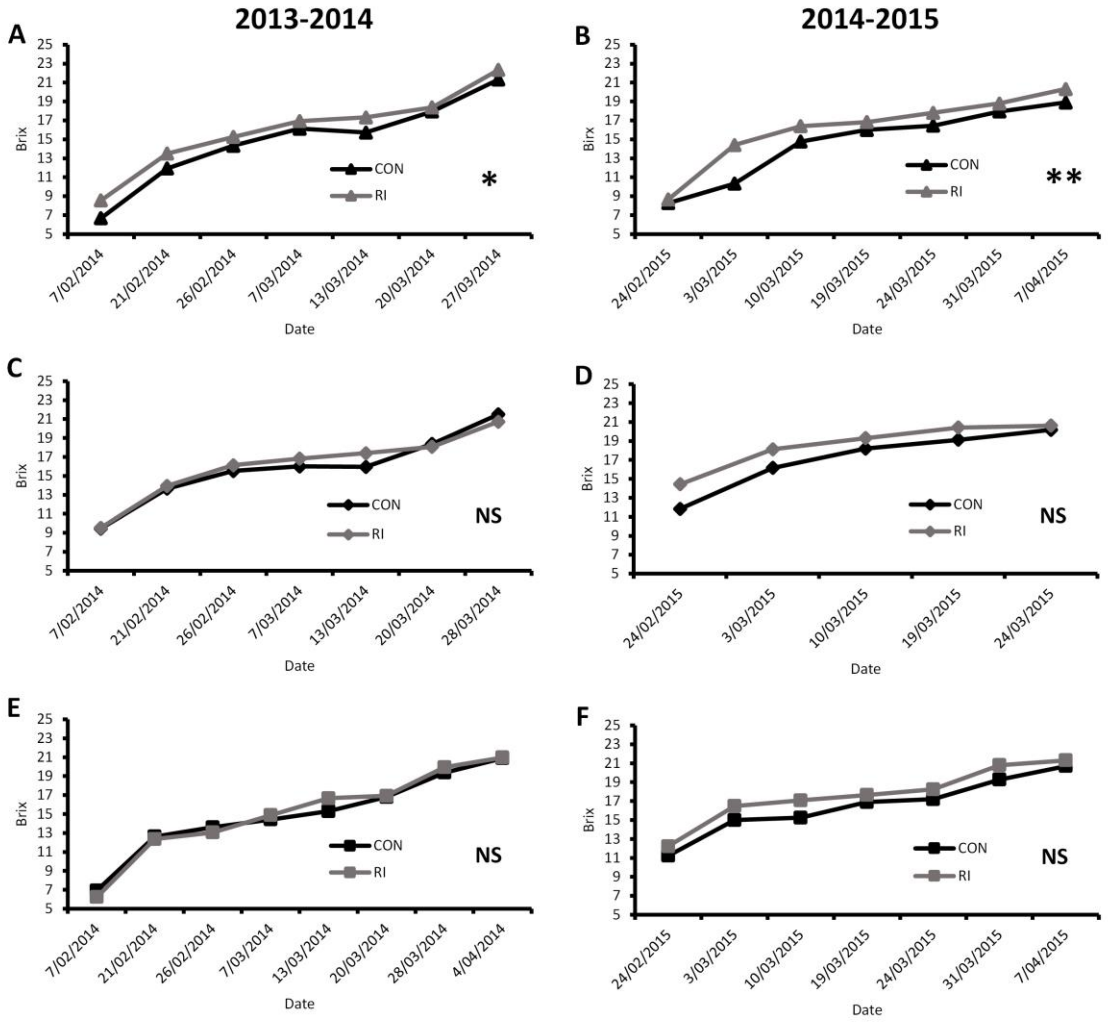


**Figure 27 Evolution of Brix over the two seasons at all sites. For any given date, each value represents the average of four replicates. Charts correspond to: WH (A, B), GB5 (C, D), GB10 (E, F). Differences between CON and RI were calculated based on mean area under the curve from all measurements over the season. Significances are: * $p < 0.050$; ** $p < 0.010$; NS non-significant results ($p \geq 0.050$).**

The two-ways ANOVAs reported no differences in Brix between sites and treatments in the first season, whereas both of them reported differences in the second (Table 40). Also, the combined analysis did not show differences in Brix across sites when data from both seasons were analysed, but the effect of the treatment as well as differences between seasons were observed.

**Table 40 Results of two-way ANOVA on the effects of "site" (WH, GB5 and GB10), "treatment" (CON and RI), and "season" on Brix. Numbers correspond to p values.**

| Factor | Season | |
|---|---|---|
| | 2013-2014 | 2014-2015 |
| Site | 0.258 | **0.010** |
| Treatment | 0.173 | **0.010** |
| | Combined analysis | |
| Site | 0.058 | |
| Treatment | **0.041** | |
| Season | **0.023** | |

## Grape juice pH

RI did not have an effect on grape juice pH in either season, except at WH in 2014-2015 (Figure 28).

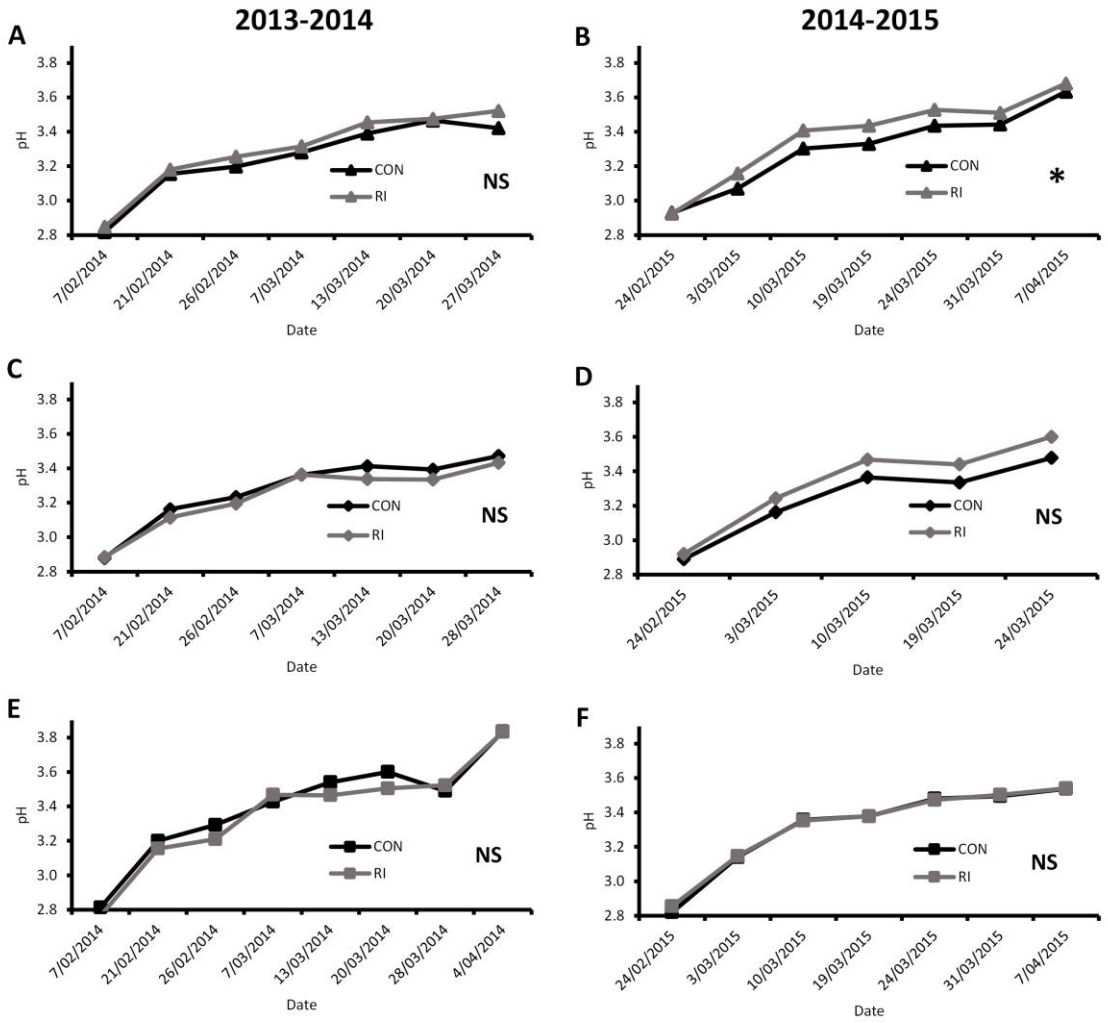


**Figure 28** **Evolution of grape juice pH over the two seasons at all sites. For any given date, each value represents the average of four replicates. Charts correspond to: WH (A, B), GB5 (C, D), GB10 (E, F). Differences between CON and RI were calculated based on mean area under the curve from all measurements over the season. Significances are: * p < 0.050; NS non-significant results (p ≥ 0.050).**

No differences between sites and treatments were observed for grape juice pH in either season or between seasons (Table 41).

**Table 41 Results of two-way ANOVA on the effects of "site" (WH, GB5 and GB10), "treatment" (CON and RI), and "season" on grape juice pH. Numbers correspond to p values.**

| Factor | Season | |
|---|---|---|
| | 2013-2014 | 2014-2015 |
| Site | 0.114 | 0.521 |
| Treatment | 0.667 | 0.185 |
| | Combined analysis | |
| Site | 0.210 | |
| Treatment | 0.448 | |
| Season | 0.145 | |

## Titratable acidity (TA)

TA was unaffected by RI in either season or any site (Figure 29).

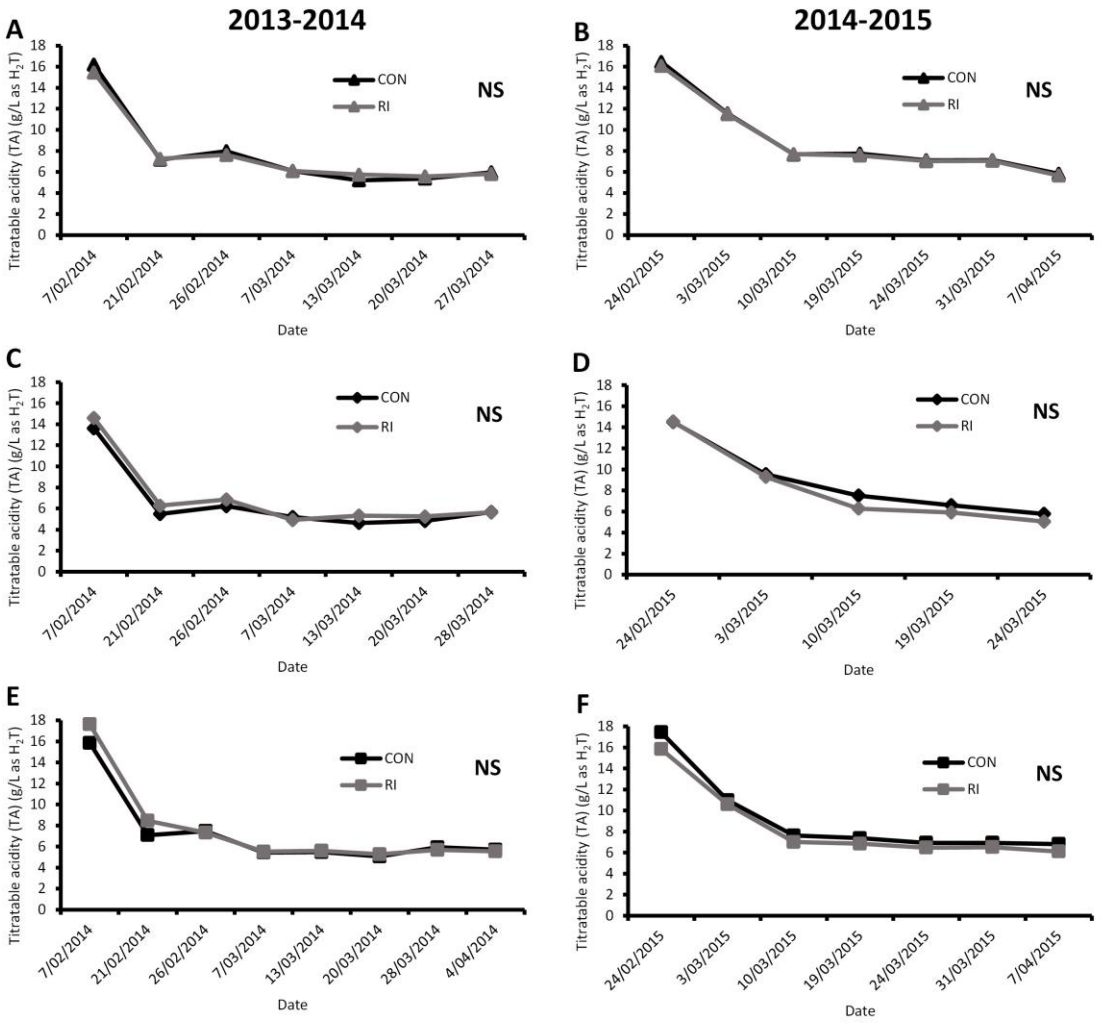


**Figure 29** **Evolution of titratable acidity (TA) over the two seasons at all sites. For any given date, each value represents the average of four replicates. Charts correspond to: WH (A, B), GB5 (C, D), GB10 (E, F). Differences between CON and RI were calculated based on mean area under the curve from all measurements over the season. NS means non-significant results ($p \geq 0.050$).**

The two-ways ANOVAs for each season did not show differences in TA between sites and treatments. Only the combined analysis reported differences across sites, but not between treatments or seasons (Table 42).

**Table 42 Results of two-way ANOVA on the effects of "site" (WH, GB5 and GB10), "treatment" (CON and RI), and "season" on titratable acidity (TA). Numbers correspond to p values.**

| Factor | Season | |
|---|---|---|
| | 2013-2014 | 2014-2015 |
| Site | 0.061 | 0.216 |
| Treatment | 0.208 | 0.122 |
| | Combined analysis | |
| Site | **0.006** | |
| Treatment | 0.130 | |
| Season | 0.149 | |

### 4.3.3 Tannin concentration by MCP in seeds and skins

#### Seed tannin concentration

No differences were found for seed tannin concentration at any site in either season (Figure 30). However, it is worth noting that seed tannin concentration followed a different trend in each season. In 2013-2014 (A, C, and E) this decreased gradually toward harvest, while in 2014-2015 (B, D, and F) this followed a similar tendency only for a few weeks after veraison, and then increased steadily until harvest.

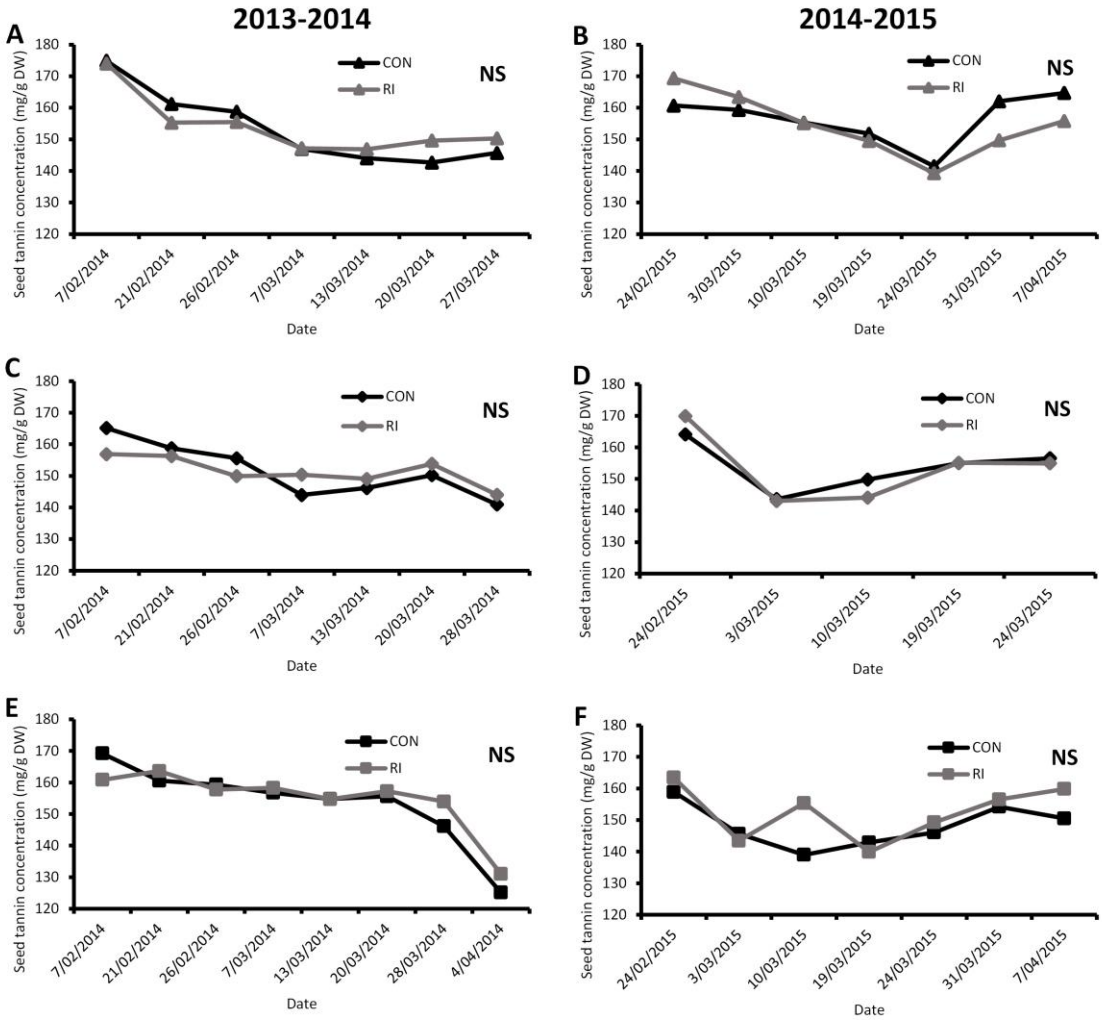


**Figure 30 Evolution of seed tannin concentration (mg/g DW epicatechin equivalents) over the two seasons at all sites. For any given date, each value represents the average of four replicates. Charts correspond to: WH (A, B), GB5 (C, D), GB10 (E, F). Differences between CON and RI were calculated based on mean area under the curve from all measurements over the season. NS means non-significant results ($p \geq 0.050$).**

Only differences in seed tannin concentration across sites were reported in the first season. No differences between other variables were found (Table 43).

**Table 43 Results of two-way ANOVA on the effects of "site" (WH, GB5 and GB10), "treatment" (CON and RI), and "season" on seed tannin concentration. Numbers correspond to p values.**

| Factor | Season | |
|---|---|---|
| | 2013-2014 | 2014-2015 |
| Site | **0.032** | 0.273 |
| Treatment | 0.175 | 0.906 |
| | Combined analysis | |
| Site | 0.502 | |
| Treatment | 0.686 | |
| Season | 0.746 | |

## Seed total phenolics

Seed total phenolics followed a similar trend as seed tannin concentration, but in this case, differences between CON and RI were observed at GB5 in 2014-2015 only. All other sites in either season showed no differences (Figure 31).

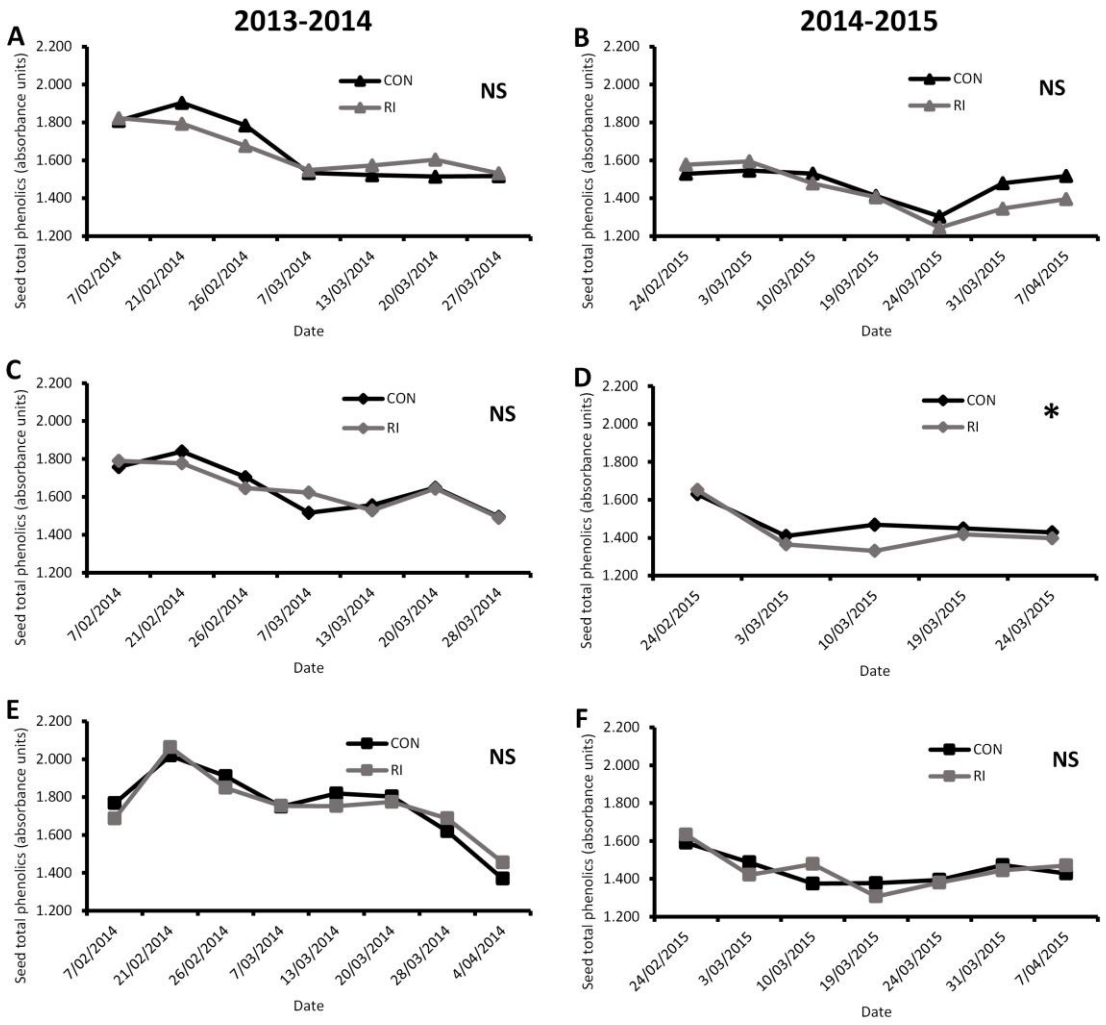


**Figure 31** **Evolution of seed total phenolics (absorbance units) over the two seasons at all sites. For any given date, each value represents the average of four replicates. Charts correspond to: WH (A, B), GB5 (C, D), GB10 (E, F). Differences between CON and RI were calculated based on mean area under the curve from all measurements over the season. Significances are: * $p < 0.050$; NS non-significant results ($p \geq 0.050$).**

Unlike for seed tannin concentration, differences in seed total phenolics between sites were reported in the first season, as well as in the overall analysis. No differences in seed total phenolics between seasons were observed (Table 44).

**Table 44 Results of two-way ANOVA on the effects of "site" (WH, GB5 and GB10), "treatment" (CON and RI), and "season" on total seed phenolics. Numbers correspond to p values.**

| Factor | Season | |
|---|---|---|
| | 2013-2014 | 2014-2015 |
| Site | **<.001** | 0.798 |
| Treatment | **0.017** | 0.149 |
| | Combined analysis | |
| Site | **0.030** | |
| Treatment | 0.117 | |
| Season | 0.199 | |

### Skin tannin concentration

The evolution of skin tannin concentration showed differences between CON and RI at WH in 2013-2014 and GB5 in 2014-2015, whereas no differences were reported at GB10 in either season (Figure 32).

Although evolution of skin tannin concentration followed a similar trend as seed tannin concentration in 2013-2014, with both CON and RI tending to decrease toward harvest, this was less pronounced than for seed tannins. Both sites at Greystone vineyard showed a similar trend as seed tannin in the second season, with this decreasing from veraison onwards and increasing just before harvest, unlike WH where both CON and RI showed a small decrease during the last week pre-harvest.

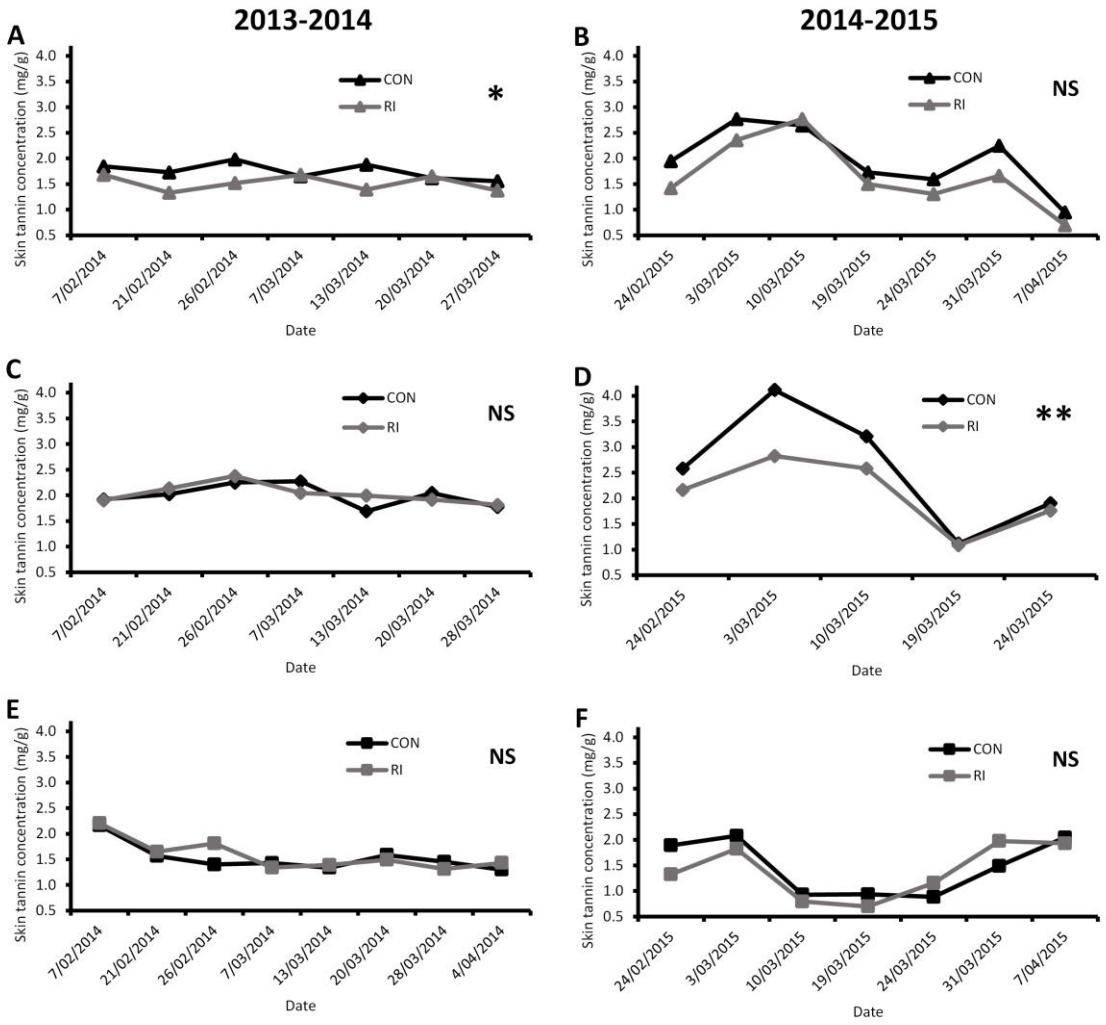


**Figure 32 Evolution of skin tannin concentration (mg epicatechin equivalents/g of homogenate) over the two seasons at all sites. For any given date, each value represents the average of four replicates. Charts correspond to: WH (A, B), GB5 (C, D), GB10 (E, F). Differences between CON and RI were calculated based on mean area under the curve**

**from all measurements over the season. Significances are: * p < 0.050; ** p < 0.010; NS non-significant results (p ≥ 0.050).**

In each season, neither site nor treatment had an effect on skin tannin concentration, but in the combined analysis differences between sites were found in skin tannin concentration. Treatment, however, had no effect on this parameter, with also no differences between seasons being reported (Table 45)

**Table 45 Results of two-way ANOVA on the effects of "site" (WH, GB5 and GB10), "treatment" (CON and RI), and "season" on skin tannin concentration. Numbers correspond to p values.**

| Factor | Season | |
|---|---|---|
| | 2013-2014 | 2014-2015 |
| Site | 0.083 | 0.061 |
| Treatment | 0.617 | 0.189 |
| | Combined analysis | |
| Site | **0.039** | |
| Treatment | 0.192 | |
| Season | 0.303 | |

### Skin total phenolics concentration

RI did not affect the seasonal evolution of skin total phenolics at any site in either season (Figure 33). Interestingly, as previously described, skin tannin concentration tended to decrease during the ripening period in 2013-2014, opposite to skin total phenolics, which increased toward harvest. On the other hand, skin total phenolics followed a similar tendency as skin tannins in 2014-2015.

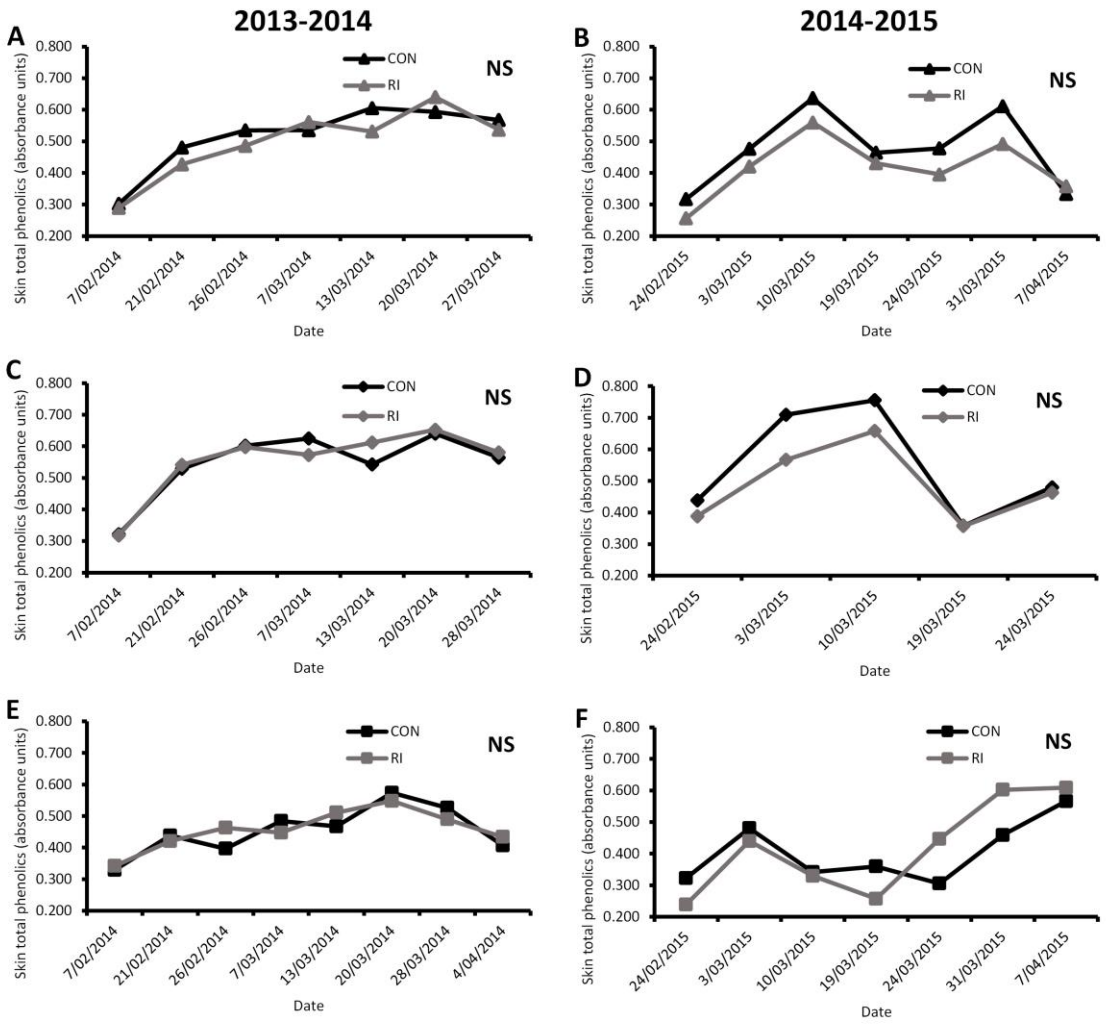


**Figure 33** **Evolution of skin total phenolics (absorbance units) over the two seasons at all sites. For any given date, each value represents the average of four replicates. Charts correspond to: WH (A, B), GB5 (C, D), GB10 (E, F). Differences between CON and RI were calculated based on mean area under the curve from all measurements over the season. NS means non-significant results ($p \geq 0.050$).**

Differences in skin total phenolics across sites were reported in the first season only, while the treatment did not affect skin total phenolics in either season. The combined analysis also found no differences between sites, treatments or seasons (Table 46).

**Table 46 Results of two-way ANOVA on the effects of "site" (WH, GB5 and GB10), "treatment" (CON and RI), and "season" on skin total phenolics (absorbance units). Numbers correspond to p values.**

| Factor | Season | |
|---|---|---|
| | 2013-2014 | 2014-2015 |
| Site | **0.021** | 0.126 |
| Treatment | 0.691 | 0.307 |
| | Combined analysis | |
| Site | 0.054 | |
| Treatment | 0.315 | |
| Season | 0.330 | |

### 4.3.4 Carbon isotope ratio ($\delta^{13}C$) in grape juice at harvest

No differences between CON and RI were found for $\delta^{13}C$ in grape juice at harvest in either season or any site (Table 47).

**Table 47 Carbon isotope ratio ($\delta^{13}C$) in grape juice at harvest during seasons 2013-2014 and 2014-2015. Each value is the average of four replicates.**

| Season 2013-2014 | | | |
|---|---|---|---|
| Treatment | Site | | |
| | WH | GB5 | GB10 |
| CON | -27.75 | -26.99 | -26.38 |
| RI | -26.95 | -26.45 | -26.39 |
| LSD 5% | 0.87 | 2.56 | 2.04 |
| p value | 0.064 | 0.627 | 0.995 |
| Season 2014-2015 | | | |
| Treatment | Site | | |
| | WH | GB5 | GB10 |
| CON | -25.57 | -25.71 | -26.35 |
| RI | -25.12 | -24.36 | -26.21 |
| LSD 5% | 1.18 | 2.57 | 2.41 |
| p value | 0.384 | 0.244 | 0.892 |

Confirming the results previously reported, no differences in grape juice $\delta^{13}C$ were found between sites, treatments and seasons in the overall analysis (Table 48)

**Table 48 Results of two-ways ANOVA on the effects of "site" (WH, GB5 and GB10), "treatment" (CON and RI), and "season" on grape juice $\delta^{13}C$ at harvest. Numbers correspond to p values.**

| Factor | Season | |
|---|---|---|
| | 2013-2014 | 2014-2015 |
| Site | 0.151 | 0.191 |
| Treatment | 0.203 | 0.217 |
| | Combined analysis | |
| Site | 0.410 | |
| Treatment | 0.167 | |
| Season | 0.608 | |

### 4.3.5 Correlation between grape juice $\delta^{13}C$ and minimum stem water potential ($\psi_s$)

A high correlation was found between grape juice $\delta^{13}C$ and minimum $\psi_s$ at all sites in both seasons, except at GB5 in 2013-2014 where the $R^2$ obtained was the lower of all sites over the two seasons, this correlation being also non-significant (Figure 34).

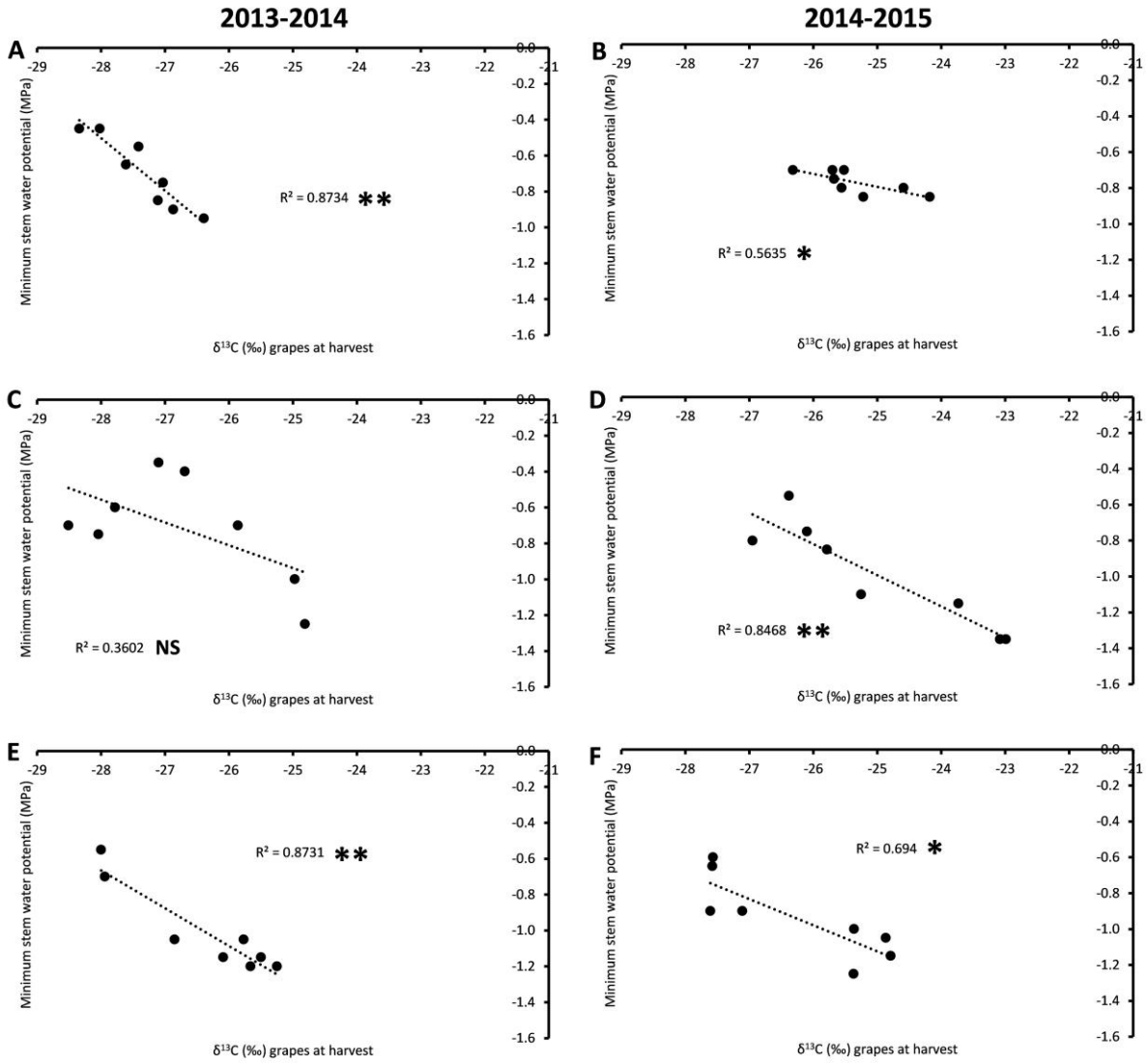


**Figure 34 Correlation between grape juice $\delta^{13}C$ (‰) at harvest and minimum stem water potential (MPa) during the 2013-2014 and 2014-2015 seasons at all sites. Each value is the average of four replicates. Charts correspond to: WH (A, B), GB5 (C, D), GB10 (D, F). Significances are: * p < 0.050; ** p < 0.010; NS non-significant results (p ≥ 0.050).**

### 4.3.6 YAN

#### Primary amino acid nitrogen (PAAN)

Differences in PAAN between CON and RI were detected at WH in 2013-2014 and GB10 in 2014-2015 (Table 49).

**Table 49 Primary amino acid nitrogen (PAAN) (mg N/L) during seasons 2013-2014 and 2014-2015. Each value is the average of four replicates.**

| Season 2013-2014 | | | |
|---|---|---|---|
| Treatment | Site | | |
| | WH | GB5 | GB10 |
| CON | 119.5 | 126.0 | 224.0 |
| RI | 165.5 | 134.5 | 192.5 |
| LSD 5% | 32.4 | 56.5 | 69.6 |
| p value | **0.013** | 0.726 | 0.311 |
| Season 2014-2015 | | | |
| Treatment | Site | | |
| | WH | GB5 | GB10 |
| CON | 261.0 | 186.8 | 247.0 |
| RI | 278.8 | 200.0 | 165.5 |
| LSD 5% | 56.2 | 96.0 | 70.9 |
| p value | 0.469 | 0.747 | **0.031** |

No differences in PAAN across sites, treatments and seasons were reported (Table 50)

**Table 50 Results of two-way ANOVA on the effects of "site" (WH, GB5 and GB10), "treatment" (CON and RI), and "season" on primary amino acid nitrogen (PAAN). Numbers correspond to p values.**

| Factor | Season | |
|---|---|---|
| | 2013-2014 | 2014-2015 |
| Site | 0.176 | 0.319 |
| Treatment | 0.765 | 0.655 |
| | Combined analysis | |
| Site | 0.442 | |
| Treatment | 0.879 | |
| Season | 0.263 | |

## Ammonia nitrogen (AN)

AN was unaffected by RI at all sites in either season (Table 51).

**Table 51 Ammonia nitrogen (mg/L) during seasons 2013-2014 and 2014-2015. Each value is the average of four replicates.**

| Season 2013-2014 | | | |
|---|---|---|---|
| Treatment | Site | | |
| | WH | GB5 | GB10 |
| CON | 43.3 | 48.2 | 55.4 |
| RI | 45.4 | 45.8 | 52.8 |
| LSD 5% | 2.40 | 5.9 | 4.21 |
| p value | 0.076 | 0.344 | 0.174 |
| Season 2014-2015 | | | |
| Treatment | Site | | |
| | WH | GB5 | GB10 |
| CON | 40.3 | 39.1 | 41.1 |
| RI | 40.8 | 32.8 | 37.5 |
| LSD 5% | 0.59 | 12.48 | 7.33 |
| p value | 0.062 | 0.259 | 0.274 |

Differences in AN between sites and treatments were not found (Table 52).

**Table 52 Results of two-ways ANOVA on the effects of "site" (WH, GB5 and GB10), "treatment" (CON and RI), and "season" on ammonia nitrogen. Numbers correspond to p values.**

| Factor | Season | |
|---|---|---|
| | 2013-2014 | 2014-2015 |
| Site | 0.065 | 0.341 |
| Treatment | 0.593 | 0.254 |
| | Combined analysis | |
| Site | 0.222 | |
| Treatment | 0.354 | |
| Season | 0.134 | |

### Yeast available nitrogen (YAN)

As found for PAAN, WH in 2013-2014 and GB10 in 2014-2015 showed differences in YAN between CON and RI (Table 53).

**Table 53 Yeast available nitrogen (YAN) (mg/L) during seasons 2013-2014 and 2014-2015. Each value is the average of four replicates.**

| Season 2013-2014 | | | |
|---|---|---|---|
| Treatment | Site | | |
| | WH | GB5 | GB10 |
| CON | 162.8 | 174.3 | 279.4 |
| RI | 210.9 | 180.3 | 245.3 |
| LSD 5% | 33.38 | 58.2 | 73.2 |
| p value | **0.012** | 0.808 | 0.297 |
| Season 2014-2015 | | | |
| Treatment | Site | | |
| | WH | GB5 | GB10 |
| CON | 301.3 | 225.9 | 288.1 |
| RI | 319.6 | 232.8 | 203.0 |
| LSD 5% | 56.3 | 106.5 | 73.1 |
| p value | 0.457 | 0.879 | **0.029** |

Following the tendency showed for PAAN and AN, no differences between sites, treatments, or seasons were found for YAN (Table 54)

**Table 54 Results of two-ways ANOVA on the effects of "site" (WH, GB5 and GB10), "treatment" (CON and RI), and "season" on yeast available nitrogen (YAN). Numbers correspond to p values.**

| Factor | Season | |
|---|---|---|
| | 2013-2014 | 2014-2015 |
| Site | 0.163 | 0.304 |
| Treatment | 0.805 | 0.604 |
| | Combined analysis | |
| Site | 0.424 | |
| Treatment | 0.832 | |
| Season | 0.219 | |

### 4.3.7 Grape juice amino acids

Due to the volume of information, results from each site will be reported in separate tables. The results are listed in order of elution.

Overall, berries from plants in RI at WH showed the higher concentration of amino acids in either season (Table 55). In 2013-2014, differences between CON and RI were found for GLU, SER, THR, ARG, VAL, MET, LYS, LEU, and PRO, whereas only LYS showed differences in 2014-2015.

**Table 55 Grape juice amino acid concentration (µmol/L) at Waipara Hills during the 2013-2014 and 2014-2015 seasons. Each value is the average of four replicates.**

| Season 2013-2014 | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Treatment | Amino acid (µmol/L) | | | | | | | | | | | | | | | | | | | |
| | ASP | GLU | CYS | ASN | SER | GLN | HIS | GLY | THR | ARG | ALA | TYR | VAL | MET | TRP | PHE | ILE | LYS | LEU | PRO |
| CON | 217 | 195 | n.r | 0 | 495 | 498 | 161 | 23 | 876 | 3,487 | 1,527 | 3 | 198 | 60 | 64 | 83 | 135 | 80 | 160 | 1,638 |
| RI | 276 | 254 | n.r | 11 | 665 | 687 | 181 | 33 | 1,217 | 5,027 | 2,001 | 13 | 288 | 89 | 83 | 109 | 199 | 88 | 253 | 2,065 |
| LSD 5% | 107 | 58 | n.r | 13 | 113 | 282 | 20 | 11 | 283 | 1,345 | 494 | 12 | 77 | 28 | 32 | 41 | 77 | 7 | 77 | 410 |
| p value | 0.225 | **0.048** | n.r | 0.080 | **0.011** | 0.153 | 0.055 | 0.066 | **0.025** | **0.031** | 0.058 | 0.102 | **0.029** | **0.041** | 0.204 | 0.176 | 0.088 | **0.030** | **0.026** | **0.043** |
| Season 2014-2015 | | | | | | | | | | | | | | | | | | | | |
| Treatment | Amino acid (µmol/L) | | | | | | | | | | | | | | | | | | | |
| | ASP | GLU | CYS | ASN | SER | GLN | HIS | GLY | THR | ARG | ALA | TYR | VAL | MET | TRP | PHE | ILE | LYS | LEU | PRO |
| CON | 156 | 387 | n.r | 15 | 721 | 1,085 | 291 | 50 | 1,617 | 4,443 | 2,608 | 24 | 590 | 144 | 157 | 231 | 447 | 0 | 565 | 2,905 |
| RI | 172 | 425 | n.r | 19 | 779 | 1,090 | 298 | 55 | 1,746 | 5,295 | 2,833 | 17 | 596 | 151 | 152 | 234 | 426 | 90 | 539 | 3,187 |
| LSD 5% | 55 | 171 | n.r | 26 | 147 | 499 | 43 | 16 | 396 | 1,287 | 702 | 33 | 191 | 43 | 52 | 91 | 162 | 73 | 206 | 435 |
| p value | 0.494 | 0.606 | n.r | 0.667 | 0.372 | 0.984 | 0.736 | 0.547 | 0.457 | 0.156 | 0.464 | 0.642 | 0.947 | 0.720 | 0.838 | 0.941 | 0.768 | **0.024** | 0.768 | 0.164 |

n.r: not reported

Only LYS in the first season showed differences between CON and RI at GB5, while no amino acids reportes differences in 2014-2015 (Table 56).

**Table 56 Grape juice amino acid concentration (µmol/L) at Greystone block 5 during the 2013-2014 and 2014-2015 seasons. Each value is the average of four replicates.**

| Season 2013-2014 | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Treatment | Amino acid (µmol/L) | | | | | | | | | | | | | | | | | | | |
| | ASP | GLU | CYS | ASN | SER | GLN | HIS | GLY | THR | ARG | ALA | TYR | VAL | MET | TRP | PHE | ILE | LYS | LEU | PRO |
| CON | 153 | 276 | 3 | 24 | 617 | 850 | 180 | 39 | 1,211 | 4,287 | 2,013 | 27 | 390 | 111 | 99 | 160 | 283 | 76 | 355 | 3,155 |
| RI | 157 | 318 | 17 | 36 | 584 | 659 | 127 | 44 | 1,149 | 3,528 | 1,648 | 42 | 401 | 115 | 66 | 136 | 299 | 30 | 354 | 3,344 |
| LSD 5% | 71 | 168 | 15 | 33 | 235 | 481 | 78 | 26 | 513 | 1,823 | 759 | 37 | 309 | 96 | 53 | 100 | 247 | 31 | 298 | 2,034 |
| p value | 0.901 | 0.564 | 0.059 | 0.409 | 0.740 | 0.368 | 0.145 | 0.615 | 0.776 | 0.348 | 0.283 | 0.352 | 0.936 | 0.933 | 0.172 | 0.583 | 0.885 | **0.010** | 0.994 | 0.828 |
| Season 2014-2015 | | | | | | | | | | | | | | | | | | | | |
| Treatment | Amino acid (µmol/L) | | | | | | | | | | | | | | | | | | | |
| | ASP | GLU | CYS | ASN | SER | GLN | HIS | GLY | THR | ARG | ALA | TYR | VAL | MET | TRP | PHE | ILE | LYS | LEU | PRO |
| CON | 93 | 216 | n.r | 0 | 556 | 872 | 288 | 37 | 1,250 | 2,900 | 1,928 | 9 | 419 | 112 | 162 | 116 | 247 | 0 | 305 | 3,376 |
| RI | 124 | 220 | n.r | 4 | 679 | 929 | 298 | 42 | 1,554 | 3,302 | 1,764 | 12 | 532 | 148 | 183 | 126 | 356 | 29 | 460 | 3,654 |
| LSD 5% | 37 | 75 | n.r | 11 | 277 | 873 | 97 | 19 | 751 | 1,667 | 654 | 24 | 516 | 137 | 111 | 135 | 478 | 72 | 568 | 1,085 |
| p value | 0.086 | 0.907 | n.r | 0.356 | 0.318 | 0.877 | 0.813 | 0.504 | 0.360 | 0.577 | 0.564 | 0.757 | 0.612 | 0.547 | 0.648 | 0.866 | 0.594 | 0.356 | 0.527 | 0.554 |

n.r: not reported

At GB10, differences between CON and RI were found for ASN, HIS, PHE and LYS in the first season, and SER, HIS, GLY, VAL, MET, TRY, PHE, ILE, LEU, and PRO in 2014-2015 (Table 57)

**Table 57 Grape juice amino acid concentration (µmol/L) at Greystone block 10 during the 2013-2014 and 2014-2015 seasons. Each value is the average of four replicates.**

| Season 2013-2014 | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Treatment | Amino acid (µmol/L) | | | | | | | | | | | | | | | | | | | |
| | ASP | GLU | CYS | ASN | SER | GLN | HIS | GLY | THR | ARG | ALA | TYR | VAL | MET | TRP | PHE | ILE | LYS | LEU | PRO |
| CON | 326 | 394 | 17 | 64 | 840 | 1,335 | 149 | 52 | 1,570 | 5,417 | 2,535 | 11 | 549 | 132 | 72 | 256 | 448 | 44 | 544 | 2,466 |
| RI | 270 | 320 | 0 | 40 | 733 | 844 | 108 | 41 | 1,271 | 4,212 | 2,109 | 11 | 413 | 86 | 56 | 202 | 323 | 30 | 384 | 2,177 |
| LSD 5% | 85 | 109 | 24 | 21 | 176 | 594 | 33 | 12 | 338 | 1,628 | 671 | 4 | 141 | 53 | 31 | 53 | 173 | 13 | 198 | 793 |
| p value | 0.158 | 0.149 | 0.138 | **0.032** | 0.188 | 0.090 | **0.025** | 0.069 | 0.073 | 0.120 | 0.172 | 0.915 | 0.056 | 0.080 | 0.241 | **0.046** | 0.128 | **0.042** | 0.095 | 0.407 |
| Season 2014-2015 | | | | | | | | | | | | | | | | | | | | |
| Treatment | Amino acid (µmol/L) | | | | | | | | | | | | | | | | | | | |
| | ASP | GLU | CYS | ASN | SER | GLN | HIS | GLY | THR | ARG | ALA | TYR | VAL | MET | TRP | PHE | ILE | LYS | LEU | PRO |
| CON | 221 | 417 | n.r | 33 | 829 | 1,695 | 305 | 47 | 1,809 | 5,311 | 2,734 | 21 | 594 | 119 | 127 | 294 | 418 | 60 | 518 | 3,173 |
| RI | 107 | 381 | n.r | 0 | 527 | 432 | 229 | 24 | 1,125 | 2,655 | 1,386 | 0 | 309 | 50 | 58 | 107 | 188 | 0 | 239 | 2,267 |
| LSD 5% | 149 | 291 | n.r | 68 | 292 | 1.940 | 70 | 22 | 705 | 3,147 | 1,404 | 24 | 183 | 58 | 62 | 175 | 151 | 86 | 201 | 800 |
| p value | 0.110 | 0.775 | n.r | 0.274 | **0.045** | 0.162 | **0.036** | **0.043** | 0.055 | 0.085 | 0.057 | 0.068 | **0.009** | **0.027** | **0.034** | **0.040** | **0.010** | 0.135 | **0.015** | **0.032** |

n.r: not reported

When the effect of the site and treatment was determined for each season, only GLU in the second season showed differences across sites, while the rest of the amino acids were not altered by either the site or treatment (Table 58).

**Table 58 Results of two-way ANOVA on the effects of "site" (WH, GB5 and GB10), "treatment" (CON and RI) during the 2013-2014 and 2014-2015 seasons on grape juice amino acid concentration. Numbers correspond to p values.**

| Season 2013-2014 | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Factor | Amino acid | | | | | | | | | | | | | | | | | | | |
| | ASP | GLU | CYS | ASN | SER | GLN | HIS | GLY | THR | ARG | ALA | TYR | VAL | MET | TRP | PHE | ILE | LYS | LEU | PRO |
| Site | 0.136 | 0.229 | 0.673 | 0.167 | 0.283 | 0.311 | 0.451 | 0.257 | 0.422 | 0.722 | 0.405 | 0.061 | 0.185 | 0.442 | 0.671 | 0.083 | 0.167 | 0.249 | 0.197 | 0.062 |
| Treatment | 0.949 | 0.850 | 0.933 | 0.976 | 0.915 | 0.491 | 0.388 | 0.815 | 0.975 | 0.883 | 0.750 | 0.198 | 0.875 | 0.864 | 0.572 | 0.531 | 0.813 | 0.399 | 0.788 | 0.656 |
| Season 2014-2015 | | | | | | | | | | | | | | | | | | | | |
| Factor | Amino acid | | | | | | | | | | | | | | | | | | | |
| | ASP | GLU | CYS | ASN | SER | GLN | HIS | GLY | THR | ARG | ALA | TYR | VAL | MET | TRP | PHE | ILE | LYS | LEU | PRO |
| Site | 0.605 | **0.029** | n.r | 0.623 | 0.747 | 0.931 | 0.714 | 0.440 | 0.764 | 0.538 | 0.447 | 0.562 | 0.648 | 0.412 | 0.237 | 0.488 | 0.552 | 0.862 | 0.547 | 0.424 |
| Treatment | 0.677 | 0.932 | n.r | 0.587 | 0.789 | 0.451 | 0.549 | 0.681 | 0.809 | 0.713 | 0.460 | 0.366 | 0.686 | 0.804 | 0.587 | 0.462 | 0.682 | 0.696 | 0.732 | 0.798 |

n.r: not reported

From the combined analysis, including data from all sites, treatments and seasons, the results indicated that amino acid concentration did not vary across sites, with the treatment also not having an effect. Moreover, only ASN and TYR showed differences between seasons (Table 59).

**Table 59 Results of two-way ANOVA on the combined effects of "site" (WH, GB5 and GB10), "treatment" (CON and RI), and "season" (2013-2014 and 2014-2015) on grape juice protein amino acids content. Numbers correspond to p values.**

| Combined analysis | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Factor | Amino acid | | | | | | | | | | | | | | | | | | | |
| | ASP | GLU | CYS | ASN | SER | GLN | HIS | GLY | THR | ARG | ALA | TYR | VAL | MET | TRP | PHE | ILE | LYS | LEU | PRO |
| Site | 0.288 | 0.166 | 0.673 | 0.439 | 0.647 | 0.776 | 0.491 | 0.999 | 0.868 | 0.654 | 0.664 | 0.397 | 0.909 | 0.762 | 0.205 | 0.443 | 0.897 | 0.601 | 0.892 | 0.199 |
| Treatment | 0.818 | 0.876 | 0.933 | 0.761 | 0.889 | 0.447 | 0.391 | 0.878 | 0.860 | 0.769 | 0.532 | 0.995 | 0.740 | 0.820 | 0.453 | 0.462 | 0.715 | 0.963 | 0.734 | 0.992 |
| Season | 0.448 | 0.795 | 0.933 | **0.015** | 0.682 | 0.509 | 0.893 | 0.215 | 0.767 | 0.715 | 0.427 | **0.028** | 0.621 | 0.828 | 0.848 | 0.492 | 0.665 | 0.474 | 0.796 | 0.393 |

Taurine concentration in berry juice at harvest showed differences at WH in 2014-2015 only (Table 60).

**Table 60 Taurine concentration in berry juice (µmol/L) during seasons 2013-2014 and 2014-2015. Each value is the average of four replicates.**

| Season 2013-2014 | | | |
|---|---|---|---|
| Treatment | Site | | |
| | WH | GB5 | GB10 |
| CON | 1,013 | 1,310 | 1,338 |
| RI | 1,233 | 1,114 | 1,330 |
| LSD 5% | 237 | 383 | 366 |
| p value | 0.064 | 0.258 | 0.958 |
| Season 2014-2015 | | | |
| Treatment | Site | | |
| | WH | GB5 | GB10 |
| CON | 1,448 | 847 | 1,519 |
| RI | 1,683 | 950 | 1,178 |
| LSD 5% | 165 | 110 | 407 |
| p value | **0.013** | 0.062 | 0.086 |

No differences in berry juice taurine content across sites, treatments and seasons were observed in the overall analysis (Table 61).

**Table 61 Results of two-way ANOVA on the effects of "site" (WH, GB5 and GB10), "treatment" (CON and RI), and "season" (2013-2014 and 2014-2015) on grape juice taurine content. Numbers correspond to p values.**

| Factor | Season | |
|---|---|---|
| | 2013-2014 | 2014-2015 |
| Site | 0.491 | 0.164 |
| Treatment | 0.970 | 0.995 |
| | Combined analysis | |
| Site | 0.276 | |
| Treatment | 0.988 | |
| Season | 0.976 | |

### 4.3.8 PCA on grapevine physiology and berry parameters for both seasons

The PCA indicated that the first two components explained 80.55% of the variability (Figure 35). The first component explained 61.9%, while the second factor 18.65% of the variability in the dataset. There were clear differences between treatments in WH and GB5, which is evidenced by the separation between points for WHC and WHRI, and GB5C and GB5RI. On the other hand, GB10 did not show differences between CON and RI, with both points being located close each other, although it was clearly separated from the other two sites.

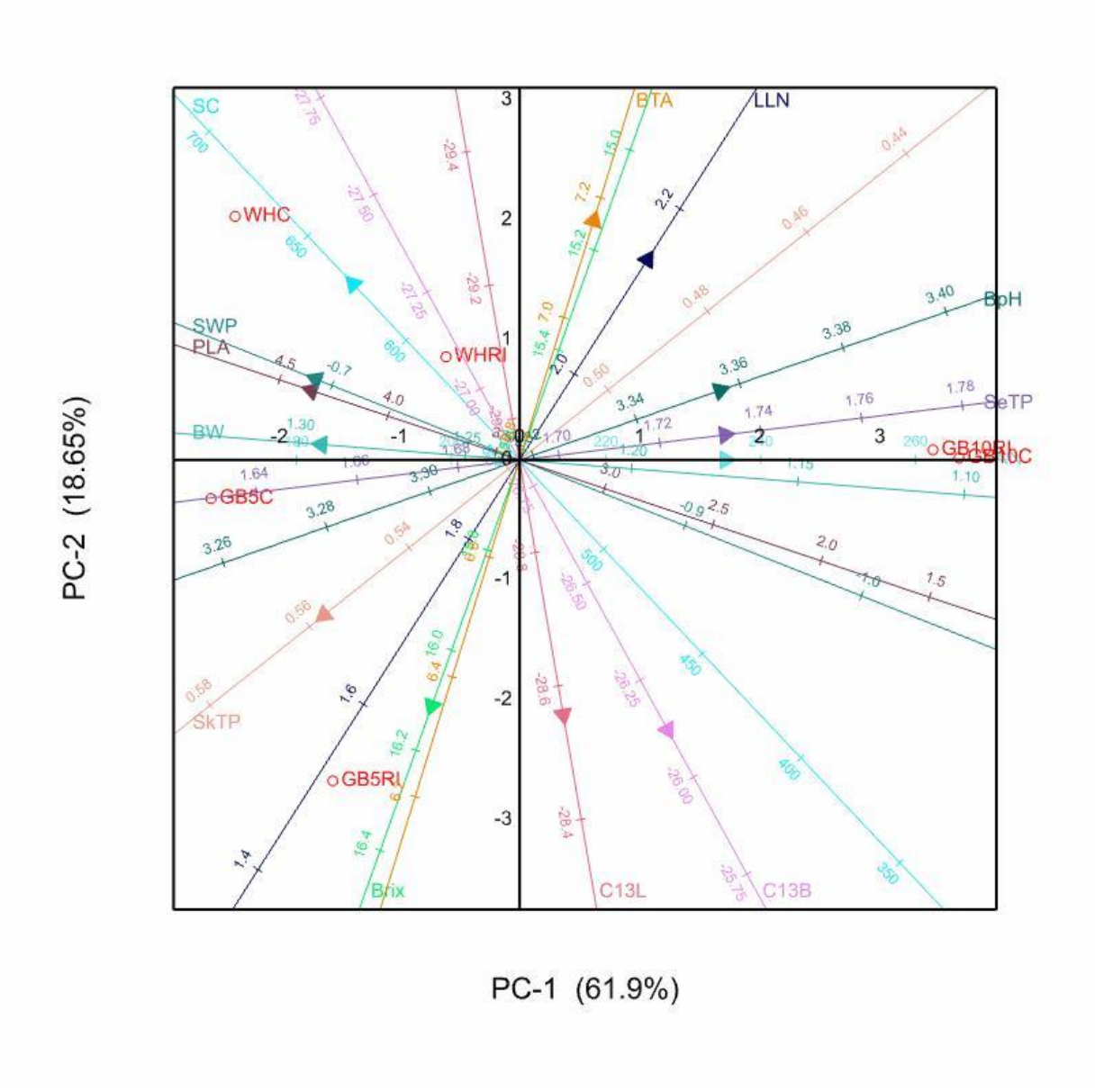


**Figure 35** **Principal component analysis (PCA) of grapevine physiology and berry parameter means in the 2013-2014 season. Each point of the PCA represents a combination of site/treatment: WHC, Waipara Hills Control; WHRI, Waipara Hills Reduced Irrigation; GB5C, Greystone Block 5 Control; GB5RI, Greystone Block 5 Reduced irrigation; GB10C, Greystone Block 10 Control; GB10RI, Greystone Block 10 Reduced Irrigation. Nomenclature used for parameters: PLA, primary leaf area; SWP, stem water potential; SC, stomatal conductance; LLN, leaf layer number; C13L, leaf $\delta^{13}C$; BW, berry weight; Brix, grape juice Brix; BpH, berry juice pH; BTA, berry juice TA; SeTP, seed total**

**phenolics; SkTP, skin total phenolics; YAN, yeast available nitrogen; C13B, grape juice $\delta^{13}C$.**

WHC and WHRI were grouped on the upper left plan of the plot, GB5C and GB5RI in the lower left plan, while both CON and RI at GB10 were grouped in the right of the plot, which highlighted the differences between sites found in this study.

The first component had a positive association with YAN and seed total phenolics (SeTP), while this was negatively loaded with berry weight (BW). The second component had a positive association with berry TA (BTA), and was negatively loaded with leaf $\delta^{13}C$ (C13L). Berry TA and leaf layer number were negatively correlated with Brix.

In the second season, the PCA diagram indicated that the first component represents 54.97% of the total variation, while the second component 21.49%. Therefore, both components accounted 76.47% of the total variability in the data (Figure 36). There were clear differences between treatments at all sites, especially for GB5 and GB10 where CON and RI were located in different planes of the plot.

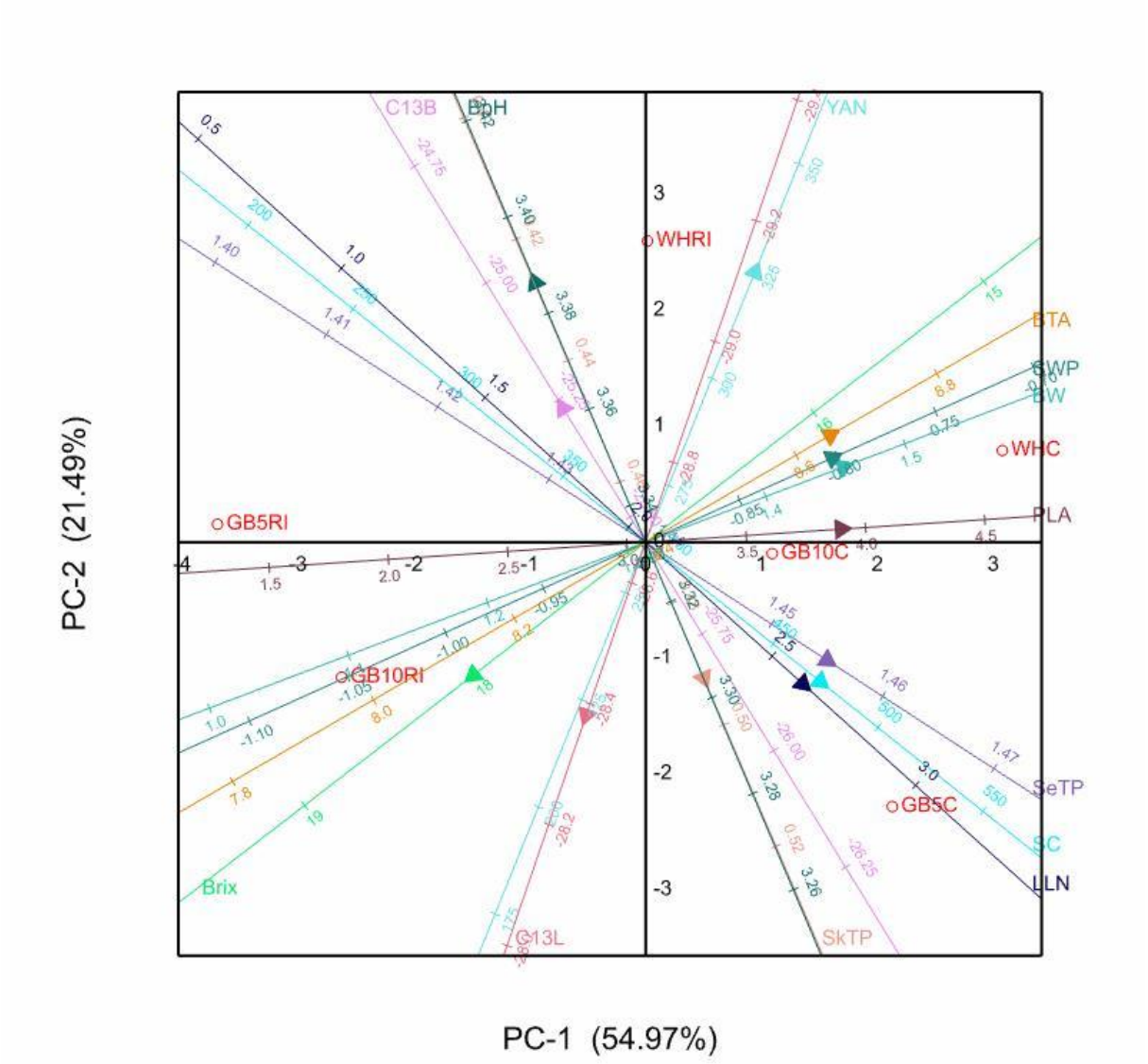


**Figure 36** **Principal component analysis (PCA) of grapevine physiology and berry parameter means in the 2014-2015 season. Each point of the PCA represents a combination of site/treatment: WHC, Waipara Hills Control; WHRI, Waipara Hills Reduced Irrigation; GB5C, Greystone Block 5 Control; GB5RI, Greystone Block 5 Reduced irrigation; GB10C, Greystone Block 10 Control; GB10RI, Greystone Block 10 Reduced Irrigation. Nomenclature used for parameters: PLA, primary leaf area; SWP, stem water potential; SC, stomatal conductance; LLN, leaf layer number; C13L, leaf $\delta^{13}C$; BW, berry weight; Brix, grape juice Brix; BpH, berry juice pH; BTA, berry juice TA; SeTP, seed total phenolics; SkTP, skin total phenolics; YAN, yeast available nitrogen; C13B, grape juice $\delta^{13}C$.**

In general, the RI treatments tended to be grouped in the left side of the plot, while the control treatments in the right.

The first component was positively correlated with primary leaf area (PLA), berry weight (BW), and stem water potential (SWP). On the other hand, this was negatively correlated with Brix. PC-2 has a positive correlation with berry pH (BpH) and YAN, and a negative correlation with leaf $\delta^{13}C$ (C13L). Berry weight and stem water potential have a negative correlation with Brix, which may suggest that vines with lower stem water potential tended to have smaller berries, with higher Brix, especially at WH. This is similar to the results shown in the previous analyses.

## 4.4 Discussion

Unfortunately, the frost registered early in the second season caused a shortage of grapes for the two companies collaborating with this research, which meant data for yield, number of clusters, and cluster weight could not be collected in 2014-2015. Therefore, only data for berry weight are presented from both seasons.

### Berry weight

Berry weight was reduced by RI at all sites, except at WH in 2013-2014 and GB10 in 2014-2015. These differences developed well after veraison in the first season, whereas they were evident from veraison onwards in 2014-2015. These findings are in concordance with a number of research groups (Deloire *et al.* 2004, Ginestar *et al.* 1998, Romero *et al.* 2013), where berries from vines under water stress showed lower weight during ripening. Water deficit generally limits berry size since this inhibits cell division and cell expansion, especially when applied during the first phase of rapid berry enlargement (Conde *et al.* 2007, Roby and Matthews 2004, Romero and Martinez-Cutillas 2012). However, changes to berry size after veraison due to water deficit could also be the result of other mechanisms. From veraison onwards, the berry is connected to the vine primarily via phloem (Findlay *et al.* 1987), therefore, a reduction in berry size could also be a consequence of a decrease in photosynthesis (Chaves *et al.* 2010). This is described by Van Leeuwen *et al.* (2009), who proposed that stomatal closure under water stress restricts photosynthesis, which may impact dry matter production. In this study, as irrigation started at fruit-set or before, the lower berry weight obtained in RI vines can be attributed to both limited cell division and cell expansion during the first phase of berry growth and a theoretical lower dry matter production due to partial stomatal closure described in the previous chapter (Table 21).

The large amount of precipitations registered during the ripening period in 2013-2014 highlighted the sensitivity of berries under RI to re-watering. This phenomenon was observed in the second

season as well, but as the amount of rain was lower and it was less pronounced than in the previous season. Berries pre-veraison are more responsive to fluctuations in vine water status, whereas post-veraison berries are much less subject to such variations (Creasy and Lombard 1993). Creasy *et al.* (1993) reported that berry water uptake declines at veraison due to rupturing of xylem vessels in the peripheral vascular tissues of the berry. However, it has been proposed that xylem flow after veraison is reduced, but not totally eliminated (Greenspan *et al.* 1994). In addition, Keller *et al.* (2015) have suggested that xylem vessels remaining after veraison recirculate surplus phloem-derived water out of the berry. Under prolonged water stress, berry shrinkage occur when xylem efflux plus berry transpiration exceed phloem influx (Bondada and Shutthanandan 2012). This may partially explain the loss of weight in berries under RI since those vines had more exposed fruit, which may lead to higher transpiration rates. Yet, the reason why berries in water-stressed vines were more responsive to rainfall before harvest remains unclear. Although the berry cuticle has shown a high permeability for water uptake, a recent experiment has suggested that this occurs mainly through the berry pedicel and receptacle (Becker and Knoche 2011). It is speculated that water received from rainfall directly on berry surface was absorbed immediately through those mechanisms, while the higher absorption rate found in shrivelled berries might be explained by their higher osmotic potential as a consequence of higher solute concentration due to dehydration, although the latter was not evaluated.

### Seed weight

Seeds in berries from vines under RI appeared to show a slightly lower fresh weight than those in CON in either season, but this was not significant. Seed fresh weight tended to decrease toward to harvest, reaching its minimum at harvest, with the same trend being found for seed water content. This suggested that the reduction in seed fresh weight was mainly due to the decrease in seed moisture, since seed dry weight remained nearly constant during the ripening period. These results are consistent with those of other studies where similar trends have been described, though there is little research published in this area. Thus, Kennedy *et al.* (2000b) reported a decrease in seed fresh weight toward harvest, as well as small changes in seed dry weight during the two months pre-harvest in cv. Shiraz. The difference between fresh and dry weight in such study is equivalent to the seed water content reported here, which followed the same tendency as in this study. Ristic and Iland (2005) described similar results as those of Kennedy *et al.* (2000b), but they evaluated these parameters from the beginning of seed development. The authors indicated that the maximum seed fresh weight is reached at veraison and this starts to decrease through the ripening period as seed water loss increases. However, Pastor del Rio and Kennedy (2006) described that maximum fresh weight is reached by one to two weeks before veraison.

**Brix, pH and TA through the ripening period**

Differences in Brix and pH were found only at WH, while TA was not altered at any site in either season. The differences in Brix and pH observed at WH are consistent with the lower soil profile available water described for this site, which although receiving higher volumes of water through irrigation in both seasons, it was the only site showing the physiological effect of reducing irrigation by 50% under the conditions of this study. Pellegrino *et al.* (2014) reported that water deficit reduced berry size in Cabernet Sauvignon, but this had a little impact on Brix, as found at GB5 and GB10. However, the results of Brix evolution in this study differs from others reported elsewhere (Etchebarne *et al.* 2010, Ginestar *et al.* 1998) in which non-stressed vines showed higher Brix than those under water restricted conditions. Ginestar *et al.* (1998) attributed the lower Brix found under water stress to a reduction in photosynthesis rate. This is supported by other research groups who indicated that severe stress during ripening can curtail berry sugar accumulation due to a decrease in photosynthesis and sugar export from the leaves (Peyrot des Gachons *et al.* 2005, Rogiers *et al.* 2004, Santesteban and Royo 2006). Nonetheless, the author also pointed out that if the water deficit is mild enough to restrict shoot and root growth more than photosynthesis, there is more sugar available for other sinks (e.g. grapes), which may lead to a higher sugar accumulation in those organs. This might explain in part the slightly higher sugar accumulation in berries under RI reported here, despite those vines showed lower stomatal conductance as described in the previous chapter (Table 21). Moreover, berries in plants under water stress are usually smaller and also shrink due to dehydration (Greenspan *et al.* 1994). Such loss of water concentrates berry solutes, which induces an apparent gain of Brix. However, as discussed in the previous chapter, there was also an early senescence of leaves in the cluster zone in plants under RI, and their carbon, nitrogen, polysaccharides and other nutrients were likely remobilized and used to sustain the metabolism of other organs (e.g. young leaves, clusters, or roots), so it is theorised that part of those could have also contributed to berry sugar accumulation.

Berry pH was increased by RI at WH in 2014-2015 only. RI did not alter this at GB5 and GB10 in either season. The results at WH agree with those in Ginestar *et al.* (1998), who described a similar trend over the ripening season, with berries under different restricted irrigation treatments showing higher pH than the well-watered controls. Etchebarne *et al.* (2010) in contrast, found that soil water availability did not alter berry pH, similar to the results at GB5 and GB10. Boulton (1980) suggested that nutrient remobilisation from other organs into the berries may cause an undesirable increase in fruit $K^+$ that may lead an increase in juice and wine pH. Ginestar *et al.* (1998) proposed that the higher pH under water restricted conditions found in their study may be a consequence of a greater cluster exposure, which may have increased berry temperature, inducing a higher respiration of malic acid. In this study, both $K^+$ remobilisation from senescent leaves and higher berry respiration

rate due to higher bunch exposure may explain the differences in berry pH found under RI. Regarding TA, this was not affected by RI. Similar results are described in the review of Chaves *et al.* (2010), where most of the literature cited by the authors reported no changes in TA from water-stressed vines. The same tendency was found by Ginestar *et al.* (1998), with no changes in TA being reported through the ripening season between different irrigation treatments.

**Tannin and total phenolic concentration in seeds and skins**

No differences in seed tannin concentration between CON and RI were observed in either season or any site. However, though no differences were detected within each site, differences between sites were found in the 2013-2014 season. Moreover, different trends have been observed between seasons, where seed tannin concentration at all sites in 2013-2014 tended to decrease toward harvest, whereas in the next season, values at all sites decreased after veraison, but tended to increase before harvest. Harbertson *et al.* (2002), using bovine serum albumin (BSA) precipitation, reported a similar trend as here in 2013-2014, with seed tannin concentration peaking at veraison and declining toward harvest. In the same research, the authors investigated the seasonal seed tannin accumulation in Cabernet Sauvignon over two seasons finding that this followed a different pattern in two consecutive years, as in this study. As described before, seeds in both seasons reached their maximum fresh weight at veraison, which did coincide with their maximum tannin concentration, as reported elsewhere (Adams 2006, Kennedy *et al.* 2000b). Moreover, the decline in seed extractable tannin during ripening has been associated to seed colour changes through ripening (Adams 2006, Ristic and Iland 2005). It is known that biosynthesis of flavan-3-ol monomers (catechin, epicatechin, and epicatechin gallate) in seeds decrease after veraison (Kennedy *et al.* 2000b), which coincides with a seed colour change from green to brown as a consequence of tannin oxidation and seed coat dehydration (Adams 2006, Ristic and Iland 2005). However, the effect of water stress on the dynamic of tannin accumulation over ripening has been little studied. In Cabernet Sauvignon, Kennedy *et al.* (2000a) found that a reduction in irrigation affected the pattern of flavan-3-ol monomers through ripening, where seeds from vines under restricted irrigation showed lower tannin concentration than those either well or double-irrigated.

Despite seed total phenolics following a similar trend as seed tannins, the overall analysis highlighted the differences between sites in the first season and when the two seasons were combined. Tannin is one of the components of the total phenolics measurement, which includes free anthocyanins, flavonols, phenolic acids, and other UV-absorbing materials (Mercurio *et al.* 2010). This may explain in part the similar patterns found between seed tannin concentration and total phenolics found here. Pastor del Rio and Kennedy (2006) found that seed flavan-3-ol monomers in Pinot noir reach their maximum concentration at veraison, then declining toward harvest. Tannin is the most abundant class of poly-phenolic compounds in seeds and skins (Adams 2006), and therefore, the similar trends

found between seed tannin and seed total phenolics may be better attributed to changes in seed tannin concentration than any other phenolic compounds.

Skin tannin concentration tended to decrease toward harvest in either season, but this was less pronounced in 2013-2014, where this remained fairly constant between veraison and harvest. On the other hand, this showed higher values near veraison in the second season, which then decreased during ripening, then rose a small amount before harvest at GB5 and GB10. Differences in skin tannin accumulation between CON and RI were found at WH in 2013-2014 and GB5 in 2014-2015. The combined analysis indicated differences between sites for the dynamic of skin tannin concentration. Despite the results reported by Harbertson *et al.* (2002) not being from an irrigation study, the authors observed a similar trend than in 2013-2014 for skin tannin concentration in Pinot noir, where this remained nearly constant between veraison and harvest. This also agree with the findings of Sternad Lemut *et al.* (2013a), who showed that flavan-3-ols concentration reaches its maximum after fruit-set, but starts to decrease before veraison, remaining more or less constant during ripening. Cortell *et al.* (2008) found a higher skin tannin concentration in grapes from low vigour vines, compared to those with medium and high vigour, but as the differences in vigour in their study were not a consequence of water restriction, it is difficult to compare their results with those reported here.

In Pinot noir, however, and depending on the skin phenolic compound in question, some reach their maximum concentration before veraison, tending to decline toward harvest, whereas other compounds peak between veraison and harvest (Sternad Lemut *et al.* 2013a). It is known that the dynamic of phenolic compounds accumulation can be altered by different environmental factors such as temperature, water status, nutrient status, among others, making it difficult to identify only one factor as responsible of the different accumulation patterns and the variations across sites. Thus, a correlation between skin phenolic compounds at harvest and bunch exposure has been described elsewhere. Rustioni *et al.* (2011) reported that berry skins in well-exposed bunches showed a higher anthocyanin concentration than those shaded. The authors also highlighted the differences in anthocyanin accumulation pattern between the three sites of their experiment, which are similar to those reported here. However, a higher cluster exposure may have as a consequence a rise of the berry temperature up to levels in which some metabolic processes are inhibited. For example, anthocyanin production increases up to an optimum berry temperature of 30°C, whereas this is inhibited above 35°C (Kliewer 1977, Spayd *et al.* 2002). Tannin concentration has been also described as increasing with a rise in temperature, while flavonol synthesis seems to be less sensitive to temperature, but more responsive to UV light instead (Ferrandino *et al.* 2012, Pastor del Rio and Kennedy 2006). A more specific analysis (e.g. HPLC) may contribute to clarify the effect of the factors previously described on tannin and total phenolic accumulation in this study.

### Carbon isotope ratio ($\delta^{13}C$) in grape juice at harvest

There were no differences in grape juice $\delta^{13}C$ between CON and RI in either season or any site. No differences were also found between sites, treatments or seasons. As described in Gaudillère *et al.* (2002), sucrose is translocated from leaves to fruit and converted to glucose and fructose during berry ripening, so $\delta^{13}C$ in berry sugars should integrate the photosynthetic activity during that period. As berry $\delta^{13}C$ is usually measured at harvest, this has been proposed as a good technique to evaluate the accumulated effect of water stress during the season (Van Leeuwen *et al.* 2010). Under the conditions of this study, $\delta^{13}C$ in grape juice showed higher values than in primary leaves, which agree with the results of De Souza *et al.* (2003) and De Souza *et al.* (2005b). De Souza *et al.* (2003) suggested that the higher values of $\delta^{13}C$ in grapes may be due to that carbon in berries is derived from photosynthesis occurring after veraison, when the effect of water stress is more pronounced, so any change in photosynthesis rate should be reflected on berry $\delta^{13}C$. In contrast, as discussed in the previous chapter and elsewhere, leaves are formed early in the season before any water deficit is experienced, then a lower leaf $\delta^{13}C$ ratio is expected under field conditions (Santesteban *et al.* 2015). Hence, the lack of differences between CON and RI in grape juice $\delta^{13}C$ may suggest that although vines under RI showed lower leaf area and lower stomatal conductance, especially in the second season, the RI treatment did not result in a decline in photosynthesis rate. These results support those obtained for grapevine physiology, and therefore, it is speculated that vines under RI did compensate for the lower water availability by either increasing the photosynthesis rate of the remaining leaves or mobilising carbohydrate reserves from other vine organs to accumulate sugars in the berries under adverse conditions.

$\delta^{13}C$ has been described as a good integrative indicator of water stress under field conditions (De Souza *et al.* 2005b, Gomez-Alonso and Garcia-Romero 2010, Herrero-Langreo *et al.* 2013), but it is known that there are differences in $\delta^{13}C$ measured on grape sugar between varieties grown under similar conditions. For instance, Gaudillère *et al.* (2002) reported a large variation in grape sugar $\delta^{13}C$ between varieties cultivated in France grafted on the same rootstock and cultivated under the same conditions. In their study, grape $\delta^{13}C$ values ranging from -21.6‰ (cv. Riesling) to -24.9‰ (cv. Muscat de Hambourg) were reported, where Pinot noir showed a grape sugar $\delta^{13}C$ of -23.6‰, higher than any value reported here. Recently, Santesteban *et al.* (2015), integrating data on berry $\delta^{13}C$ and pre-dawn and midday stem water potential from different varieties and conditions, proposed a classification for the level of water deficit experimented by the vines between veraison and harvest based on their berry sugar $\delta^{13}C$. Using this classification, all values in the first season of this study corresponded to “weak or nil” water deficit. In 2014-2015, both CON and RI at WH and CON at GB5 showed values that can be classified as “weak to moderate” water deficit, while RI at GB5 was the only over the two seasons and sites showing a value that can be classified as “moderate to severe”

water deficit. In contrast, GB10 reported similar results in either season, where all of them corresponded to "weak or nil" water deficit. Knowing that Pinot noir tends to have lower grape $\delta^{13}C$ than other varieties (Gaudillère *et al.* 2002), this classification can be taken only as a reference since it is not specifically for Pinot noir.

### Correlation between grape juice $\delta^{13}C$ and minimum stem water potential ($\psi_s$)

Grape juice $\delta^{13}C$ showed to be well correlated with the minimum stem water potential of the season, although no differences were found either for $\psi_s$ or grape juice $\delta^{13}C$ when individually analysed. These high correlations agree with those reported elsewhere for other varieties (De Souza *et al.* 2003, Santesteban *et al.* 2012, Van Leeuwen *et al.* 2009). De Souza *et al.* (2003) reported a significant correlation between pre-dawn leaf water potential, another important plant water status indicator, and $\delta^{13}C$ measured in grape berries from different irrigation treatments in cv. Moscatel ($R^2 = 0.68$). Santesteban *et al.* (2012) found a high correlation between berry $\delta^{13}C$ and minimum $\psi_s$ in cv. Tempranillo, similar to those reported here. Despite their evaluations having been carried out in two sites, one non-irrigated and other under different irrigation treatments, the $R^2$ obtained were 0.68 and 0.70, highlighting the close relationship between these parameters, regardless of soil characteristics or irrigation treatments. In Saint-Emilion, Van Leeuwen *et al.* (2009) also studied the relationship between grape sugar $\delta^{13}C$ and minimum $\psi_s$ in cv. Merlot, finding a similar correlation ($R^2 = 0.69$) than those described here and other similar studies.

Although there were no treatment differences within these factors, the fact that are significantly correlated and that the relationship is similar to that reported in the literature suggests the measurements were accurately representing the physiological state of the vines.

### YAN and amino acid content

Although differences in YAN were observed only at WH in 2013-2014 and GB10 in 2014-2015, in general, vines in RI at WH and GB5 showed higher values in either season, while the opposite was found at GB10. As AN was unaffected by RI, the differences in YAN were due to the differences in amino acid content, confirmed by HPLC.

Most of the nitrogenous compounds in berry juice (50-90%) correspond to free amino acids. The remainder is composed of ammonium ions, peptides, proteins, nitrates and trace amounts of vitamins, nucleotides and amines (Bell and Henschke 2005, Van Heeswijck *et al.* 2001). YAN has two main components: primary amino acids and ammonium. Despite ammonium concentration in grape juice being proportionally lower than amino acids, it is still an important component of YAN because it is the preferred nitrogen source for yeast due to its fast assimilation (Bell and Henschke 2005). Berry juice contains about thirty amino acids (Conde *et al.* 2007), but only some of them are assimilated by yeasts during fermentation. PRO and ARG have been described as the most

predominant amino acids in grape juice, but PRO is not considered as part of the assimilable fraction due to it not being metabolised by yeasts (Bell and Henschke 2005, Van Heeswijck *et al.* 2001).

Under the conditions of this study, ammonia nitrogen was not affected by RI in either season or any site. Differences across sites and seasons were also not found. The effect of water stress on ammonia nitrogen has varied results in the literature. For example, De Royer Dupré *et al.* (2014) showed that water stress induced a higher ammonium accumulation in berries of Grenache noir at one site, whereas the same treatment at a different site reported the opposite results. Schreiner *et al.* (2013) concluded that limited N supply can alter ammonia nitrogen content in grape juice and De Royer Dupré *et al.* (2014) showed that water stress can limit soil water uptake and therefore nitrogen uptake, leading to low berry juice ammonium concentration. Based on this evidence, that no differences were found between treatments, sites or seasons might suggest that RI did not affect either water or N uptake from the soil. May be also other factors affecting berry ammonia nitrogen content that have not been studied. For example, as data reporting the effect of water stress on this was not found specifically for Pinot noir, the variety-related factors cannot be discarded as influencing berry ammonia accumulation. Also, as the differences in soil nitrogen content were not evaluated, this may have an influence in the lack of differences in berry ammonia nitrogen at any site or any season.

Primary amino acids analysed by enzymatic kit reported higher values for concentrations in berries under RI at WH and GB5 in both seasons, although this was significant only at WH in 2013-2014. In contrast, CON at GB10 showed higher amino acid concentration than RI in both seasons, being this significant in the second season. Most of the higher values of primary amino acids reported by enzymatic kit coincide with higher concentrations of ARG reported from the HPLC analysis, with GB5 in the 2013-2014 season the only exception. A great proportion (60-80%) of the total amino acid concentration in grape juice is make up by ARG and PRO (Bell and Henschke 2005). However, their concentration in berries has shown high sensitivity to several factors such as water stress (De Royer Dupré *et al.* 2014), N supply (Schreiner *et al.* 2014), presence or absence of cover crops (Gouthu *et al.* 2012), UV radiation (Gregan *et al.* 2012), as well as a natural variability between varieties (Stines *et al.* 2000). Some studies have demonstrated that ARG is the major amino acid in berries of Pinot noir, followed by PRO (Lee and Schreiner 2010, Schreiner *et al.* 2014). Both CON and RI in either season at WH agree with this, as well as GB5 in 2013-2014 and GB10 in 2014-2015. However, in 2014-2015 both CON and RI at GB5 reported higher PRO accumulation with respect to ARG, results that agree with those of Stines *et al.* (2000). In most of the cases, the third highest concentration was reported for ALA, and then GLU.

Water stress did alter the accumulation of some amino acids in berries of Pinot noir. For example, PRO concentration was increased by RI at WH in 2013-2014, whereas the opposite trend was found at GB10 in 2014-2015. PRO was not affected at GB5 in either season. The function of free PRO in grape berries remains speculative and Van Heeswijck *et al.* (2001) have suggested that it might act as an osmolyte that protect berry cells from the changes in osmotic pressure caused by accumulating hexose sugars during ripening. Most of the PRO is accumulated after veraison, reaching a peak prior to harvest and then tending to decline until harvest (Bell and Henschke 2005, Berdeja *et al.* 2014). GLN is transported from leaves to the berry via phloem, where it is converted into GLU by aminotransferases. PRO is synthesised from GLU via two interconnected pathways known as the glutamate and ornithine pathways (Van Heeswijck *et al.* 2001). Its production from GLU may involve hydroxide ($OH^-$) release, which might contribute to the rise in juice pH in berries with high PRO concentration (Smith and Raven 1979). Samples in this study reporting high PRO concentration also reported high values of GLU and GLN.

ARG concentration was affected by RI at WH in 2014-2015 only. Gouthu *et al.* (2012) have reported Pinot noir as an ARG accumulating variety, which means that its concentration in berries tends to increase right up until harvest, unlike Cabernet Sauvignon or Chardonnay in which ARG content generally stabilises and/or declines during ripening, ending up at lower values than PRO at harvest (Bell and Henschke 2005). Most of the findings in this research confirmed Pinot noir as an ARG accumulating variety. ARG plays a role as the major N storage compound in grape berries (Berdeja *et al.* 2014, Stines *et al.* 2000). This is synthesised from GLN, as is PRO (Stines *et al.* 2000, Van Heeswijck *et al.* 2001), and its variation under water stress may be associated with its potential use as a precursor of polyamides (Berdeja *et al.* 2014), its mobilization to storage organs (e.g. roots) (Bell and Henschke 2005), or its conversion to PRO since their metabolism may be linked, with ARG acting as a precursor for at least part of the PRO accumulated in grape berries (Van Heeswijck *et al.* 2001).

Taurine, a non-protein amino acid, has been rarely described in higher plants. Jacobsen and Smith (1968) reported that until their study was published, taurine had been identified in pollen of five dicotyledonous genera, whereas no taurine was found in plant of *Pinus* or *Malus* genera, as well as in potatoes. During the 80's and 90's, with improvement in extraction and analysis techniques, taurine started to be described in other species such as sea weed (Kataoka and Ohnishi 1986), beans (Pasantes-Morales and Flores 1991), clovers, pine, tomato, lingonberry or cowberry (*Vaccinium vitis-idaea*), and even *Saccharomyces cerevisiae* (Lähdesmäki 1986). More recently, taurine has been found in seeds and seedlings of the genus *Lens* (Rozan *et al.* 2001), cactus pear fruit (Fernández-López *et al.* 2010), flowers of *Cucurbita pepo* L. (Nepi 2014, Nepi *et al.* 2012), and microalgae (Tevatia *et al.* 2015). Interestingly, taurine has been also described in Shanxi aged vinegar, a traditional Chinese vinegar made from several kinds of cereal (Chen *et al.* 2013). The authors speculated that

the taurine detected may come from the raw material fermented to obtain the vinegar. As its presence in plants has been rarely reported, taurine metabolism in higher plants is understudied. In microalgae, Tevatia *et al.* (2015) concluded that taurine is metabolised through the "serine/sulphate pathway", but its biosynthesis in higher plants needs to be investigated. Its role in plant physiology has also not been studied, but as suggested by Tevatia *et al.* (2015), taurine likely functions as an osmolyte. Moreover, as described in Vranova *et al.* (2011) and Lee (2015), taurine has also a role in protecting cell membranes and as a useful anti-stress agent.

The presence of taurine in grapes has not been reported in the literature. All samples analysed in this study reported the presence of taurine in berry juice of Pinot noir, which likely constitute the first time that this non-protein amino acid is reported in *Vitis vinifera* L.

## 4.5 Conclusions

The responses of Pinot noir berries to water stress were varied, with a few parameters being affected, while the majority remained unaltered. In general, the main effect of RI on berry parameters was a reduction of berry weight. Brix and pH were altered by the RI treatment at WH only, with no differences being detected at GB5 and GB10. These results suggest that site-related factors, such as soil profile available water and irrigation management are relevant in conditioning the effect of water stress on basic berry parameters, in that changes in Brix and pH were observed only at WH, the site having the lower soil profile available water and higher volume of water applied by irrigation over the two seasons. In contrast, the two sites having higher soil profile available water (GB5 and GB10), but lower irrigation volume, did not show differences for these parameters either in a wetter (2013-2014) or dry (2014-2015) season.

On the other hand, TA was unaffected at all sited in either season. RI did not have an effect on seed fresh weight, seed dry weight, or seed water content, showing that seeds are less sensitive to a reduction in soil available water either in a wet or dry season. Tannin concentration and phenolic compounds were little affected by RI either in seeds or skins. $\delta^{13}C$ in grape juice and ammonia nitrogen were not altered by RI at any site or season. Among the 20 amino acids analysed, only few of them were either affected by RI or influenced by the site. In addition, taurine was found in grape juice, likely the first time in which this compound has been described in *Vitis vinifera* L.

When data of grapevine physiology and grapes were combined, the PCA analyses found clear differences between sites, even when no differences were observed between CON and RI within the same site. This indicated that the effect of a reduction of the irrigation by 50% under field conditions is influenced by the site characteristics, soil profile available water one of the most important. Finally, knowing the effect of a 50% reduction in the commercial irrigation will contribute to improve the

water use efficiency in Waipara, since RI did not cause important changes in Pinot noir berry composition. However, the changes in berry weight and juice pH have to be analysed more carefully due to their potential impact in winemaking.

# Chapter 5

# The effect of reduced irrigation on wine composition at three Pinot noir vineyards with contrasting soils

## 5.1 Introduction

In the previous chapters, water stress was shown to affect plant physiology and grape composition in various ways, which might be reflected in differences in wine composition among sites and treatments.

Several studies have investigated the differences between wines produced in the same area (Cliff and Dever 1996, Hakimi-Rezaei and Reynolds 2010, Rutan *et al.* 2014), while others have gone further in trying to explain the edapho-climatic differences across a region and their link to wine characteristics (Burns 2012, Costantini *et al.* 2012, Van Leeuwen *et al.* 2004). However, studies aiming to evaluate the effect of reducing irrigation on wine composition under different field conditions and its importance in determining effects associated with site characteristics such as water availability are less abundant in the literature.

Ledderhof *et al.* (2014) evaluated the effect of water status on sensory profile of Ontario Pinot noir and noted, among other conclusions, that the so-called "terroir effect" may be partially attributed to differences in vine water status across the four vineyards in their study. It has been suggested that differences in vine water status can influence canopy density and consequently fruit light exposure (Robinson *et al.* 2014 and literature therein), inducing variations in grape characteristics (Medrano *et al.* 2015, Oliveira *et al.* 2003, Tomás *et al.* 2014), and therefore, wine composition.

The objective of this study was to characterise the differences between wines made from grapes harvested from vines under reduced irrigation and those normally irrigated in three Pinot noir vineyards in Waipara over the 2013-2014 season.

## 5.2 Materials and methods

### 5.2.1 Experimental design

The experimental design, reduced irrigation treatments, and edapho-climatic conditions of each site and season have been described in Chapter 2. Please refer there for more details.

### 5.2.2 Winemaking

Grapes from each replicate (24 total) were manually crushed and de-stemmed in the Lincoln University winery. Five kg of grapes per replicate were placed into 10 L plastic buckets. Then, 50 mg/L $SO_2$ as potassium metabisulfite was added and $CO_2$ gas was poured in the headspace to protect the must from oxidation. Thereafter, lids were fixed to the buckets and these were placed in a cool room at 4 °C for 48 hours, and then left at room temperature for one day until inoculation. The must was inoculated with Enoferm Burgundy (BGY™; Lallemand Australia PTY LTD, South Australia) at 25 g/hL, and moved to a 28 °C room. No acids or yeast nutrients were added. Caps were punched down three times per day, and fermentations monitored daily for temperature and soluble solids by hydrometry.

Five days after inoculation, once fermentations reached dryness (determined by hydrometry), wines were left on skins for one more day. Then, wines were pressed by decanting the wine and squeezing the remained skins and seeds by hand until no further wine was recovered. About 2.5 L of wine was obtained from each replicate, which was transferred into 3 L water bottles (PET), capped and placed in a cool room at 4 °C for 2 days for settling. Wines were then racked and heavy lees removed. Racking was repeated one more time. Wines after racking were moved to a 21 °C room and inoculated with malolactic culture at 0.006 g/L (Vinoflora® Oenos; Chr. Hansen, Denmark). Malolactic fermentation progress was followed by paper chromatography as described in Iland *et al.* (2004) and checked at the end by enzymatic analysis kits (Vintessential Laboratories, Australia), according to the manufacturer's instructions.

Wines were racked a third time at bottling with the addition of $SO_2$ to bring each wine to 35 mg/L free $SO_2$. Unfiltered wines were bottled in 375 ml bottles under $N_2$, closed with screw cap, and stored at room temperature in the dark.

### 5.2.3 Wine pH and titratable acidity

Wine pH was measured under gentle stirring using a Suntex pH/mV/temperature meter SP-701 (Suntex, Taiwan) with a Eutech Instruments probe (EC 620133; Eutech Instruments Pte Ltd, Singapore) as described in Iland *et al.* (2004). Before analysis, the pH meter was calibrated using two standard buffer solutions of pH 4.0 and 7.0. Wine titratable acidity (TA) was determined by titration to pH 8.2 using 0.1 M NaOH (LabServ, 97% min; Biolab (Australia) Ltd.). The results were reported in g/L as tartaric acid ($H_2T$), and calculated as described in Iland *et al.* (2004):

$$\text{Titratable acidity } (g/L \text{ as } H_2T) = 75 * \text{molarity of NaOH} * \frac{\text{Titre value (mL)}}{\text{Volume of juice (mL)}}$$

### 5.2.4 Wine tannin concentration by MCP

The 1 mL assay using sample aliquots of 25 µL as described by Mercurio *et al.* (2007) was used to determine the tannin concentration of wine. Methylcellulose solution (0.04% w/v, 1500 cP viscosity at 2%, M-0387, Sigma-Aldrich, USA) and saturated ammonium solution (Sigma-Aldrich, Auckland) were prepared as described in Mercurio *et al.* (2007). Absorbances at 280 nm were measured using a Shimadzu UV-1800 Spectrophotometer (Shimadzu Corporation, Kyoto, Japan), using 1 cm pathlength methacrylate disposable cuvettes.

### 5.2.5 Wine colour by Somers' method

The method described in Iland *et al.* (2004) and originally proposed by Somers and Evans (1977) was used to determine wine colour density, colour hue, degree of red pigment colouration, estimate of $SO_2$ resistant pigments, total red pigments, total phenolics and total anthocyanins of the wines. Solutions of $CH_3CHO$ (>99.5%; BDH Laboratories Supplies, England), $NaS_2O_5$ (Unilab; Ajax Chemicals Pty Limited, AUS), and HCl (~37%; Fisher Scientific, UK) were prepared as indicated by Iland *et al.* (2004). Absorbance values were measured using a Shimadzu UV-1800 Spectrophotometer (Shimadzu Corporation, Kyoto, Japan), using 1cm pathlength methacrylate disposable cuvettes.

### 5.2.6 Wine colour by CIELab method

CIELab coordinates were used to determine wine lightness (L), chroma (C), hue angle (h), red-greenness (a) and yellow-blueness (b), colour intensity, and tonality as described in Ayala *et al.* (1997). Absorbance values between 380 to 780 nm were measured using a Shimadzu UV-1800 Spectrophotometer (Shimadzu Corporation, Kyoto, Japan), using a 2mm pathlength quartz cuvette (Starna Pty Ltd, NSW, Australia) as suggested by the OIV (International Organisation of Vine and Wine 2014).

Data were processed with the MSCV® software (Ayala *et al.* 2012) and a graphic representation of the wine colour was obtained using Corel PHOTO-PAINT®12 for comparisons.

### 5.2.7 Wine aroma compounds by GC-MS

The methods described by Tomasino *et al.* (2015) were adapted to determine the concentration of aroma compounds in the wines. As originally detailed by the authors, the HS-SPME (head space solid phase micro extraction) extraction and subsequent quantitative analysis by GC-MS was carried out using three different methods selected to achieve the desired separation and the sensitivity needed for accurate quantitation of each compound. Tomasino *et al.* (2015) reported these as method 1, 2 and 3, so the same nomenclature will be used here.

Method 1 was used to identify esters, alcohols and one aromatic aldehyde. The compounds successfully identified (19 total) were: ethyl acetate, ethyl isobutyrate, ethyl butanoate, ethyl isovalerate, isoamyl acetate, ethyl pentanoate, isoamyl alcohol, ethyl hexanoate, hexyl acetate, ethyl lactate, hexanol, *trans*-3-Hexen-1-ol, ethyl heptanoate, *cis*-3-Hexen-1-ol, 1-Heptanol, ethyl octanoate, benzaldehyde, ethyl decanoate and phenylethyl alcohol. The method modifications with respect to Tomasino *et al.* (2015) included the use of a diluent solution of 5 g/L tartaric acid in deionised water adjusted to pH 3.5 (standards and wine samples), which replaces the deionised water described in the original method. Both wine samples and standards were diluted 10-fold using this diluent. Also, working standards were made up in dearomatized wine rather than 14% aqueous ethanol solution. For this, 100 mL of Pinot noir wine was rotary evaporated (Buchi Rotavapor-R, Switzerland) at 30 °C for 2 hours under a vacuum of 100 kPa. Once dearomatized, the wine was then reconstituted in deionised water with 100 % HPLC grade ethanol added to a strength of 14 %. The pH of the dearomatized wine was adjusted to pH 3.5 as described in Song *et al.* (2015b).

All wine samples and standards were run on a Shimadzu QP2010 GC-MS (Shimadzu Scientific Instruments, Japan) equipped with a CTC Combi-Pal auto sampler (CTC-Analytics, Switzerland). A dual column setup with Restek columns (Restek, USA) Rtx-wax 30m x 0.25mm ID x 0.5µm film thickness and Rxi-1ms 15m x 0.25mm ID x 0.5µm film thickness in series, was used. All samples and standards were held on a cooler tray at 8 °C until analysed. The HS-SPME conditions were as follows: 10 minute incubation of the sample vial at 60 °C with agitation of 500 rpm, followed by extraction at 60 °C for 60 minutes using static sampling of the headspace with a 2 cm Stableflex DVB/CAR/PDMS fibre (p/n 57348-U, 50/30 µm thickness, 24 gauge, Supelco, USA). The sample preparation and chromatographic separation conditions used followed that described by Tomasino *et al.* (2015).

Method 2 was used to quantify low concentration compounds. These were: linalool, citronellol, 2-Phenylethyl acetate, β-Damascenone, geraniol, ethyl hydrocinnamate, β-Ionone and ethyl cinnamate. The instrument, GC columns and HS-SPME conditions used were the same as those mentioned in method 1 with the sample preparation and chromatographic separation conditions detailed in Tomasino *et al.* (2015). Working standards were made up using dearomatized wine, the same as for method 1. Also, the same diluent (tartaric acid, pH 3.5) was used to dilute 10-fold samples and standards.

Method 3 involved the analysis of seven volatile fatty acids: acetic acid, isobutyric acid, butanoic acid, isovaleric acid, 2-Methylbutanoic acid, hexanoic acid and octanoic acid. Tomasino *et al.* (2015) suggested the use of a separate method for volatile fatty acids due to the specific SPME fibre extraction conditions needed for acids with low vapour pressures. The instrument and GC columns used were the same as method 1. The HS-SPME conditions used were as follows: 10 minute

incubation of the sample vial at 60 °C with agitation of 500 rpm, followed by extraction at 60 °C for 30 minutes using static sampling of the headspace with a 2 cm Stableflex DVB/CAR/PDMS fibre as in method 1 and 2. Unlike methods 1 and 2, the working standards were prepared in 14% ethanol as originally described by Tomasino *et al.* (2015) along with the sample preparation and chromatographic separation conditions. Samples and standards were diluted 10-fold with the same diluent as described in method 1.

All wine samples were analysed in duplicate for all three methods. Composite standard solutions stored at -20 °C were used to make the working standards required to create calibration curves. Composite deuterated internal standards were added to all samples and standards. Spiked samples were run alongside each set of wine samples to check the accuracy of each method. The quantification parameters for the three methods are described in Appendix E.

### 5.2.8 Statistical analyses

One-way analyses of variance (ANOVA) and, equivalently, the least significant difference (LSD) test were used to determine statistical differences between CON and RI for each site at the 5% level ($p < 0.05$) for the data. Then, to evaluate the average treatment difference across sites, the two treatment means for each site were put into a randomised complete block design ANOVA with blocking factor "site" and treatment factor "treatment" with LSD test at 5% for wine pH, TA, tannin and total phenolics concentration, wine colour by Somers' and CIELab methods, and each aroma compound.

Residual plots, including plots of residuals against fitted-value and histogram of residuals were obtained for each set of data to evaluate whether data need any square root or logarithm transformation. Based on this, no transformations were carried out.

All means, LSD 5%, and p-values for all two-way ANOVAs of this chapter can be found in Appendix D.

For the GC-MS results only, a canonical variate analysis (CVA) was carried out on all data, separated by group of compounds. This method produces a "dimensional representation that highlights as accurately as possible the differences that exist between the subsets of data" (Tomasino 2011), and has been successfully used to obtain differences for Pinot noir aroma profiles among different wine regions in New Zealand (Tomasino 2011, Tomasino *et al.* 2013). Here, data from the four groups of compounds (acids, alcohols, esters, and monoterpenes, norisoprenoids and aldehydes) were analysed separated by treatment and site, aiming to obtain the differences between wines produced in the 2013-2014 season. To differentiate treatments and sites, the six groups of data were labelled as: WHC, WHRI, GB5C, GB5RI, GB10C and GB10RI. Prior to the CVA analyses, all data was standardised.

Aiming to integrate wine data with grapevine physiology and grape parameters, a principal component analysis (PCA) was performed on the same five grapevine physiology and eight grape measurements used for the PCA reported in the previous chapter for the 2013-2014 season, but this time including seven wine parameters.

All the analyses were performed using Genstat 18 (GenStat for Windows, VSN International Limited, UK).

## 5.3 Results

### 5.3.1 Wine pH

RI did not alter wine pH at all sites (Table 62).

**Table 62 Wine pH. Each value is the average of four replicates.**

| Treatment | Site | | |
|---|---|---|---|
| | WH | GB5 | GB10 |
| CON | 3.97 | 3.81 | 4.14 |
| RI | 4.10 | 3.74 | 4.17 |
| LSD 5% | 0.15 | 0.25 | 0.23 |
| p value | 0.100 | 0.477 | 0.796 |

The two-way ANOVA also did not report differences in wine pH between sites and treatments (Table 63)

**Table 63 Results of two-way ANOVA on the effects of "site" (WH, GB5 and GB10), "treatment" (CON and RI) on wine pH. Numbers correspond to p values.**

| Factor | Season |
|---|---|
| | 2014-2015 |
| Site | 0.063 |
| Treatment | 0.725 |

### 5.3.2 Wine TA

The results in Table 64 indicate that the differences in wine TA between CON and RI were found at WH only, whereas this was unaltered at GB5 and GB10.

**Table 64 Wine titratable acidity (TA, g/L). Each value is the average of four replicates.**

| Treatment | Site | | |
|---|---|---|---|
| | WH | GB5 | GB10 |
| CON | 4.30 | 4.17 | 3.46 |
| RI | 3.89 | 4.16 | 3.55 |
| LSD 5% | 0.27 | 0.35 | 0.41 |
| p value | **0.009** | 0.957 | 0.583 |

As for wine pH, the two-way ANOVA did not report differences in wine TA between sites and treatments (Table 65).

**Table 65 Results of two-way ANOVA on the effects of "site" (WH, GB5 and GB10), "treatment" (CON and RI) on wine TA. Numbers correspond to p values.**

| Factor | Season |
|---|---|
| | 2014-2015 |
| Site | 0.121 |
| Treatment | 0.558 |

### 5.3.3 Wine tannin concentration and total phenolics content

#### Wine tannin concentration

No differences in wine tannin concentration at bottling were observed between CON and RI at any site (Table 66).

**Table 66 Wine tannin concentration (mg/L epicatechin equivalents). Each value is the average of four replicates**

| Treatment | Site | | |
|---|---|---|---|
| | WH | GB5 | GB10 |
| CON | 208.2 | 372.7 | 358.5 |
| RI | 244.8 | 404.9 | 363.9 |
| LSD 5% | 56.2 | 98.7 | 104.8 |
| p value | 0.162 | 0.455 | 0.903 |

Although differences within each site were not observed, differences in wine tannin concentration across sites were observed. No differences between treatments were reported (Table 67).

**Table 67 Results of two-way ANOVA on the effects of "site" (WH, GB5 and GB10), "treatment" (CON and RI) on wine tannin concentration. Numbers correspond to p values.**

| Factor | Season |
|---|---|
| | 2014-2015 |
| Site | **0.009** |
| Treatment | 0.126 |

### 5.3.4 Wine colour by Somers' method

The results in Table 68 indicate that differences in wine colour evaluated using Somers' method were detected at WH only, whereas no parameters were altered by RI at GB5 and GB10.

**Table 68 Wine colour by Somers' method. Each value is the average of four replicates**

| Treatment | Waipara hills | | | | | | |
|---|---|---|---|---|---|---|---|
| | Wine colour density (a.u.)* | Wine colour hue (a.u.) | Degree of red pigment colouration (%) | Estimate of $SO_2$ resistant pigments (a.u.) | Total red pigments (a.u.) | Total phenolics (a.u.) | Total anthocyanins (mg/L) |
| CON | 2.725 | 0.901 | 13.9 | 0.517 | 10.352 | 19.988 | 189.8 |
| RI | 3.491 | 0.892 | 13.4 | 0.677 | 13.761 | 24.532 | 252.6 |
| LSD 5% | 0.430 | 0.081 | 1.2 | 0.087 | 2.072 | 3.603 | 39.2 |
| p value | **0.005** | 0.790 | 0.345 | **0.004** | **0.007** | **0.021** | **0.008** |
| Treatment | Greystone block 5 | | | | | | |
| | Wine colour density (a.u.) | Wine colour hue (a.u.) | Degree of red pigment colouration (%) | Estimate of $SO_2$ resistant pigments (a.u.) | Total red pigments (a.u.) | Total phenolics (a.u.) | Total anthocyanins (mg/L) |
| CON | 3.001 | 0.773 | 15.4 | 0.509 | 11.110 | 20.997 | 205.2 |
| RI | 3.209 | 0.735 | 17.3 | 0.511 | 10.605 | 20.619 | 195.1 |
| LSD 5% | 1.081 | 0.111 | 4.6 | 0.133 | 2.018 | 4.307 | 37.6 |
| p value | 0.655 | 0.430 | 0.337 | 0.965 | 0.563 | 0.837 | 0.533 |
| Treatment | Greystone block 10 | | | | | | |
| | Wine colour density (a.u.) | Wine colour hue (a.u.) | Degree of red pigment colouration (%) | Estimate of $SO_2$ resistant pigments (a.u.) | Total red pigments (a.u.) | Total phenolics (a.u.) | Total anthocyanins (mg/L) |
| CON | 2.683 | 1.070 | 14.8 | 0.531 | 8.711 | 21.124 | 156.5 |
| RI | 2.726 | 1.082 | 14.7 | 0.536 | 8.838 | 20.871 | 158.9 |
| LSD 5% | 0.911 | 0.099 | 3.4 | 0.184 | 1.323 | 4.028 | 21.4 |
| p value | 0.910 | 0.778 | 0.925 | 0.949 | 0.823 | 0.883 | 0.797 |

*a.u.: absorbance units

Wines made from grapes under RI showed higher colour density, estimate $SO_2$ resistant pigments, total red pigments, total phenolics, and total anthocyanins than those in CON at WH when evaluated by Somers' method. However, no parameters as part of this evaluation were affected by RI at GB5 and GB10 (Table 68)

The two-way ANOVA for each parameter reported differences between sites only for wine colour hue. On the other hand, no differences between treatments were reported for any of the parameters (Table 69).

**Table 69 Results of two-way ANOVA on the effects of "site" (WH, GB5 and GB10), "treatment" (CON and RI) on wine colour by Somers' method. Numbers correspond to p values.**

| Factor | Somers' colour evaluation | | | | | | |
|---|---|---|---|---|---|---|---|
| | Wine colour density (a.u.) | Wine colour hue (a.u.) | Degree of red pigment colouration (%) | Estimate of $SO_2$ resistant pigments (a.u.) | Total red pigments (a.u.) | Total phenolics (a.u.) | Total anthocyanins (mg/L) |
| Site | 0.400 | **0.006** | 0.193 | 0.498 | 0.286 | 0.760 | 0.267 |
| Treatment | 0.261 | 0.499 | 0.620 | 0.396 | 0.493 | 0.505 | 0.501 |

### 5.3.5 Wine colour by CIELab method

As for wine colour by Somers' method, wine colour evaluated by the CIELab method reported differences between CON and RI at WH only (Table 70). In this case, differences at WH corresponded to: wine luminosity, chroma, blueness, and colour intensity. No differences were observed at GB5 and GB10 for any parameter.

**Table 70 Wine colour by CIELab method. Each value is the average of four replicates.**

| Treatment | Waipara hills | | | | | | |
|---|---|---|---|---|---|---|---|
| | Luminosity (L) | Chroma (C) | Hue angle (h) | Redness (a) | Blueness (b) | Colour intensity | Tonality |
| CON | 45.12 | 51.01 | 18.80 | 48.24 | 16.48 | 2.678 | 0.754 |
| RI | 36.33 | 55.78 | 22.57 | 51.48 | 21.38 | 3.520 | 0.766 |
| LSD 5% | 4.68 | 3.64 | 4.22 | 3.49 | 4.06 | 0.429 | 0.091 |
| p value | **0.004** | **0.018** | 0.071 | 0.063 | **0.026** | **0.003** | 0.758 |
| Treatment | Greystone block 5 | | | | | | |
| | Luminosity (L) | Chroma (C) | Hue angle (h) | Redness (a) | Blueness (b) | Colour intensity | Tonality |
| CON | 43.50 | 58.9 | 15.34 | 56.7 | 15.79 | 2.865 | 0.614 |
| RI | 41.45 | 57.7 | 13.79 | 55.9 | 13.81 | 3.027 | 0.576 |
| LSD 5% | 8.90 | 10.73 | 4.75 | 9.5 | 7.09 | 0.896 | 0.087 |
| p value | 0.593 | 0.785 | 0.456 | 0.846 | 0.521 | 0.672 | 0.324 |
| Treatment | Greystone block 10 | | | | | | |
| | Luminosity (L) | Chroma (C) | Hue angle (h) | Redness (a) | Blueness (b) | Colour intensity | Tonality |
| CON | 48.17 | 45.93 | 24.48 | 41.7 | 19.0 | 2.521 | 0.900 |
| RI | 48.20 | 46.44 | 25.38 | 41.9 | 20.0 | 2.546 | 0.916 |
| LSD 5% | 11.69 | 8.13 | 5.27 | 7.40 | 5.37 | 0.828 | 0.109 |
| p value | 0.996 | 0.883 | 0.690 | 0.963 | 0.671 | 0.943 | 0.732 |

Among the parameters considered in the CIELab method, differences across sites have been observed for wine redness and tonality. The other parameters did not report differences either between sites or treatments (Table 71).

**Table 71 Results of two-way ANOVA on the effects of "site" (WH, GB5 and GB10), "treatment" (CON and RI) on wine colour by CIELab method. Numbers correspond to p values.**

| Factor | Somers' colour evaluation | | | | | | |
|---|---|---|---|---|---|---|---|
| | Luminosity (L) | Chroma (C) | Hue angle (h) | Redness (a) | Blueness (b) | Colour intensity | Tonality |
| Site | 0.259 | 0.061 | 0.061 | **0.021** | 0.312 | 0.359 | **0.009** |
| Treatment | 0.308 | 0.531 | 0.567 | 0.550 | 0.581 | 0.307 | 0.866 |

For a better understanding of the differences in wine colour, the CIELab method allows the creation of a graphical representation of this based on the numerical parameters. Thus, in Figure 37 can be observed that the wines from grapes under RI showed a slightly deeper and intense colour than those from CON, especially at WH and GB5. It is difficult to notice the differences between treatments at GB10.

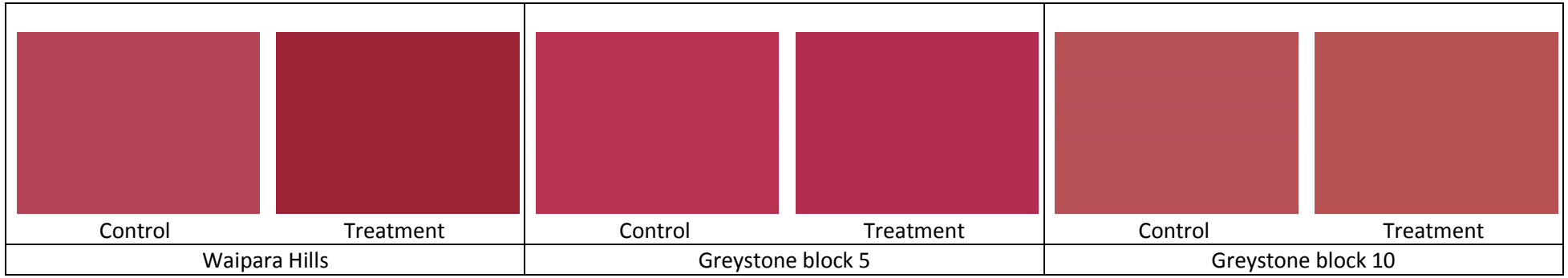


**Figure 37 Graphical comparison of the wine colours by CIELab method in the 2013-2014 season. These were created using Corel PHOTO-PAINT®12, based on the information in Ayala *et al.* (2012). Each represent the average of four replicates.**

### 5.3.6 Aroma compound concentrations by GCMS

The results of all three methods were re-grouped into four groups to facilitate the statistical analyses, as described below (in alphabetical order):

Acids: 2-Methylbutanoic acid, acetic acid, butanoic acid, hexanoic acid, isobutyric acid, isovaleric acid and octanoic acid.

Alcohols: 1-Heptanol, *cis*-3-Hexen-1-ol, hexanol, isoamyl alcohol, phenylethyl alcohol and *trans*-3-Hexen-1-ol.

Esters: 2-Phenylethyl acetate, ethyl acetate, ethyl butanoate, ethyl cinnamate, ethyl decanoate, ethyl heptanoate, ethyl hexanoate, ethyl hydrocinnamate, ethyl isobutyrate, ethyl isovalerate, ethyl lactate, ethyl octanoate, ethyl pentanoate, hexyl acetate and isoamyl acetate.

Monoterpenes, norisoprenoids and aldehydes: citronellol, geraniol, linalool, β-Damascenone, β-Ionone and benzaldehyde.

**Acids**

The results in Table 72 show that RI altered volatile acids concentration at WH only, with 2-Methyl butanoic acid and isovaleric acid showing lower concentration of these compounds in wines made from grapes under RI.

**Table 72 Volatile acids concentration in wines. Each value is the average of four replicates.**

| Treatment | Waipara hills | | | | | | |
|---|---|---|---|---|---|---|---|
| | 2-Methylbutanoic acid | Acetic acid | Butanoic acid | Hexanoic acid | Isobutyric acid | Isovaleric acid | Octanoic acid |
| CON | 750.3 | 481,870.9 | 931.2 | 874.5 | 6,830.3 | 833.5 | 489.9 |
| RI | 656.8 | 502,248.7 | 1,012.6 | 788.2 | 6,156.6 | 702.5 | 402.0 |
| LSD 5% | 88.4 | 35,192.9 | 98.4 | 111.7 | 886.1 | 100.4 | 180.4 |
| p value | **0.041** | 0.206 | 0.090 | 0.107 | 0.112 | **0.019** | 0.278 |
| Treatment | Greystone block 5 | | | | | | |
| | 2-Methylbutanoic acid | Acetic acid | Butanoic acid | Hexanoic acid | Isobutyric acid | Isovaleric acid | Octanoic acid |
| CON | 700.8 | 529,751.4 | 1,505.2 | 872.0 | 7,081.3 | 717.1 | 612.9 |
| RI | 733.4 | 555,251.8 | 1,353.4 | 822.1 | 7,477.8 | 744.7 | 543.8 |
| LSD 5% | 110.4 | 53,546.3 | 248.6 | 126.2 | 870.6 | 47.7 | 120.2 |
| p value | 0.498 | 0.288 | 0.186 | 0.371 | 0.308 | 0.207 | 0.209 |
| Treatment | Greystone block 10 | | | | | | |
| | 2-Methylbutanoic acid | Acetic acid | Butanoic acid | Hexanoic acid | Isobutyric acid | Isovaleric acid | Octanoic acid |
| CON | 872.0 | 535,429.1 | 1,533.2 | 843.3 | 9,015.1 | 786.2 | 522.7 |
| RI | 926.8 | 545,012.4 | 1,606.0 | 848.3 | 9,635.8 | 841.8 | 535.8 |
| LSD 5% | 199.2 | 37,185.4 | 129.9 | 188.4 | 1,043.7 | 126.4 | 242.4 |
| p value | 0.526 | 0.552 | 0.219 | 0.950 | 0.196 | 0.323 | 0.899 |

Acetic acid and butanoic acid showed differences across sites, whereas treatment did not have an overall effect on any volatile acid compound (Table 73).

**Table 73 Results of two-way ANOVA on the effects of "site" (WH, GB5 and GB10), "treatment" (CON and RI) on volatile acids concentration. Numbers correspond to p values.**

| Factor | Volatile acid | | | | | | |
|---|---|---|---|---|---|---|---|
| | 2-Methylbutanoic acid | Acetic acid | Butanoic acid | Hexanoic acid | Isobutyric acid | Isovaleric acid | Octanoic acid |
| Site | 0.118 | **0.020** | **0.043** | 0.874 | 0.053 | 0.594 | 0.139 |
| Treatment | 0.969 | 0.059 | 0.992 | 0.241 | 0.801 | 0.810 | 0.262 |

## Alcohols

RI reduced phenylethyl alcohol concentration at WH. On the other hand, hexanol concentration at GB5 was increased by the RI treatment. No alcohol compounds were altered at GB10 (Table 74).

**Table 74 Alcohols concentration in wines. Each value is the average of four replicates.**

| Treatment | Waipara hills | | | | | |
|---|---|---|---|---|---|---|
| | 1-Heptanol | *cis*-3-Hexen-1-ol | Hexanol | Isoamyl alcohol | Phenylethyl alcohol | *trans*-3-Hexen-1-ol |
| CON | 51.2 | 51.2 | 3,373.8 | 231,222.8 | 41,752.3 | 127.8 |
| RI | 53.9 | 49.4 | 3,503.9 | 212,791.0 | 34,319.8 | 136.6 |
| LSD 5% | 6.7 | 11.1 | 281.8 | 18,573.1 | 4,956.8 | 16.0 |
| p value | 0.357 | 0.710 | 0.301 | 0.051 | **0.010** | 0.230 |
| Treatment | Greystone block 5 | | | | | |
| | 1-Heptanol | *cis*-3-Hexen-1-ol | Hexanol | Isoamyl alcohol | Phenylethyl alcohol | *trans*-3-Hexen-1-ol |
| CON | 44.4 | 40.2 | 2,333.5 | 195,343.0 | 30,351.2 | 99.6 |
| RI | 44.2 | 43.1 | 2,501.7 | 189,348.2 | 28,687.2 | 96.8 |
| LSD 5% | 4.3 | 5.9 | 149.7 | 15,359.7 | 5,689.7 | 22.4 |
| p value | 0.901 | 0.275 | **0.033** | 0.376 | 0.501 | 0.771 |
| Treatment | Greystone block 10 | | | | | |
| | 1-Heptanol | *cis*-3-Hexen-1-ol | Hexanol | Isoamyl alcohol | Phenylethyl alcohol | *trans*-3-Hexen-1-ol |
| CON | 45.7 | 57.6 | 2,817.9 | 196,102.0 | 25,846.8 | 87.3 |
| RI | 44.9 | 55.3 | 2,831.4 | 210,056.2 | 29,308.2 | 90.9 |
| LSD 5% | 6.7 | 6.2 | 100.5 | 36,704.6 | 6,751.7 | 9.9 |
| p value | 0.798 | 0.395 | 0.755 | 0.388 | 0.256 | 0.400 |

Four out of six volatile alcohols (1-Heptanol, *cis*-3-Hexen-1-ol, hexanol and *trans*-3-Hexen-1-ol) showed differences across sites, while the treatment did not affect the concentration of any alcohol compounds considered in this analysis (Table 75).

**Table 75 Results of two-way ANOVA on the effects of "site" (WH, GB5 and GB10), "treatment" (CON and RI) on alcohols concentration. Numbers correspond to p values.**

| Factor | Alcohols | | | | | |
|---|---|---|---|---|---|---|
| | 1-Heptanol | *cis*-3-Hexen-1-ol | Hexanol | Isoamyl alcohol | Phenylethyl alcohol | *trans*-3-Hexen-1-ol |
| Site | **0.041** | **0.036** | **0.006** | 0.228 | 0.194 | **0.016** |
| Treatment | 0.640 | 0.838 | 0.155 | 0.747 | 0.611 | 0.439 |

## Esters

Wines made from grapes under RI showed higher concentration of ethyl acetate and ethyl butanoate, and lower concentration of ethyl heptanoate and ethyl isovalerate compared to CON at WH. Ethyl decanoate was the only ester compound showing differences between CON and RI at both GB5 and GB10 (Table 76).

**Table 76 Esters concentration in wines. Each value is the average of four replicates.**

| Treatment | 2-Phenylethyl acetate | Ethyl acetate | Ethyl butanoate | Ethyl cinnamate | Ethyl decanoate | Ethyl heptanoate | Ethyl hexanoate | Ethyl hydrocinnamate | Ethyl isobutyrate | Ethyl isovalerate | Ethyl lactate | Ethyl octanoate | Ethyl pentanoate | Hexyl acetate | Isoamyl acetate |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Waipara hills** | | | | | | | | | | | | | | | |
| CON | 27.1 | 64,278.1 | 238.3 | 1.2 | 140.9 | 4.2 | 399.6 | 0.6 | 116.3 | 9.7 | 48,041.9 | 542.2 | 1.4 | 15.5 | 481.8 |
| RI | 21.3 | 75,324.4 | 287.6 | 1.0 | 136.4 | 3.6 | 377.3 | 0.6 | 97.3 | 8.2 | 45,849.1 | 535.5 | 1.4 | 17.7 | 425.1 |
| LSD 5% | 5.9 | 9,075.9 | 41.7 | 0.4 | 26.7 | 0.4 | 54.7 | 0.1 | 19.2 | 1.4 | 5,241.3 | 150.6 | 0.1 | 8.8 | 224.9 |
| p value | 0.054 | **0.025** | **0.028** | 0.254 | 0.696 | **0.006** | 0.357 | 0.439 | 0.052 | **0.037** | 0.345 | 0.917 | 0.249 | 0.562 | 0.560 |
| **Greystone block 5** | | | | | | | | | | | | | | | |
| CON | 55.6 | 99,726.4 | 449.6 | 1.0 | 212.3 | 4.1 | 504.5 | 0.8 | 139.9 | 11.5 | 57,052.2 | 760.9 | 1.4 | 56.2 | 1397.1 |
| RI | 57.5 | 94,393.6 | 379.3 | 0.8 | 161.5 | 4.4 | 470.8 | 0.8 | 151.0 | 12.5 | 57,571.8 | 679.9 | 1.4 | 53.0 | 1148.2 |
| LSD 5% | 28.8 | 12,510.5 | 79.1 | 0.3 | 46.5 | 0.7 | 72.6 | 0.3 | 25.4 | 2.1 | 12,115.8 | 112.2 | 0.2 | 27.6 | 487.6 |
| p value | 0.882 | 0.337 | 0.072 | 0.375 | **0.037** | 0.482 | 0.292 | 0.922 | 0.323 | 0.289 | 0.920 | 0.128 | 0.491 | 0.787 | 0.258 |
| **Greystone block 10** | | | | | | | | | | | | | | | |
| CON | 22.8 | 94,284.7 | 428.9 | 2.1 | 141.9 | 3.5 | 454.6 | 1.5 | 141.3 | 9.9 | 58,155.9 | 613.4 | 1.7 | 8.5 | 349.2 |
| RI | 27.2 | 93,665.9 | 441.3 | 2.1 | 188.0 | 3.6 | 456.8 | 1.5 | 143.8 | 10.4 | 57,873.6 | 655.5 | 1.6 | 10.3 | 439.2 |
| LSD 5% | 9.7 | 17,593.6 | 94.0 | 0.9 | 40.1 | 0.7 | 113.7 | 0.6 | 26.9 | 1.6 | 4,367.6 | 193.6 | 0.3 | 4.8 | 194.7 |
| p value | 0.318 | 0.934 | 0.759 | 0.858 | **0.031** | 0.746 | 0.964 | 0.989 | 0.824 | 0.519 | 0.880 | 0.614 | 0.796 | 0.391 | 0.302 |

Differences between sites were found for 2-Phenylethyl acetate, ethyl cinnamate, ethyl hexanoate, ethyl hydrocinnamate, ethyl lactate, hexyl acetate and isoamyl acetate. No esters reported an overall effect of the treatment (Table 77).

**Table 77 Results of two-way ANOVA on the effects of "site" (WH, GB5 and GB10), "treatment" (CON and RI) on esters concentration. Numbers correspond to p values.**

| Factor | Esters | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2-Phenylethyl acetate | Ethyl acetate | Ethyl butanoate | Ethyl cinnamate | Ethyl decanoate | Ethyl heptanoate | Ethyl hexanoate | Ethyl hydrocinnamate | Ethyl isobutyrate | Ethyl isovalerate | Ethyl lactate | Ethyl octanoate | Ethyl pentanoate | Hexyl acetate | Isoamyl acetate |
| Site | **0.020** | 0.074 | 0.096 | **0.003** | 0.501 | 0.327 | **0.032** | **<.001** | 0.115 | 0.155 | **0.012** | 0.104 | 0.059 | **0.004** | **0.029** |
| Treatment | 0.973 | 0.761 | 0.942 | 0.072 | 0.923 | 0.707 | 0.233 | 0.545 | 0.862 | 0.988 | 0.503 | 0.712 | 0.889 | 0.890 | 0.540 |

## Monoterpenes, norisopreids and aldehydes

The two norisoprenoids included in this analysis reported differences between CON and RI at WH. For both compounds, RI tended to increase their concentration.

At GB5 and GB10, no monoterpene, norisoprenoids or aldehyde was affected by RI (Table 78).

**Table 78 Monoterpenes, norisoprenoids and aldehydes concentration in wines. Each value is the average of four replicates.**

| Treatment | Waipara hills | | | | | |
|---|---|---|---|---|---|---|
| | Citronellol | Geraniol | Linalool | β-Damascenone | β-Ionone | Benzaldehyde |
| CON | 9.3 | 5.7 | 35.4 | 8.8 | 0.9 | 35.7 |
| RI | 9.6 | 5.9 | 36.2 | 10.1 | 1.0 | 60.3 |
| LSD 5% | 1.4 | 0.6 | 3.5 | 1.1 | 0.1 | 27.6 |
| p value | 0.621 | 0.472 | 0.613 | **0.029** | **0.006** | 0.072 |
| Treatment | Greystone block 5 | | | | | |
| | Citronellol | Geraniol | Linalool | β-Damascenone | β-Ionone | Benzaldehyde |
| CON | 7.2 | 5.1 | 27.7 | 8.1 | 0.8 | 28.2 |
| RI | 7.2 | 5.2 | 28.1 | 7.7 | 0.8 | 30.1 |
| LSD 5% | 1.3 | 0.5 | 5.5 | 1.2 | 0.2 | 11.2 |
| p value | 0.948 | 0.640 | 0.837 | 0.500 | 0.266 | 0.969 |
| Treatment | Greystone block 10 | | | | | |
| | Citronellol | Geraniol | Linalool | β-Damascenone | β-Ionone | Benzaldehyde |
| CON | 9.3 | 5.8 | 34.1 | 11.9 | 0.8 | 139.6 |
| RI | 9.5 | 5.8 | 34.7 | 11.8 | 0.8 | 109.8 |
| LSD 5% | 1.4 | 0.8 | 3.9 | 2.0 | 0.1 | 84.6 |
| p value | 0.723 | 0.829 | 0.708 | 0.868 | 0.817 | 0.421 |

Differences between sites were reported for the three monoterpenes (citronellol, geraniol and linalool), as well as for β-Damascenone. Linalool was also the only compound showing the effect of the treatment among all the aroma compounds considered in this research (Table 79).

**Table 79 Results of two-way ANOVA on the effects of "site" (WH, GB5 and GB10), "treatment" (CON and RI) on monoterpenes, norisoprenoids and aldehydes. Numbers correspond to p values.**

| Factor | Alcohols | | | | | |
|---|---|---|---|---|---|---|
| | Citronellol | Geraniol | Linalool | β-Damascenone | β-Ionone | Benzaldehyde |
| Site | **0.003** | **0.030** | **<.001** | **0.048** | 0.220 | 0.068 |
| Treatment | 0.144 | 0.428 | **0.016** | 0.649 | 0.976 | 0.950 |

In addition, for a better understanding of the results obtained here, Table 80 shows the perception thresholds and concentrations of the same aroma compounds in New Zealand Pinot noir from different regions, which correspond to the same data showed in the literature review (Table 1).

**Table 80 Range of concentrations for aroma compounds recently found in Pinot noir wine from different regions in New Zealand. All results are reported in µg/L**

| Aroma compound | Perception threshold | Olfactory description | Waipara | Central Otago | | | Marlborough | Martinborough |
|---|---|---|---|---|---|---|---|---|
| | | | Tomasino (2011) | Imre *et al.* (2012) | Rutan *et al.* (2014) | Tomasino (2011) | Tomasino (2011) | Tomasino (2011) |
| *Acids* | | | | | | | | |
| 2-Methylbutanoic acid | 3,000 [a] | Cheese | nr | nr | nr | nr | nr | nr |
| Acetic acid | 200,000 [a] | Vinegar | 349,000 - 702,000 | nr | nr | 415,000 - 690,000 | 553,000 - 874,000 | 516,000 - 707,000 |
| Butanoic acid | 10,000 [a] | Cheese | 290 - 716 | nr | 1,026 – 1,845 | 209 - 755 | 314 - 562 | 325 - 715 |
| Hexanoic acid | 3,000 [a] | Sweat, cheese | 1,141 - 1,941 | 640 - 680 | 712 – 1,217 | 1,104 - 1,744 | 1,142 - 1,497 | 1,169 – 1,700 |

| Aroma compound | Perception threshold | Olfactory description | Waipara | Central Otago | | | Marlborough | Martinborough |
|---|---|---|---|---|---|---|---|---|
| | | | Tomasino (2011) | Imre *et al.* (2012) | Rutan *et al.* (2014) | Tomasino (2011) | Tomasino (2011) | Tomasino (2011) |
| Isobutyric acid | 2,300 [b] | Rancid | nr | nr | 389 - 895 | nr | nr | nr |
| Isovaleric acid | 33.4 [c] | Parmesan, sweat | nr | nr | 275 - 665 | nr | nr | nr |
| Octanoic acid | 500 [b,c] | Fatty, rancid | 665 – 2,002 | 1,300 – 1,700 | 911 – 1,302 | 760 – 1,157 | 724 – 1,092 | 726 – 1,067 |
| *Alcohols* | | | | | | | | |
| 1-Heptanol | 2,500 [h] | Herbal, leafy, green | 19.3 – 270.3 | nr | nr | 25.0 – 246.8 | 30.0 – 115.9 | 12.2 – 171.0 |
| *cis*-3-Hexen-1-ol | 400 [a, b, c] | Cut grass, leafy | 39.2 – 115.8 | 35.7 – 42.4 | 22 - 43 | 24.2 – 82.4 | 30.0 – 64.6 | 33.6 – 65.4 |
| Hexanol | 8,000 [a, b, c] | Toasted, green | 2,000 – 4,700 | 568 - 607 | 809 – 1,272 | 2,400 – 3,700 | 2,300 – 3,300 | 1,900 – 3,500 |
| Isoamyl alcohol | 30,000 [b, c] | Fusel, alcoholic | nr | nr | 104,295 – 150,538 | nr | nr | nr |
| Phenylethyl alcohol | 14,000 [c] | Floral, rose | nr | nr | 68,719 – 134,980 | nr | nr | nr |
| *trans*-3-Hexen-1-ol | 8,000 [f] | Vegetable | 56.9 – 107.9 | 9.6 – 12.2 | 18 - 35 | 66.1 – 126.5 | 72.0 – 123.6 | 60.5 – 92.6 |
| *Esters* | | | | | | | | |
| 2-Phenylethyl acetate | 250 [a] | Fruity, floral, honey | nr | 11.8 – 12.3 | 11.6 – 18.1 | nr | nr | nr |
| Ethyl acetate | 12,270 [b] | Sweet fruity | nr | nr | nr | nr | nr | nr |
| Ethyl butanoate | 20 [a, b, c] | Fruity, strawberry | 134.9 – 271.0 | 30.0 – 32.4 | 75 - 153 | 116.4 – 289.4 | 164.8 – 286.2 | 165.8 – 339.9 |
| Ethyl cinnamate | 1.1 [b, c] | Fruity, cherry, plum | 0.8 – 3.0 | 0.36 – 0.71 | 1.6 – 4.1 | 1.8 – 3.1 | 0.8 – 2.8 | 1.2 – 7.2 |

| Aroma compound | Perception threshold | Olfactory description | Waipara | Central Otago | | | Marlborough | Martinborough |
|---|---|---|---|---|---|---|---|---|
| | | | Tomasino (2011) | Imre *et al.* (2012) | Rutan *et al.* (2014) | Tomasino (2011) | Tomasino (2011) | Tomasino (2011) |
| Ethyl decanoate | 200 [b,c] | Fruity, waxy | 171.9 – 940.3 | 17 - 23 | 164 - 207 | 190.3 – 971.3 | 259.0 – 518.5 | 154.2 – 629.5 |
| Ethyl heptanoate | 220 [g] | Fruity, pineapple | 3.1 – 9.2 | nr | nr | 3.6 – 8.1 | 2.6 – 4.0 | 3.2 – 5.9 |
| Ethyl hexanoate | 14 [b, c] | Fruity, strawberry | 299.3 – 559.4 | 41 - 45 | 312 - 372 | 320.7 – 557.4 | 334.9 – 409.9 | 339.2 – 593.8 |
| Ethyl hydrocinnamate | 1.6 [b, c] | Fruity, balsamic | nr | 10.3 – 11.5 | 1.11 – 2.31 | nr | nr | nr |
| Ethyl isobutyrate | 15 [c] | Fruity, sweet | nr | nr | 25 - 54 | nr | nr | nr |
| Ethyl isovalerate | 3 [c] | Fruity, sweet | nr | nr | 27 - 49 | nr | nr | nr |
| Ethyl lactate | 154,000 [b] | Lactic, raspberry | nr | nr | 134,921 – 191,724 | nr | nr | nr |
| Ethyl octanoate | 580 [b] | Sweet, fruity | 442.6 – 874.3 | 60.1 – 69.5 | 318 - 384 | 415.6 – 763.6 | 437.8 – 598.0 | 410.2 – 642.5 |
| Ethyl pentanoate | 1.5 [e] | Fruity, orange | 1.6 – 3.1 | nr | nr | 1.1 – 4.3 | 1.3 – 3.1 | 1.4 – 3.4 |
| Hexyl acetate | 1,500 [d] | Fruity, green apple | nr | 9.4 – 9.9 | 10.6 – 18.6 | nr | nr | nr |
| Isoamyl acetate | 30 [b, c] | Banana, pear | 148.5 – 244.4 | nr | 189 - 254 | 160.0 – 377.5 | 216.7 – 370.2 | 151.3 – 297.9 |
| *Monoterpenes, norisoprenoids and aldehydes* | | | | | | | | |
| Citronellol | 100 [a, b] | Citronella | nr | 1.7 – 2.3 | 6.9 – 11.1 | nr | nr | nr |
| Geraniol | 20 [b] – 30 [a, c] | Floral, fruity, citrus | 0 – 4.8 | 13 - 26 | 12.4 – 16.2 | 0 – 3.3 | 0 – 2.4 | 0 – 2.1 |
| Linalool | 15 [a], 25 [b], 25.2 [c] | Citrus, orange, floral | 77.1 – 170.1 | 1.2 – 1.4 | 2.25 – 5.37 | 41.4 – 146.6 | 84.5 – 167.2 | 62.5 – 142.8 |
| β - Damascenone | 0.05 [a, b, c] | Rose | 0.7 – 3.3 | 4.8 – 5.6 | 4.0 – 5.4 | 1.0 – 4.4 | 1.6 – 3.4 | 0.6 – 3.7 |

| Aroma compound | Perception threshold | Olfactory description | Waipara | Central Otago | | | Marlborough | Martinborough |
|---|---|---|---|---|---|---|---|---|
| | | | Tomasino (2011) | Imre *et al.* (2012) | Rutan *et al.* (2014) | Tomasino (2011) | Tomasino (2011) | Tomasino (2011) |
| β - Ionone | 0.09 [b, c] | Berry, violets | 0.1 – 0.5 | 0.19 – 0.21 | 0.29 – 0.42 | 0.3 – 0.6 | 0.3 – 0.7 | 0.1 – 0.6 |
| Benzaldehyde | 2,000 [i] | Almond, sweet | 10.8 – 66.0 | nr | 10.2 – 18.6 | 5.1 – 11.0 | 7.7 – 39.3 | 10.2 – 32.5 |

nr: not reported

[a] Guth (1997); [b] Escudero *et al.* (2007); [c] Ferreira *et al.* (2000); [d] Li *et al.* (2008); [e] Genovese *et al.* (2007); [f] Dunlevy *et al.* (2009); [g] Zea *et al.* (2001); [h] Ferreira *et al.* (2000) from Tomasino (2011); [i] Escudero *et al.* (2007) from Rutan *et al.* (2014).

### 5.3.7 Canonical analyses of aroma compounds

**Acids**

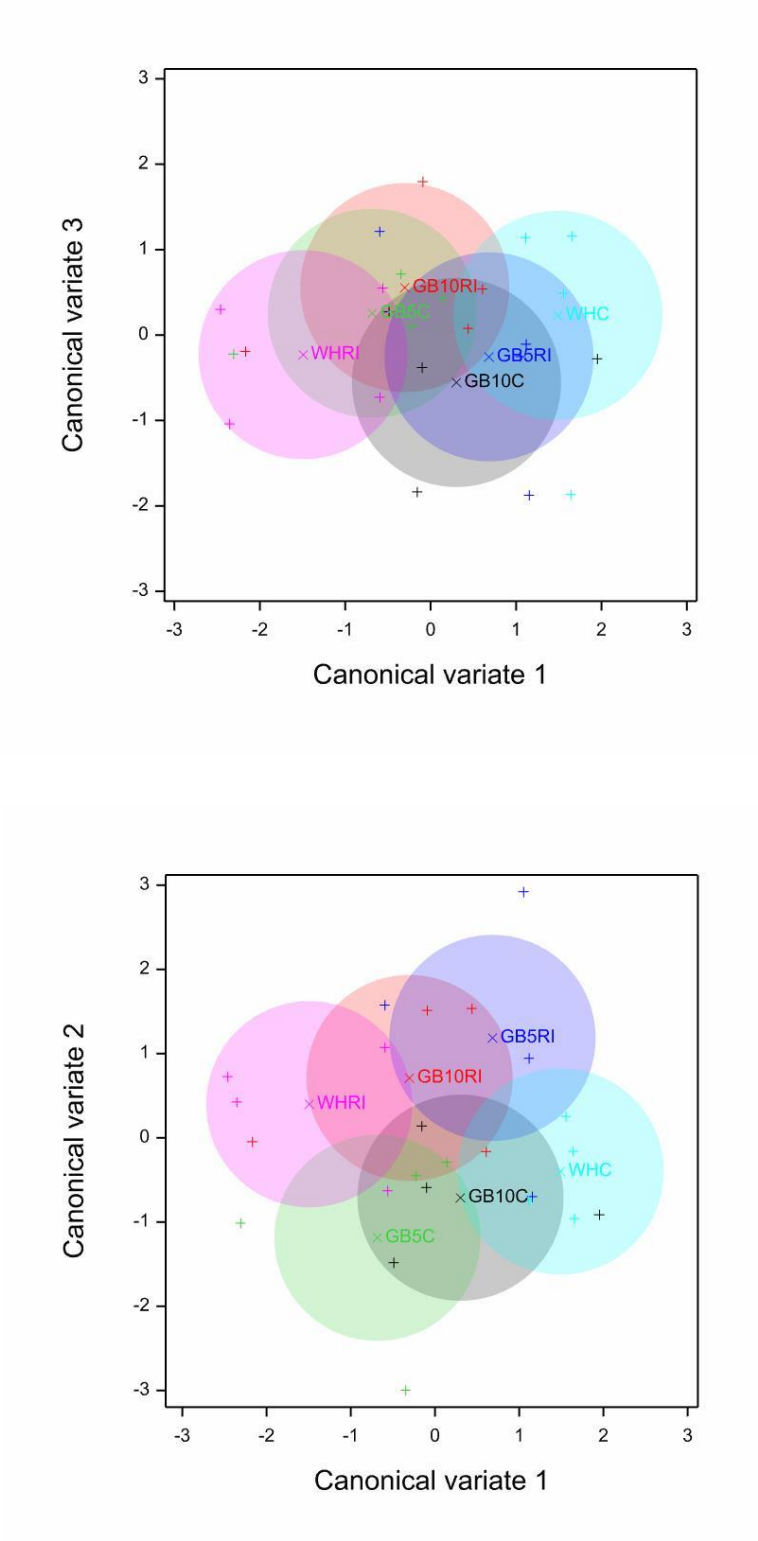


**Figure 38** **Separation of Pinot noir wines by site and treatment using canonical variate analysis based on volatile acid concentrations. Circles represent 95% confidence intervals surrounding the means. Canonical variate 1 (CV1) and CV2 are plotted in the lower chart and CV1 and CV3 are plotted in the upper chart. Abbreviations represent to a**

**combination of site/treatment: WHC, Waipara Hills Control; WHRI, Waipara Hills Reduced Irrigation; GB5C, Greystone Block 5 Control; GB5RI, Greystone Block 5 Reduced irrigation; GB10C, Greystone Block 10 Control; GB10RI, Greystone Block 10 Reduced Irrigation. Each cross symbol correspond to a replicate for each site/treatment combination.**

As can be observed in Figure 38 that the different irrigation treatments for sites WH and GB5 could be separated by canonical variate analysis of the volatile data, while this was not that evident at GB10. For WH, irrigation treatments were differentiated by CV1, and GB5 by CV1 and CV2. The first three variates explained 52.70%, 39.22% and 8.07% of the total variance, respectively. None of the three variates was statistically significant at $p < 0.05$.

The between group distances reported in Table 88 (Appendix D) confirmed the better separation for acids at WH and GB5, compared to GB10. Thus, the largest separation between treatments at the same site was reported for WH with 3.122, followed by GB5 with 2.785. On the other hand, the separation between CON and RI at GB10 was 1.902, and as shown in Figure 38, both circles representing the 95% confidence intervals overlap either plotting CV1 and CV2 or CV1 and CV3.

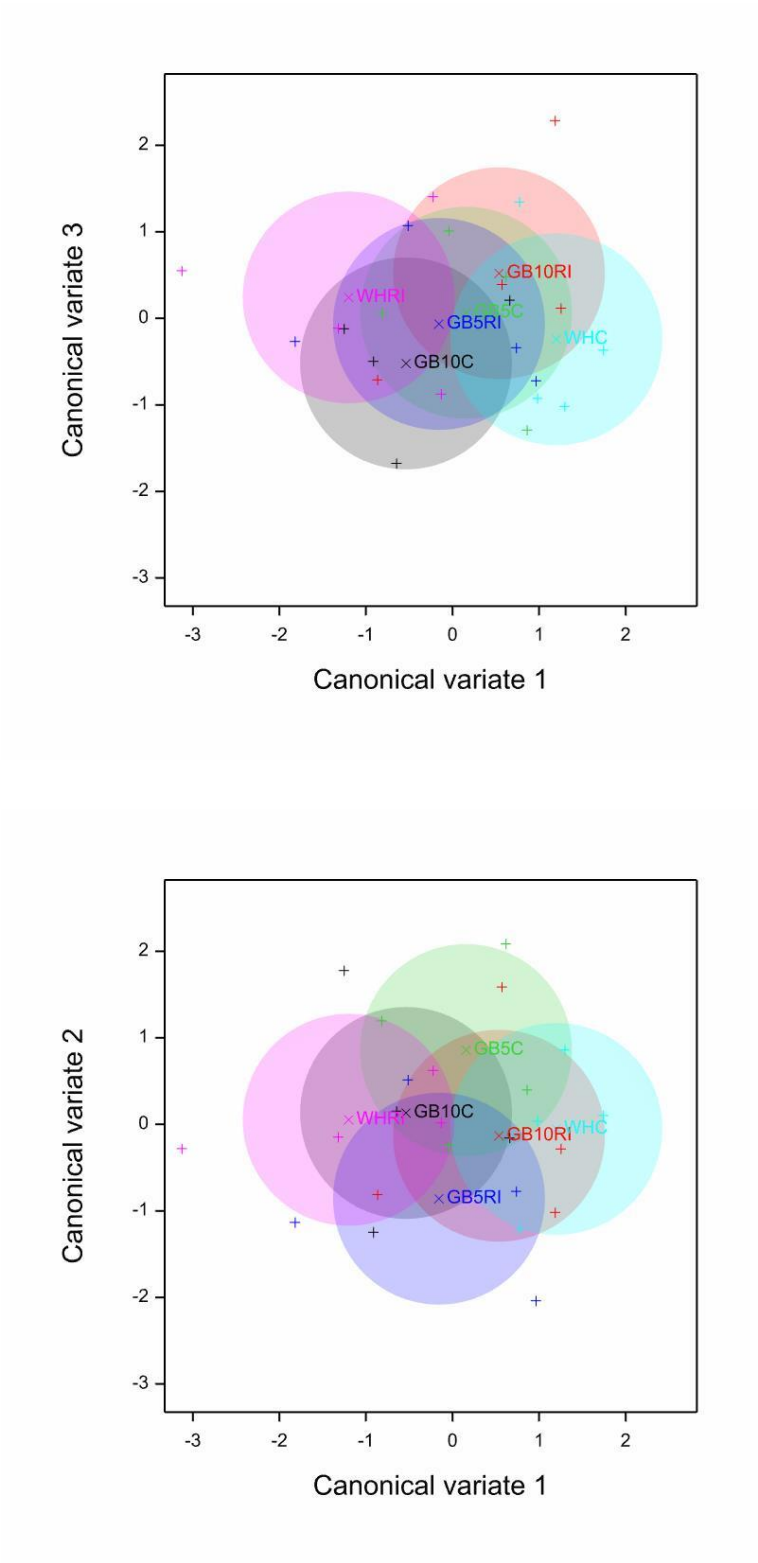


**Figure 39** **Separation of Pinot noir wines by site and treatment using canonical variate analysis based on alcohol concentrations. Circles represent 95% confidence intervals surrounding the means. Canonical variate 1 (CV1) and CV2 are plotted in the lower chart and CV1 and CV3 are plotted in the upper chart. Abbreviations represent to a combination of site/treatment: WHC, Waipara Hills Control; WHRI, Waipara Hills Reduced Irrigation; GB5C, Greystone Block 5 Control; GB5RI, Greystone Block 5 Reduced irrigation; GB10C, Greystone Block 10 Control; GB10RI, Greystone Block 10**

**Reduced Irrigation. Each cross symbol correspond to a replicate for each site/treatment combination.**

Figure 39 shows that the separation between sites and treatments by CVA using the volatile alcohol data was not as great as that using the volatile acids data. Although it was very small at WH, either plotting CV1 and CV2 or CV1 and CV3, all sites and treatments overlap each other, which was evidenced by the smaller between group distances found between CON and RI at all sites. None of the three variates was statistically significant at $p < 0.05$. WH registered the biggest between groups distance with 2.450, despite both 95% confidence interval circles show a small overlap. As well as for acids, the separation between CON and RI at GB5 accounted the second largest distance with 1.750, followed by GB10 with 1.519 (Table 89, Appendix D). In addition, CV1, CV2 and CV3 explained 61.63%, 26.64% and 11.73% of the total variance.

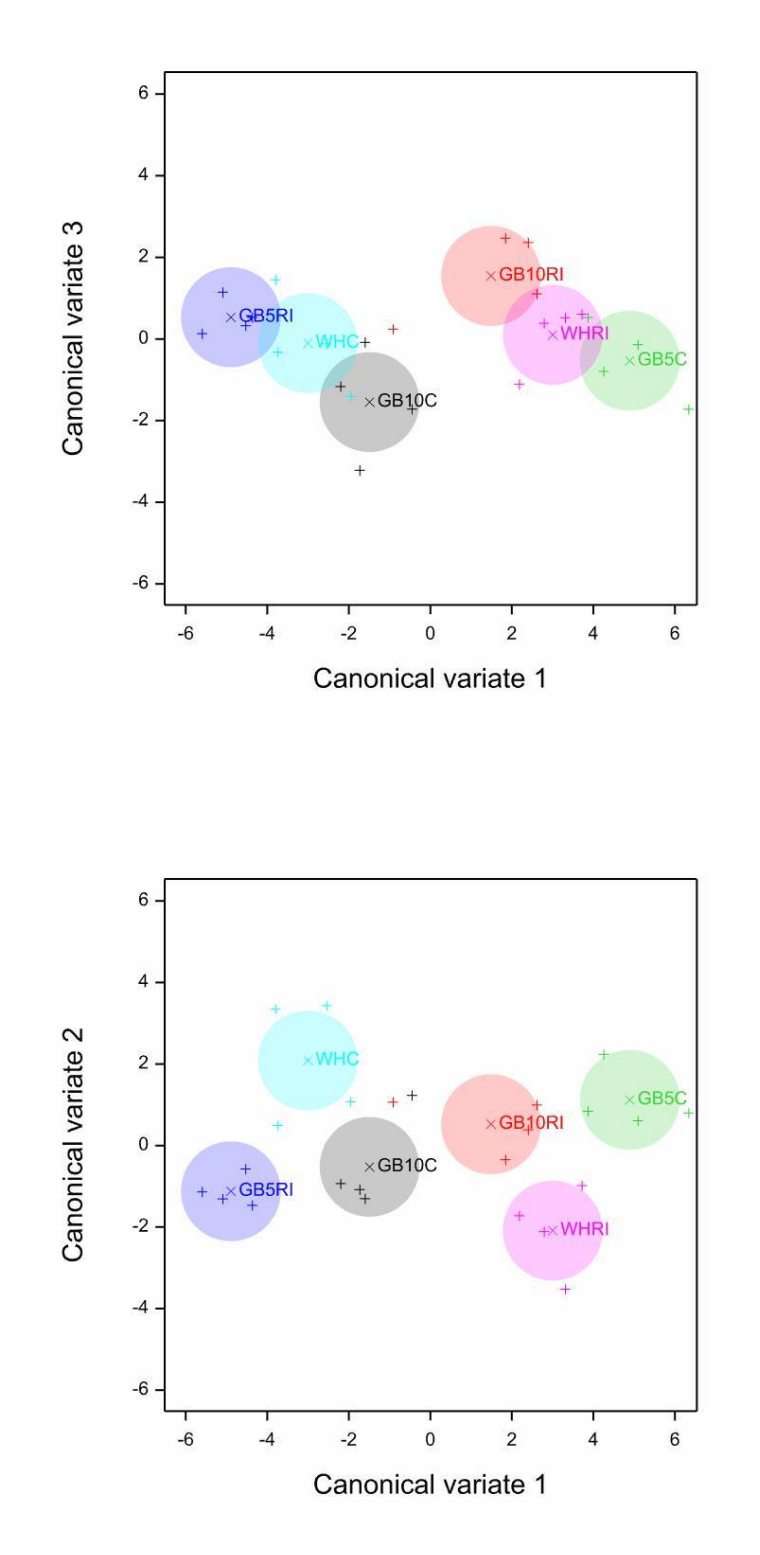


**Figure 40** **Separation of Pinot noir wines by site and treatment using canonical variate analysis based on ester concentrations. Circles represent 95% confidence intervals surrounding the means. Canonical variate 1 (CV1) and CV2 are plotted in the lower chart and CV1 and CV3 are plotted in the upper chart. Abbreviations represent to a combination of**

**site/treatment: WHC, Waipara Hills Control; WHRI, Waipara Hills Reduced Irrigation; GB5C, Greystone Block 5 Control; GB5RI, Greystone Block 5 Reduced irrigation; GB10C, Greystone Block 10 Control; GB10RI, Greystone Block 10 Reduced Irrigation. Each cross symbol correspond to a replicate for each site/treatment combination.**

Clear separation between sites and treatments was obtained by CVA using the volatile esters data (Figure 40). All sites and treatments were well differentiated by CV1 and CV2, which explained 80.42% and 13.45% of the total variance, while CV3 explained 6.13%. Volatile esters data showed the clearest separation among sites and treatments of all groups of aroma compounds, with none of the confidence interval circles overlapping each other. As same as for other groups of compounds, none of the three variates was statistically significant at $p < 0.05$.

Regarding between groups separation within the same site, CON and RI at GB5 showed the largest between groups distance with 10.095, followed by WH with 7.318 and GB10 with 4.419 (Table 90, Appendix D).

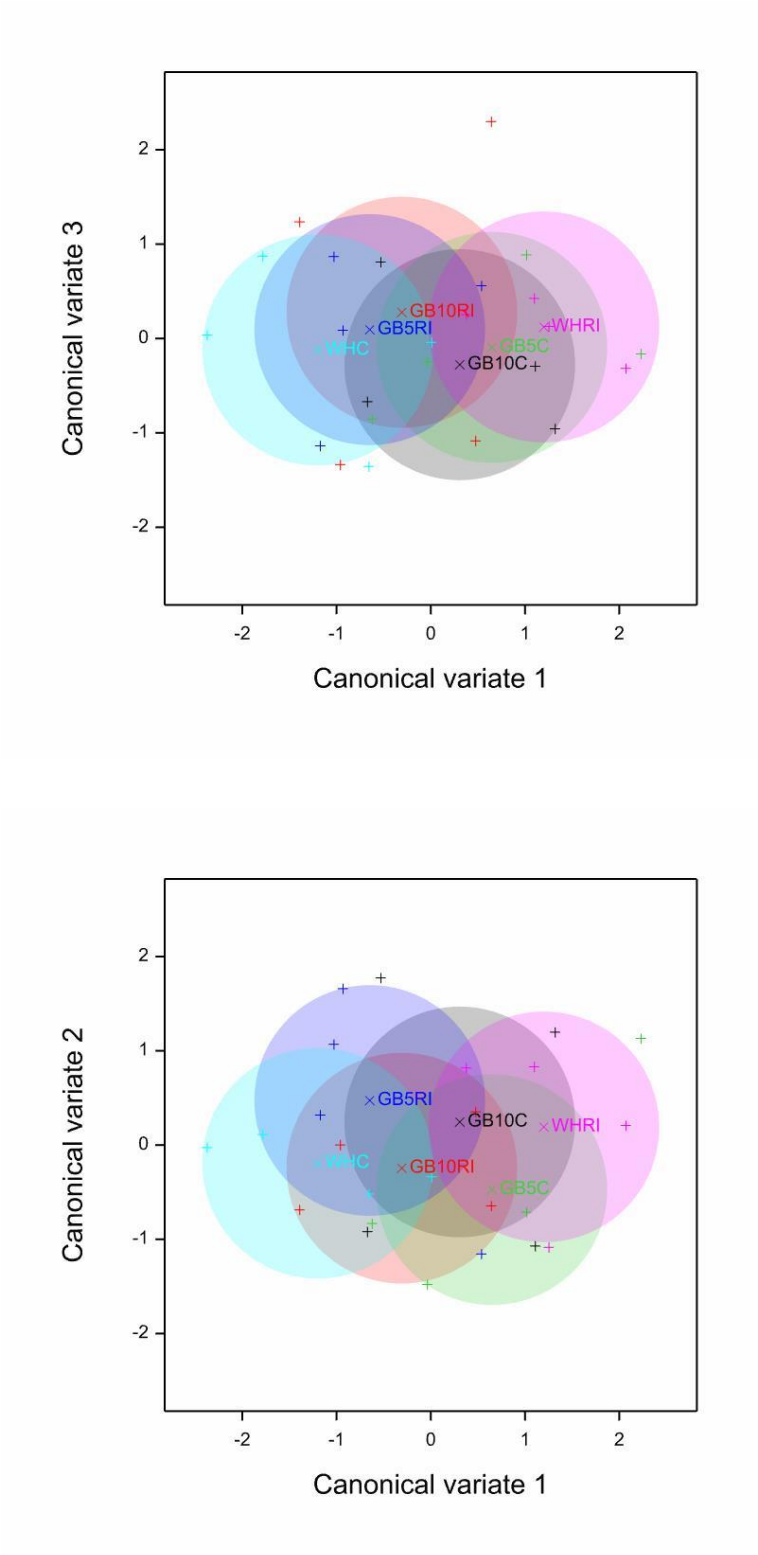


**Figure 41** **Separation of Pinot noir wines by site and treatment using canonical variate analysis based on monoterpenes, norisoprenoids and aldehyde concentrations. Circles represent 95% confidence intervals surrounding the means. Canonical variate 1 (CV1) and CV2 are plotted in the lower chart and CV1 and CV3 are plotted in the upper chart.**

**Abbreviations represent to a combination of site/treatment: WHC, Waipara Hills Control; WHRI, Waipara Hills Reduced Irrigation; GB5C, Greystone Block 5 Control; GB5RI, Greystone Block 5 Reduced irrigation; GB10C, Greystone Block 10 Control; GB10RI, Greystone Block 10 Reduced Irrigation. Each cross symbol correspond to a replicate for each site/treatment combination.**

As can be observed in Figure 41, CVA did not report a clear separation between sites and treatments for monoterpenes, norisoprenoids and aldehydes. All 95% confidence interval circles for the same site overlap each other, even when CV1 was plotted with CV3. CV1 explained 82.24% of the total variance, whereas CV2 13.51% and CV3 4.24%. None of them was statistically significant at $p < 0.05$.

The distance between CON and RI at WH was the largest among all the sites with 2.442, then GB5 with 1.615, and finally GB10 with 0.9626 (Table 91, Appendix D).

### 5.3.8 PCA on grapevine physiology, grapes and wine parameters

Principal component analysis (PCA) was performed on grapevine physiology, grapes, and wine evaluations using the same sets of data as in Figure 35 and including some wine parameters.

As can be observed in Figure 42, PC-1 explained 57.37% of the variability, while PC-2 20.76%. Both components accounted 78.13% of the total variability in the data.

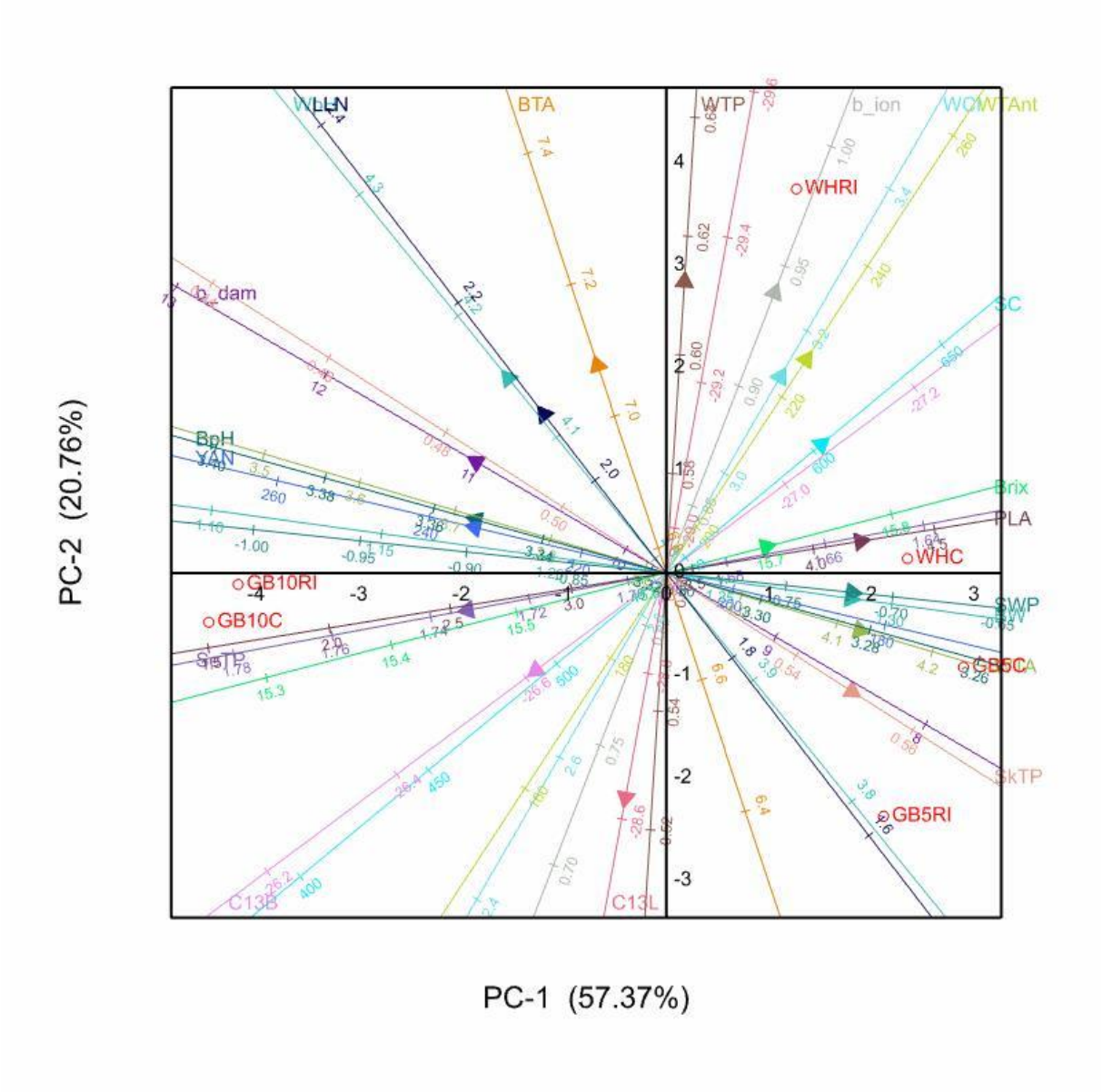


**Figure 42** **Principal component analysis (PCA) of grapevine physiology, berry, and wine parameter means in the 2013-2014 season. Each point of the PCA represents a combination of site/treatment: WHC, Waipara Hills Control; WHRI, Waipara Hills Reduced Irrigation; GB5C, Greystone Block 5 Control; GB5RI, Greystone Block 5 Reduced irrigation; GB10C, Greystone Block 10 Control; GB10RI, Greystone Block 10 Reduced Irrigation. Nomenclature used for parameters: PLA, primary leaf area; SWP, stem water potential; SC, stomatal conductance; LLN, leaf layer number; C13L, leaf**

**$\delta^{13}C$; BW, berry weight; Brix, grape juice Brix; BpH, berry juice pH; BTA, berry juice TA; SeTP, seed total phenolics; SkTP, skin total phenolics; YAN, yeast available nitrogen; C13B, grape juice $\delta^{13}C$; WpH, wine pH; WTA, wine TA; WTP, wine total phenolics; WTAnt, wine total anthocyanins; WCI, wine colour intensity; b-dam, wine β-Damascenone concentration; b-ion, wine β-Ionone concentration.**

There were clear differences between treatments for WH and GB5, whereas both GB10C and GB10RI were located very close each other, as in Figure 35 when only data of grapevine physiology and grapes were plotted. There was also a clear separation between sites with both WHC and WHRI being grouped in the right upper plan of the plot, GB5C and GB5RI in the lower right, and GB10C and GB10RI in the lower left plane of the plot.

Primary leaf area (PLA), Wine TA (WTA), stem water potential (SWP) and berry weight (BW) were positively correlated with PC-1, whereas this was negatively correlated with seed total phenolics (SeTP) and YAN. PC-2 was positively correlated with wine total phenolics (WTP) and negatively correlated with leaf $\delta^{13}C$ (C13L).

## 5.4 Discussion

### Wine pH and TA

Wine pH was not affected by RI at any site, with no differences between sites also being observed. Ledderhof *et al.* (2014) found no differences in wine pH in three out of four sites when comparing low and high vigour zones in 2009, which agrees with the lack of differences observed here between CON and RI at all sites. Sipiora *et al.* (2005) reported differences for a series of parameters between wines made from Pinot noir grapes under standard irrigation and those with supplemented irrigation (three times the standard irrigation). Their findings in the 1990 season indicated that the irrigation treatment had a significant effect on wine pH, with wines under supplemented irrigation showing higher pH than those from standard irrigation. These results are opposite than those found here.

Differences in wine TA were found at WH only, whereas this was unaltered by RI at GB5 and GB10. No differences in wine TA across sites were reported. Intrigliolo and Castel (2009) found that wines made with grapes of cv. Tempranillo under different partial rootzone drying (PRD) regimes did not show differences in TA over two seasons, similar to those at GB5 and GB10, but opposite to that at WH. No differences in wine TA were also reported by Sipiora *et al.* (2005) in a standard and

supplemented irrigation trial in Pinot noir, which underlined the small influence of the irrigation regime on wine TA.

Regarding spatial variability, the findings in this study reported no differences in wine pH and TA across sites, which agree with results of Imre *et al.* (2012), who did not observe differences in wine pH and TA in Pinot noir wines made from three different sites in Central Otago, New Zealand. However, although Imre *et al.* (2012) did not find spatial variability for wine TA and pH, their results compared wines produced from three vineyards within the same region, but did not consider the intervention of any of the factors affecting grapevine physiology, such as irrigation here, which complicates making a direct comparison of those data and the results described in this study.

### Wine tannin concentration by MCP

RI did not have an effect on wine tannin concentration at any site, but differences between sites were found. Imre *et al.* (2012) reported spatial variability for Pinot noir wine tannin concentration, where significant differences were found between three sites in Central Otago, with wines made from grapes grown in the most gravelly soil had the lowest tannin content when compared to those from clayey and coluvial schist gravelly soils, which is in agreement with the results found here where tannin content at WH, a sandy-gravelly soil, produced the wines with the lower tannin content among all sites.

Various factors have been demonstrated to have a significant effect on wine tannin concentration. Thus, Song *et al.* (2014) reported significant differences in Pinot noir wine tannin concentration between vine vigour levels, where wine tannin concentration increased as vine vigour declined. Song *et al.* (2015a) also demonstrated that bunch exposure and UV radiation had a significant effect on modifying wine tannin concentration. This is confirmed by Kemp *et al.* (2011), who demonstrated that leaf removal has shown to affect wine tannin concentration in Pinot noir grown in Waipara. However, the differences in primary leaf area found at GB5 (the only site showing differences between CON and RI), which could be linked to a higher bunch exposure in the vines under RI, did not result in a higher wine tannin concentration. This confirmed that there are other external factors influencing wine tannin concentration than water stress under field conditions.

It is known that different environmental factors can alter the concentration of tannin in the fruit, which are extracted during winemaking. However, wine tannin concentration can be highly influenced by the winemaking style and technique (Harbertson *et al.* 2008). As described in the previous chapter, seed and skin tannin concentration tended to decrease toward harvest. Hanlin *et al.* (2010) suggested that this decrease in tannin concentration through ripening is the result of

tannin association with cell wall material, which leads to reduce tannin extractability. Hence, grape tannin content is somewhat related to wine tannin concentration. The yeast strain selected for Pinot noir alcoholic fermentation can also modify wine tannin content, as described in Carew *et al.* (2013). In this study, all replicates were fermented using the same yeast strain and vinified following the same protocol, so the differences between sites described here can be attributed to environmental factors influencing this parameter under the conditions of each particular site.

### Wine colour by Somers' method

Differences due to irrigation treatment were found at WH only. Wine colour density, estimate of $SO_2$ resistant pigments, total red pigments, total phenolics and total anthocyanins were affected by RI at WH, whereas wine colour hue was the only parameter showing differences between sites.

Reduced irrigation has shown to affect wine colour under field conditions. In Cabernet Sauvignon, regulated deficit irrigation at different intensities affected wine colour parameters over three seasons in Australia (Edwards and Clingeleffer 2013). When compared to a control treatment, wines produced from vines under deficit irrigation showed consistently higher colour density, total anthocyanins, and total phenolics. In Pinot noir, irrigation regime has demonstrated to alter wine colour parameters. For example, Sipiora *et al.* (2005) studied the effect of different irrigation regimes combined with different potassium fertilization doses, reporting that over two seasons the fertilization treatments did not affect wine colour density and total anthocyanins, while the differences in irrigation had a significant effect on those parameters. Ledderhof *et al.* (2014) also investigated the effect of vine water status on a series of wine parameters across four sites over two seasons. They reported that total anthocyanins, wine colour density, and wine colour hue did not vary within the same site when compared low and high water status zones at different sites, which agree with the results found here at GB5 and GB10. Regarding spatial variability, Imre *et al.* (2012) observed differences in wine colour density, total red pigments, and monomeric pigments between wines made from three different sites in Central Otago. Cortell *et al.* (2007) also showed differences between two sites in Pinot noir wine colour density and hue. The results in this study showed differences across sites only for colour hue, which is in agreement the results of Cortell *et al.* (2007). Under the conditions of the 2013-2014 season, WH was the only site showing differences between CON and RI for wine colour variables by Somers' method, which coincide with the lower soil profile available water described for that site. This site also received more than double of the water applied to GB5 and GB10 by irrigation over the season, which confirmed that the effect of reducing irrigation by 50% on Pinot noir wine colour is higher in a lower water holding capacity soil, even when irrigated more often and using higher volumes of water.

**Wine colour by CIELab method**

As for wine colour by Somers' method, only WH showed differences between CON and RI for wine luminosity, chroma, blueness, and colour intensity. Among all parameters evaluated, only wine redness reported differences across sites.

This method was created to evaluate the chromatic characteristics of a wine attempting to imitate real observers with regards to their sensation of colour (International Organisation of Vine and Wine 2014). Unlike Somers' method, the CIELab method evaluate spectrophotometrically a series of wine colour parameters without the intervention of any chemical reagent, giving an approximation to the wine colour that can be potentially appreciated by an observer. This method has been used to differentiate the effect of leaf removal (Sternad Lemut *et al.* 2013b), microoxygenation (Durner *et al.* 2010), regulated deficit irrigation (Romero *et al.* 2013) and the vinification technique (Girard *et al.* 2001) on wine colour characteristics, among others.

Interestingly, both Somers' and CIELab methods reported differences in wine colour at WH only, whereas no colour parameters were affected by RI at GB5 and GB10. This confirmed that, under the conditions of the 2013-2014 season, the effect of RI on wine colour was more pronounced in the site having a lower soil profile available water, even with this being irrigated more often and using higher volumes of water than those at the other two sites/soils. In cv. Merlot, Chacón *et al.* (2009) found that a reduced irrigation treatment decreased the redness (a) and blueness (b) of wines, which are not in agreement with the results in this study as no differences in redness and blueness were found at GB5 and GB10, but blueness was increased by RI at WH. As previously described, this method has been extensively used to evaluate differences in wine colour, however, it is difficult to find literature reporting results specifically for Pinot noir under reduced irrigation. Thus, the results of Sternad Lemut *et al.* (2013b), although being from a leaf removal trial, show the differences in Pinot noir wine colour using the CIELab method. In that study, the authors indicated that early season leaf removal (pre-flowering and berry-set) significantly increased the dimension a (redness) in young Pinot noir wines. In addition, dimension b (blueness) was lower in wines made from non-defoliated vines and L (luminosity) was not affected by any defoliation treatment.

As discussed for wine colour by Somers' and CIELab methods, the effect of water stress on wine colour is linked to a reduction of leaf area in the cluster zone, which lead to a higher cluster exposure, having a direct impact on grape colour compound synthesis, and therefore, on wine colour. The results of Sternad Lemut *et al.* (2013b) also show the effect of increasing cluster exposure on Pinot noir wine colour, even when the cluster zone is manually defoliated. In this study, although no differences in primary leaf area were found at veraison, it is speculated that leaves in the cluster

zone at WH continued dropping over the season, which resulted in a higher bunch exposure, and therefore, altering wine colour parameters.

**Aroma compounds by GCMS**

Among the seven volatile acids included in this study, only 2-methybutanoic acid and isovaleric acid reported differences between CON and RI at WH, whereas none of them was affected by RI at GB5 and GB10. It is important to note that literature reporting aroma compound concentrations in New Zealand Pinot noir is limited, as indicated in the literature review. Thus, in the literature found (Imre *et al.* 2012, Rutan *et al.* 2014, Tomasino 2011), 2-Methylbutanoic acid has not previously been reported for New Zealand Pinot noir. Here, although this showed differences at WH, its concentration at all sites and treatments was about three times lower than its perception threshold (Guth 1997). On the other hand, isovaleric acid reported concentrations above the perception threshold described by Ferreira *et al.* (2000) and higher than those previously indicated for Central Otago Pinot noir by Rutan *et al.* (2014). As isovaleric acid has been described as having a parmesan-like aroma, its influence on wine sensory characteristics needs to be evaluated. The concentration of acetic acid did not show differences between CON and RI at any site and was similar to those found by Tomasino (2011) for Waipara Pinot noir. For all results reported here and elsewhere (Imre *et al.* 2012, Rutan *et al.* 2014, Tomasino 2011), acetic acid in wines from Waipara, Central Otago, Marlborough and Martinborough are above the perception threshold indicated by Guth (1997). All butanoic acid and hexanoic acid concentrations in this study were below the perception threshold, whereas those of isobutyric acid were greater than those described in the literature (Escudero *et al.* 2007). Depending on the site and treatment, octanoic acid showed concentrations either near or above its perception threshold. The effect of all these compounds, especially those found above their perception threshold should be evaluated, as these will likely impact wine sensory characteristics.

Ugliano and Henschke (2009) indicated that wine contains a mixture of straight chain fatty acids, usually referred to as short chain (C2–C4), medium chain (C6–C10), long chain (C12–C18), and a group of branched-chain fatty acids that include 2-Methyl propanoic, 2-Methyl butanoic, and 3-Methyl butanoic acids. Among them, acetic acid, a short-chain fatty acid (C2) is responsible for >90% of the total wine volatile acidity and plays an important role in wine quality (Robinson *et al.* 2014). Some volatile fatty acids such as hexanoic, octanoic, and decanoic acids can contribute to the aroma of some white wines (Ugliano and Henschke 2009), as well as other group of fatty acids like isobutyric, isovaleric, butyric and propanoic acids, but the role of these in wine characteristics is still under study (Robinson *et al.* 2014).

It is known that acetic acid is formed as a metabolic intermediate in the synthesis of acetyl-CoA from pyruvic acid (Robinson *et al.* 2014), while straight-chain fatty acids (C4–C12) are by-products of saturated fatty acid metabolism. In addition, branched-chain fatty acids, such as 2-Methylbutanoic acid in this study, are derived from oxidation of the aldehydes formed from α-keto acids during amino acid metabolism (Ugliano and Henschke 2009). As all wines in this research followed the same protocol, it is speculated that RI did not alter the metabolic pathway of most of the fatty acid precursors, which was reflected in the no differences between treatments at GB5 and GB10 and the minor differences at WH.

For alcohol concentrations, RI reduced phenylethyl alcohol concentration at WH and increased hexanol concentration at GB5, whereas no volatile alcohols were affected at GB10. Among the six alcohols considered in this research, 1-Heptanol, *cis*-3-Hexen-1-ol, hexanol and *trans*-3-Hexen-1-ol had concentrations below the perception threshold at all sites and treatments (Table 80). On the other hand, isoamyl alcohol and phenylethyl alcohol concentrations found in wines either from CON or RI were above their perception threshold (Table 80). Isoamyl alcohol has been described as a fusel-like aroma, while phenylethyl alcohol is said to have a floral aroma. As these were found above their perception thresholds, it is speculated that these will likely have an effect on wine sensory characteristics, and therefore should be evaluated. Compared to the concentrations of the same aroma compounds reported for New Zealand Pinot noir, 1-Heptanol concentration found here is in agreement with that reported by Tomasino (2011) for Waipara, Central Otago, Marlborough and Martinborough Pinot noir. The concentration of *cis*-3-Hexen-1-ol was also similar to that found by others in New Zealand Pinot noir (Imre *et al.* 2012, Rutan *et al.* 2014, Tomasino 2011). Hexanol concentrations are within the range of those reported by Tomasino (2011), but higher than those described in Imre *et al.* (2012) and Rutan *et al.* (2014). In addition, isoamyl alcohol and phenylethyl alcohol have been reported in New Zealand Pinot noir only by Rutan *et al.* (2014). Isoamyl alcohol concentration found in wines here was higher than that described in the literature, whereas that of phenylethyl alcohol was less than half of the concentration found in Central Otago Pinot noir. *Trans*-3-Hexen-1-ol concentration was similar to those reported by Tomasino (2011), but lower than those detected in Central Otago wines by Imre *et al.* (2012) and Rutan *et al.* (2014).

Higher alcohols are formed by decarboxylation and subsequent reduction of α-keto-acids produced as intermediates of amino acids biosynthesis and catabolism (Ugliano and Henschke 2009). For example, phenylethyl alcohol is produced from phenylalanine and tyrosine (Robinson *et al.* 2014), and therefore, differences in juice amino acid concentration may explain the differences in phenylethyl alcohol found here. However, despite fermentation conditions being controlled, there are many other factors such as yeast species and strain, initial sugar, fermentation temperature, the

pH and composition of grape juice, assimilable nitrogen, aeration, level of solids, grape variety and skin contact time that affect higher alcohols formation during fermentation, which make difficult to explain the differences found here. In addition, vineyard management has also demonstrated to alter higher alcohol concentration in Pinot noir. Thus, bunch sunlight exposure has been described as affecting hexanol concentration, where wines made from sun exposed bunches showed higher concentration of this compound, compared to those shaded (Song *et al.* 2015a). This agrees with the higher hexanol concentration found in wines from RI grapes at GB5, which were more exposed to sunlight due to the lower leaf area of those vines. Song *et al.* (2015a) also indicated that both sunlight exposure and UV exclusion did not have an effect on other alcohols such as isoamyl alcohol and heptanol. These results are similar to those reported by Feng *et al.* (2015), who indicated that a leaf removal treatment did not alter 1-Hexanol, *trans*-3-Hexenol and *cis*-3-Hexenol in Pinot noir wines produced over three seasons.

Esters, the biggest group of compounds of this study (15), reported differences for four of these compounds at WH and only one at GB5 and GB10. Ethyl acetate, ethyl butanoate and ethyl isovalerate, all of them reported above their perception threshold (Table 80), showed differences between CON and RI at WH. Ethyl heptanoate concentration was also different at WH, but this was detected below the perception threshold. Ethyl decanoate was the only ester compound showing differences between CON and RI at GB5 and GB10, but its concentration at GB10 was described as being below the perception threshold, as same as RI at GB5, while CON at GB5 was the only one among all sites and treatments reported above this. At all sites and treatments, ethyl acetate, ethyl butanoate, ethyl cinnamate, ethyl hexanoate, ethyl isobutyrate, ethyl isovalerate and isoamyl acetate concentrations were all found above their perception threshold, whereas 2-Phenylethyl acetate, ethyl heptanoate, ethyl hydrocinnamate, ethyl lactate and hexyl acetate were reported as below this. All other compounds showed a concentration either above or below the perception threshold depending on the site and treatment. Most esters reported in this study have been described in the literature as having fruity, sweet, or floral aromas, therefore, those found above their perception threshold could have a positive effect on Waipara Pinot noir sensory characteristics.

Compared to the ester concentrations reported in the literature for New Zealand Pinot noir, those of 2-Phenylethyl acetate, ethyl butanoate, ethyl isobutyrate, hexyl acetate and isoamyl acetate were higher than those described by Imre *et al.* (2012), Rutan *et al.* (2014) and Tomasino (2011). In contrast, ethyl isovalerate and ethyl lactate concentrations were lower than those reported in the literature. Concentrations of ethyl cinnamate, ethyl decanoate, ethyl heptanoate, ethyl hexanoate, ethyl hydrocinnamate, ethyl octanoate, and ethyl pentanoate were found to be within the range described for the same compounds in New Zealand Pinot noir. Finally, it is the first study reporting

ethyl acetate concentration for New Zealand Pinot noir. Its olfactory description has been defined as sweet fruity, and as this was found in all samples in much higher concentration than its perception threshold, it is speculated that this may play an important role in contributing to the fruitiness of Pinot noir produced in Waipara.

Esters are considered to be synthesised by yeast through lipid and acetyl-CoA metabolism (Robinson *et al.* 2014). However, as previously discussed for other compounds, there is a series of other factors affecting esters synthesis that influence their final concentration in wine. However, some research has been developed to determine the effect of viticultural practices on ester compounds in Pinot noir. For example, Song *et al.* (2014) found that vine vigour had an influence on final ester concentrations in Pinot noir wines. Thus, wines made with grapes from ultra-low vigour vines reported the highest concentrations of ethyl acetate, ethyl propanoate, ethyl pentanoate, whereas wines produced from high vigour vines produced the lowest concentration of ethyl butanoate. In the same study, the authors indicated that ethyl octanoate, ethyl nonanoate and ethyl decanoate were unaffected by the vine vigour. UV light and sunlight exposure have also showed to affect ester concentrations in Pinot noir wine (Song *et al.* 2015a). Thus, UV exclusion resulted in an increase of ethyl cinnamate, while this was not affected by bunch exposure.

Among the group of compound formed by monoterpenes (citronellol, geraniol and linalool), norisoprenoids (β-Damascenone and β-Ionone) and aldehydes (benzaldehyde), only the two norisoprenoids showed differences at WH, with GB5 and GB10 not reporting differences for any of these. All sites and treatments registered concentrations below the perception threshold for citronellol, geraniol, and benzaldehyde, whereas those of linalool and β-Damascenone were above this. For β-Ionone, only RI at WH reported a concentration higher than its perception threshold. In addition, concentrations of β-Damascenone and β-Ionone in all samples were higher than any reported in the literature for New Zealand Pinot noir (Imre *et al.* 2012, Rutan *et al.* 2014, Tomasino 2011). β-Damascenone and β-Ionone have been described as having floral aromas (rose, violets), therefore, it is speculated that the high concentrations of these compounds found in this study will likely have a positive impact on wine sensory characteristics, and therefore should be further investigated. Interestingly, benzaldehyde concentration at GB10 showed values about 2-fold higher than those described by Tomasino (2011) for Waipara Pinot noir, whereas these at WH and GB5 are within the range reported by the author. Likely due to methodological differences, the concentrations found for geraniol, linalool and β-Damascenone are either higher or lower than those found by other authors (Imre *et al.* 2012, Rutan *et al.* 2014).

Rusjan (2010) indicated that monoterpenes and $C_{13}$-norisoprenoids precursors are synthesised in the earlier phase of berry development. Red varieties are not characterised by having high levels of terpenes, with low concentrations of linalool, citronellol, nerol found in Cabernet Sauvignon grapes (Robinson *et al.* 2014). Geraniol, nerol, citronellol, linalool and α-Terpineol, are produced by various chemical, or possibly enzymatic transformation reactions, involving isomerisations, reductions and cyclisations, but these mechanisms are still under study (Ugliano and Henschke 2009). On the other hand, it is well known that norisoprenoids are synthesised from the biodegradation of carotenoids (Oliveira *et al.* 2003, Robinson *et al.* 2014). Song *et al.* (2014) reported that Pinot noir wines made with grapes from vines showing ultra-low vigour had higher linalool, nerol and geraniol concentrations compared to those from high vigour vines. This may be due to the higher sunlight exposure of grapes from ultra-low vigour vines. This is confirmed by González-Barreiro *et al.* (2015), who indicated that linalool appeared to be more sensitive to sunlight exposure than the other terpenol compounds. Norisoprenoids have also demonstrated to be responsive to grape sunlight exposure. Song *et al.* (2014) indicated that wines from ultra-low vigour vines reported higher concentration of β-Ionone related to more open canopies and greater fruit exposure. Carotenoids, norisoprenoids' precursors, have shown to be little sensitive to water stress in cv. Touriga nacional grapes, where the differences in soil water retention capacity demonstrated to be more important in determining carotenoids concentration at harvest (Oliveira *et al.* 2003). In this study, β-Damascenone and β-Ionone showed significant differences at WH only (the soil with lowest water retention capacity), with differences between sites found for β-Damascenone.

Tomasino (2011) and the two papers published from that thesis (Tomasino *et al.* 2015, Tomasino *et al.* 2013) are the only source of information available until now on the use of canonical analysis to differentiate Pinot noir wines from different wine regions in New Zealand. For this research, that methodology was adapted to differentiate wines from grapes under CON and RI at three different sites in Waipara. Similar to the analyses presented here, Tomasino (2011) used the data of volatile fatty acids to try to differentiate wines by region, concluding that this group of compounds were not appropriate markers to establish a good between regions separation. The findings in this study indicate that volatile fatty acids performed well in differentiating CON and RI at WH and GB5, but not very well in differentiating sites. In this case, esters was the best group in differentiating sites and treatments, obtaining the largest between group distances of all compounds considered in this analysis, so it is suggested to consider them as markers for site in future research.

Finally, as found for wine colour characteristics, most of the differences between CON and RI for aroma compounds were found at WH, suggesting that the impact of reducing irrigation by 50% on wine aroma compounds is more evident in a lower profile available water soil, even when irrigated

more often and using higher volumes of water than those sites having soils with higher profile available water.

## 5.5 Conclusions

Although only one season of data was obtained for wines, the RI treatment had a significant effect on most of the parameters evaluated at WH, whereas this tended not to alter wine composition at GB5 and GB10. Wine pH was the only parameter showing no differences between RI at any site, as well as no differences across sites.

Wines made from grapes under RI at WH had, in general, higher colour intensity, red pigments concentration, total phenolics and total anthocyanins content, as well as higher luminosity, chroma and blueness. All these can be associated to the better sunlight exposure of clusters in vines under RI, but it is important to note that this wine colour enhancement was reported only at WH, which may indicate that the effect of RI on colour compounds synthesis, such as anthocyanins, is more evident in a sandy-gravelly soil, compared to the two clayey-calcareous soils.

WH, the soil with the lower profile available water, also reported differences in aroma compound concentrations, with these being not detected at the other two sites. All groups of aroma compounds had differences between CON and RI at WH, where volatile esters highlighted as the group showing the higher number of compounds reporting differences between treatments. These results confirmed the observations of wine colour, where the RI treatment had a stronger influence on aroma compounds concentration in a sandy-gravelly soil, compared to those with higher profile available water.

Finally, when all groups of aroma compounds were used to separate sites and treatments by CVA, the volatile esters group performed the best in obtaining clear separations either within the same site or between sites, which suggests using these as markers for site effects would be advantageous in future research. PCA also confirmed a clear separation between sites when grapevine physiology, grape composition and wine data were integrated, which indicated that even when either no or little differences between treatments were found, the magnitude of these little changes was highly influenced by the site characteristics, soil profile available water being one of the most important.

# Chapter 6

# General conclusions and further research

Through the chapters, edapho-climatic differences across three vineyards in Waipara, as well as the impact of a 50% reduction of the irrigation on grapevine physiology, grape characteristics and wine composition were analysed over two seasons.

The three types of soils selected for this research had different physical and chemical characteristics, although the three vineyards were located relatively close each other. This highlighted the high soil variability in Waipara, which makes this a unique place for growing grapes as well as research into soil and site effects on grape and wine composition.

The climatic differences found within the area reported valuable information about Waipara that was unknown before this study. For example, the marked differences in wind speed between three weather stations, which matched those registered by the anemometers installed at the three sites, underlined the importance of considering this parameter for technical decisions, such as irrigation, as this is an important factor in determining evapotranspiration rate, and this water balance, in vineyards. For temperature, and therefore, GDD accumulation, differences and similarities were found among the three Pinot noir vineyards selected for this study. GB5 and GB10, both located within the same property, showed the biggest differences in monthly maximum temperature, minimum temperature and GDD accumulation, whereas WH and GB10, separated by more than 5 km, reported very similar results for all of these parameters evaluated. This data set can be used in the future to compare edapho-climatic information of Waipara to those from other wine regions in New Zealand to differentiate this place from the others in an either protected designation of origin or protected geographical indication system for New Zealand wine. In addition, as the concept of "terroir" involves human, climatic and soil factors (Van Leeuwen and Seguin 2006), the uniqueness of the wines produced at the three sites can be supported, in part, by the differences reported here.

The RI treatment produced a series of interesting physiological responses when evaluated under different field conditions. Primary leaf area abscission and stomatal closure were found as the first short term adaptive responses to water scarcity at any site either in a wet (2013-2014) or dry season (2014-2015). RI was not enough to trigger other physiological responses described in the literature, such as leaf proline accumulation and changes in leaf $\delta^{13}C$. The lack of treatment effect on leaf $\delta^{13}C$, together with limited differences in root carbohydrates may indirectly indicate that photosynthesis rate was also unaffected. Photosynthesis rate evaluations are recommended to be incorporated in

future studies due to its importance in carbon balance and its role in grapevine productivity. Also, even when no differences between CON and RI were reported within the same site, between sites differences came up confirming the concept of "terroir" and highlighting the importance of the site-related characteristics on grapevine physiological responses. The results also suggested the isohydric behaviour of Pinot noir when irrigation was reduced by 50% under field conditions over two seasons. Although stem water potential was used instead of leaf water potential, this study demonstrated that Pinot noir under water stress tended to maintain grapevine water status by reducing functional primary leaf area and closing stomata in order to face the unfavourable conditions. The adaptability of the vine to relatively severe changes to irrigation management is a highlight of this work. In the future, it is suggested to establish a trial specifically to confirm the isohydric behaviour of this variety following the protocols indicated in the literature, which will contribute to understanding Pinot noir performance under water stress conditions.

Unlike grapevine physiology, grape characteristics were less affected by the RI treatment. There was an overall reduction on berry weight, which needs to be economically evaluated as this is likely to impact the volume of wine obtained in the winery. This is important when the winegrowers sell their grapes to the wineries and their profit is calculated based on the tonnes of grapes they produce, although it may not be such a concern for winery-owned vineyards. WH, the site having the lower soil profile available water, and therefore, the highest irrigation frequency and volume, was the only site where RI had an effect on berry Brix and pH, which suggested the important role of water holding capacity on regulating physiological processes that can directly impact grape characteristics and, potentially, wine composition. It also suggests that the pattern of water availability, as well as the amount, may be important in determining vine response and fruit characteristics.

Interestingly, seed parameters like seed weight, seed dry weight and seed water content were little affected by RI, which may suggest that seeds of Pinot noir remain "isolated" from the rest of the berry after veraison, even under water stress. This needs further investigation. Also, this is likely the first time that taurine has been described in berries of *Vitis vinifera* L., which opens a new area for studying its synthesis pathway, physiological functions, etc. As a comment on the general state of grapevine physiology, differences between sites were found, which indicated that grape characteristics are highly influenced by the site where the vines are planted, confirming the concept of "terroir".

As previously explained, the volume of grapes in the 2014-2015 was not enough to make wine, so the results in that chapter correspond to only one season. However, some interesting results on the effect of RI on wine composition were obtained. WH was the site showing most of the differences in

wine composition between CON and RI, which agreed with the differences found for grape characteristics at the same site. Wine composition at GB5 and GB10, however, were little affected by the treatment. Wine TA, wine colour and aroma profile by GCMS were the analyses that showed differences between CON and RI at WH. These demonstrated the high impact on wine composition that a 50% reduction of the irrigation normally applied by the viticulture manager in a soil with moderate profile available water, compared to those described as having higher profile available water. Moreover, as some aroma compounds were found in concentrations above their perception thresholds, it is suggested to set a sensory evaluation of the wines, as these compounds will likely have an impact on wine sensory characteristics. Though this study reported a significant effect of RI on some wine composition, the results from only one season make it difficult to conclude what the real effect of the treatment and the variations across sites are. In future studies, depending on the absence of early spring frosts, it is suggested to evaluate this for at least two seasons to contrast the results under different conditions.

The results obtained during a wetter (2013-2014) and a dry season (2014-2015) confirmed the adaptive responses of Pinot noir under water stress across three different field conditions. These also highlighted the importance of soil water availability, a combination of soil characteristics and irrigation management, on determining grapevine performance once irrigation starts after water stored in the soil during the winter has been utilised. Finally, as proposed by Van Leeuwen and Seguin (2006): “irrigation is likely to modify terroir expression”. The findings in this study support such a theory, as irrigation is proposed to be one of the main factors in modifying terroir expression in Pinot noir in Waipara, especially in a site having a sandy-gravelly soil and frequent irrigation as that at WH.

# Appendix A

# Leaf area validation

### 6.1.1 Primary leaf area validation

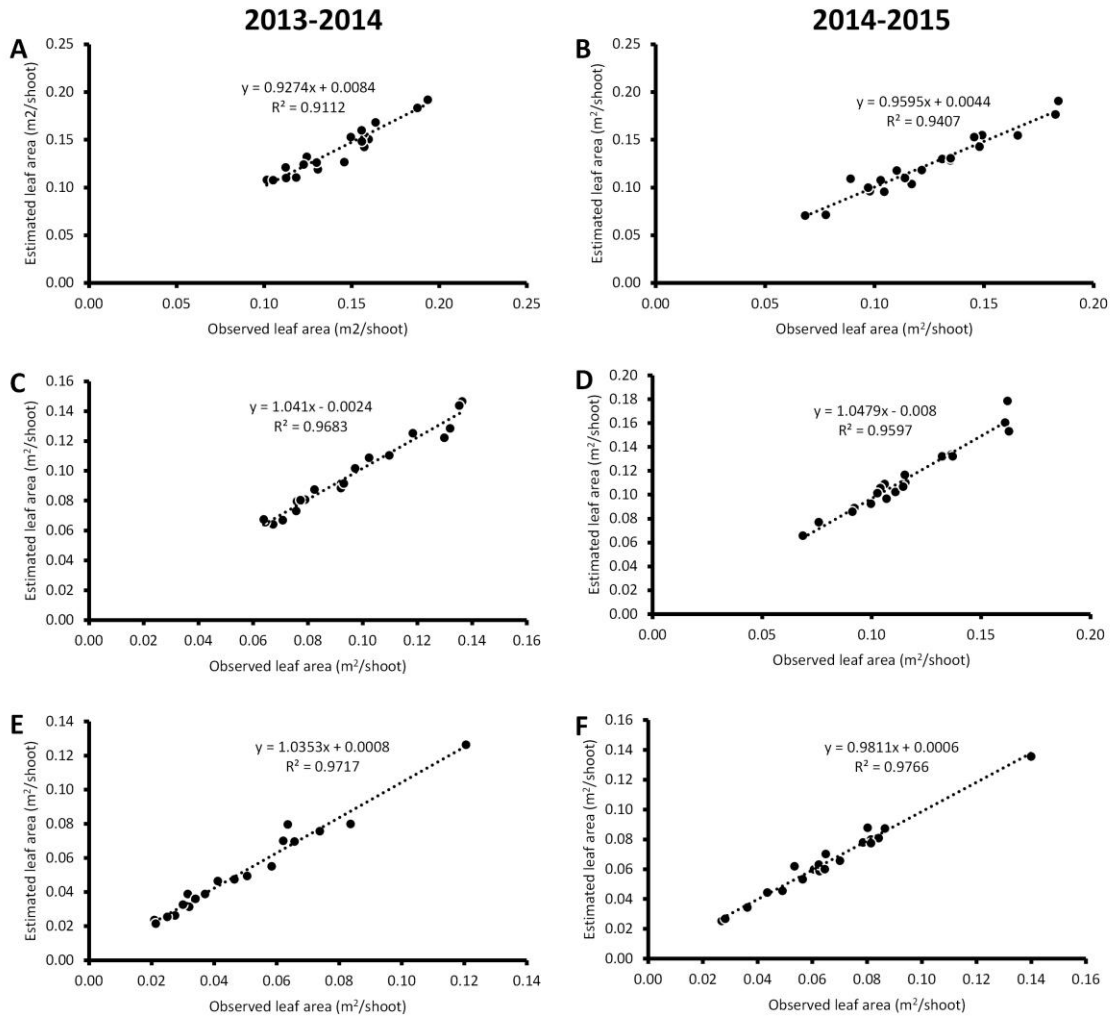


**Figure 43** **Primary leaf area validation in 2013-2014 and 2014-2015. Charts correspond to: WH (A, B), GB5 (C, D), GB10 (E, F).**

### 6.1.2 Lateral leaf area validation

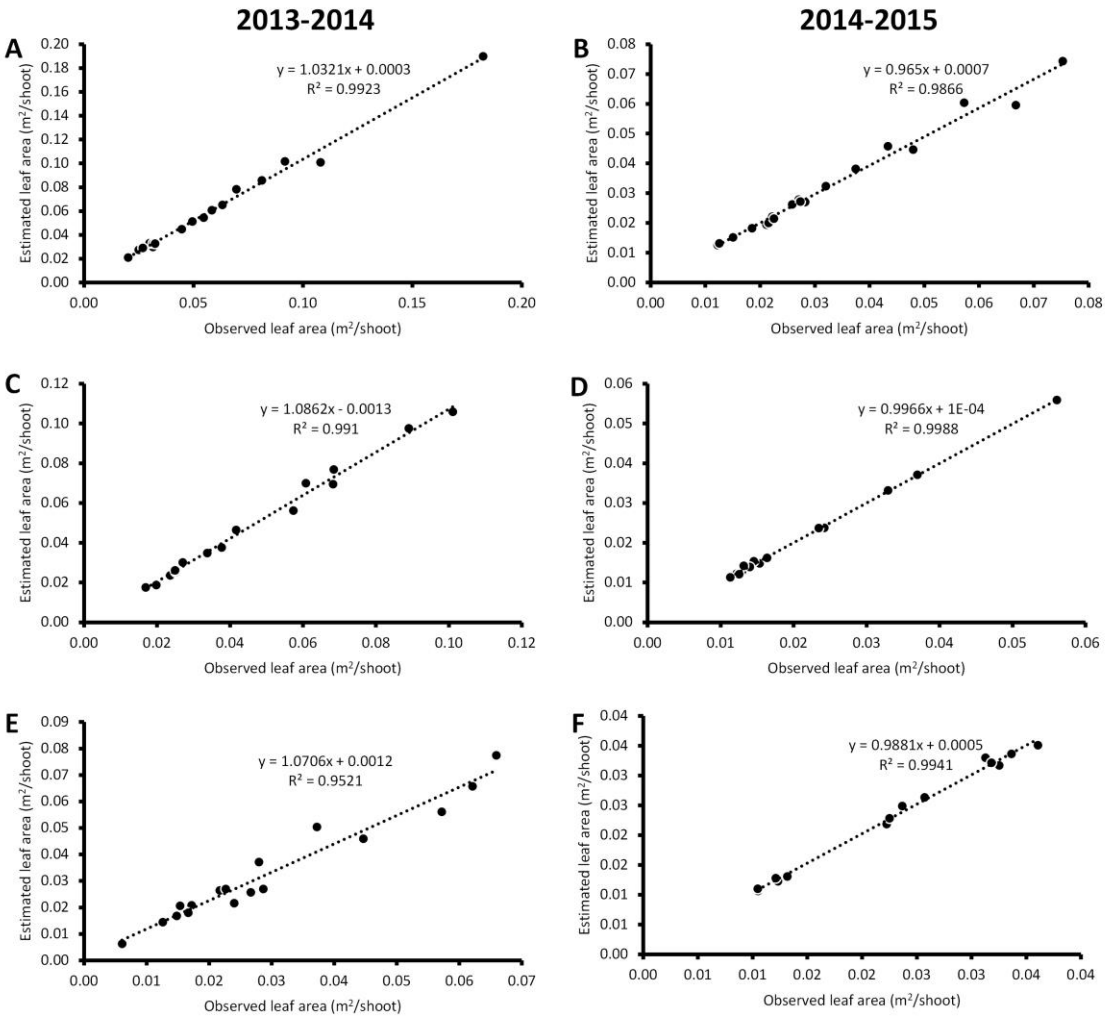


**Figure 44 Lateral leaf area validation in 2013-2014 and 2014-2015. Charts correspond to WH (A, B), GB5 (C, D), GB10 (E, F).**

# Appendix B

# Statistics Chapter 3

**Table 81** **Mean area under the curve, LSD 5%, and p values for leaf proline content, leaf osmotic potential, and estimated leaf chlorophyll content by SPAD meter (Chapter 3).**

| Evaluation | Treatment | 2013-2014 | | | 2014-2015 | | |
|---|---|---|---|---|---|---|---|
| | | WH | GB5 | GB10 | WH | GB5 | GB10 |
| Leaf proline content (Figure 20) | CON | 1.12 | 1.08 | 1.09 | 1.11 | 1.07 | 1.07 |
| | RI | 1.12 | 1.08 | 1.11 | 1.11 | 1.07 | 1.07 |
| | LSD 5% | 0.04 | 0.02 | 0.03 | 0.04 | 0.01 | 0.02 |
| | P value | 0.723 | 0.563 | 0.138 | 0.825 | 0.361 | 0.645 |
| Leaf osmotic potential ($\psi_\pi$) (Figure 21) | CON | -1.41 | -1.62 | -1.56 | -1.28 | -1.43 | -1.26 |
| | RI | -1.50 | -1.65 | -1.63 | -1.52 | -1.48 | -1.34 |
| | LSD 5% | 0.22 | 0.10 | 0.14 | 0.179 | 0.12 | 0.19 |
| | P value | 0.341 | 0.433 | 0.222 | **0.017** | 0.375 | 0.331 |
| Estimated leaf chlorophyll content by SPAD meter (Figure 22) | CON | 33.6 | 33.6 | 32.7 | 33.2 | 31.5 | 32.5 |
| | RI | 31.4 | 29.7 | 28.5 | 30.5 | 27.2 | 28.6 |
| | LSD 5% | 1.71 | 2.28 | 5.39 | 1.82 | 2.34 | 2.40 |
| | P value | **0.021** | **0.006** | 0.107 | **0.012** | **0.004** | **0.007** |

**Table 82 Results of the two-way ANOVAs on the effect of "site" and "treatment" for each season in Chapter 3 (grapevine physiology). Numbers correspond to means, LSD 5%, and p values.**

| Section | Evaluation/season | Site | | | | | Treatment | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | WH | GB5 | GB10 | LSD 5% | p value | CON | RI | LSD 5% | p value |
| Leaf area | Primary leaf area 2013-2014 | 4.33 | 4.22 | 1.63 | 1.44 | 0.023 | 3.80 | 2.98 | 1.18 | 0.095 |
| | Primary leaf area 2014-2015 | 3.95 | 3.09 | 2.19 | 1.91 | 0.113 | 4.06 | 2.10 | 1.56 | 0.033 |
| | Lateral leaf area 2013-2014 | 0.48 | 0.35 | 0.35 | 0.25 | 0.212 | 0.41 | 0.37 | 0.20 | 0.503 |
| | Lateral leaf area 2014-2015 | 0.34 | 0.30 | 0.29 | 0.26 | 0.722 | 0.45 | 0.16 | 0.21 | 0.027 |
| Point Quadrat | Percent gaps 2013-2014 | 4.32 | 5.68 | 5.00 | 8.42 | 0.805 | 4.55 | 5.46 | 6.88 | 0.626 |
| | Percent of gaps 2014-2015 | 7.20 | 12.12 | 9.85 | 20.14 | 0.643 | 3.54 | 15.91 | 16.44 | 0.084 |
| | Leaf layer number 2013-2014 | 1.92 | 1.64 | 2.13 | 1.25 | 0.414 | 2.01 | 1.78 | 1.02 | 0.436 |
| | Leaf layer number 2014-2015 | 1.86 | 2.16 | 2.16 | 1.99 | 0.788 | 2.70 | 1.41 | 1.62 | 0.076 |
| | Percent interior leaves 2013-2014 | 23.3 | 20.6 | 31.8 | 11.23 | 0.091 | 27.6 | 22.8 | 9.17 | 0.152 |
| | Percent interior leaves 2014-2015 | 19.1 | 25.1 | 26.4 | 37.89 | 0.719 | 34.1 | 13.0 | 30.93 | 0.099 |
| | Percent interior clusters 2013-2014 | 40.8 | 28.4 | 43.0 | 47.13 | 0.492 | 35.8 | 39.0 | 38.48 | 0.759 |
| | Percent interior clusters 2014-2015 | 20.8 | 38.2 | 28.7 | 47.91 | 0.452 | 47.0 | 11.4 | 39.12 | 0.059 |
| Stem water potential ($\psi_s$) | $\psi_s$ Fruit-set 2013-2014 | -0.22 | -0.28 | -0.29 | 0.11 | 0.176 | -0.28 | -0.25 | 0.09 | 0.251 |
| | $\psi_s$ Fruit-set 2014-2015 | -0.29 | -0.26 | -0.32 | 0.22 | 0.568 | -0.30 | -0.29 | 0.18 | 0.860 |
| | $\psi_s$ veraison 2013-2014 | -0.69 | -0.72 | -1.01 | 0.32 | 0.082 | -0.75 | -0.87 | 0.26 | 0.182 |
| | $\psi_s$ veraison 2014-2015 | -0.77 | -0.99 | -0.94 | 0.39 | 0.237 | -0.82 | -0.98 | 0.32 | 0.172 |
| | $\psi_s$ pre-harvest 2013-2014 | -0.41 | -0.57 | -0.64 | 0.23 | 0.087 | -0.53 | -0.55 | 0.19 | 0.800 |
| | $\psi_s$ pre-harvest 2014-2015 | -0.91 | -0.89 | -0.79 | 0.42 | 0.536 | -0.77 | -0.96 | 0.34 | 0.147 |

| Section | Evaluation/season | Site | | | | | Treatment | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | WH | GB5 | GB10 | LSD 5% | p value | CON | RI | LSD 5% | p value |
| Stomatal conductance ($g_s$) | $g_s$ veraison 2013-2014 | 623.7 | 564.9 | 439.4 | 264.2 | 0.175 | 586.8 | 498.5 | 215.7 | 0.220 |
| | $g_s$ veraison 2014-2015 | 377.6 | 392.0 | 398.2 | 187.0 | 0.894 | 482.5 | 296.1 | 152.7 | 0.034 |
| | $g_s$ pre-harvest 2013-2014 | 548.4 | 488.7 | 310.1 | 102.3 | 0.018 | 484.5 | 413.6 | 83.6 | 0.068 |
| | $g_s$ pre-harvest 2014-2015 | 337.1 | 221.8 | 304.2 | 105.2 | 0.078 | 348.9 | 226.5 | 85.9 | 0.026 |
| Leaf osmotic potential ($\psi_\pi$) | $\psi_\pi$ 2013-2014 | -1.45 | -1.64 | -1.60 | 0.10 | 0.026 | -1.53 | -1.60 | 0.08 | 0.066 |
| | $\psi_\pi$ 2014-2015 | -1.40 | -1.46 | -1.30 | 0.32 | 0.309 | -1.32 | -1.45 | 0.26 | 0.176 |
| Estimated leaf chlorophyll content by SPAD meter | Estimated leaf chlorophyll content by SPAD 2013-14 | 32.5 | 31.7 | 30.6 | 3.3 | 0.246 | 33.3 | 29.9 | 2.7 | 0.032 |
| | Estimated leaf chlorophyll content by SPAD 2014-15 | 31.8 | 29.3 | 30.6 | 2.6 | 0.105 | 32.4 | 28.8 | 2.1 | 0.018 |
| Leaf proline content | Leaf proline 2013-2014 | 1.12 | 1.08 | 1.10 | 0.04 | 0.073 | 1.10 | 1.10 | 0.03 | 0.416 |
| | Leaf proline 2014-2015 | 1.11 | 1.07 | 1.07 | 0.02 | 0.013 | 1.08 | 1.08 | 0.01 | 0.832 |
| Leaf $\delta^{13}C$ | Leaf $\delta^{13}C$ 2013-2014 | -29.24 | -28.75 | -28.82 | 2.30 | 0.676 | -28.88 | -29.00 | 1.88 | 0.817 |
| | Leaf $\delta^{13}C$ 2014-2015 | -29.07 | -28.91 | -28.02 | 1.38 | 0.139 | -28.71 | -28.63 | 1.13 | 0.791 |
| Root Carbohydrates | Root water content | 46.25 | 46.12 | 47.38 | 1.32 | 0.090 | 47.08 | 46.08 | 1.08 | 0.057 |
| | Root soluble sugars | 2.5 | 2.9 | 3.0 | 0.3 | 0.032 | 2.8 | 2.8 | 0.2 | 0.501 |
| | Root starch | 11.9 | 9.0 | 9.9 | 1.0 | 0.011 | 10.3 | 10.3 | 0.8 | 0.809 |
| Pruning weight | Pruning weight | 1.00 | 0.45 | 0.36 | 0.19 | 0.008 | 0.66 | 0.54 | 0.16 | 0.075 |

**Table 83 Results of the combined analysis in Chapter 3 (grapevine physiology). Numbers correspond to means, LSD 5%, and p values. Only results including the two seasons are presented.**

| Section | Evaluation | Site | | | | | Treatment | | | | Season | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | WH | GB5 | GB10 | LSD 5% | P value | CON | RI | LSD 5% | p value | CON | RI | LSD 5% | p value |
| Leaf area | Primary leaf area | 4.14 | 3.66 | 1.91 | 1.57 | **0.046** | 3.93 | 2.54 | 1.28 | **0.043** | -0.25 | +0.88 | 1.04 | **0.042** |
| | Lateral leaf area | 0.41 | 0.32 | 0.32 | 0.25 | 0.388 | 0.43 | 0.27 | 0.21 | 0.075 | -0.04 | +0.21 | 0.02 | **<.001** |
| Point Quadrat | Percent gaps | 5.76 | 8.90 | 7.43 | 13.25 | 0.657 | 4.04 | 10.68 | 10.82 | 0.118 | +1.01 | -10.45 | 12.92 | 0.062 |
| | Leaf layer number | 1.89 | 1.90 | 2.14 | 1.61 | 0.775 | 2.36 | 1.60 | 1.32 | 0.131 | -0.69 | +0.37 | 0.64 | **0.019** |
| | Percent interior leaves | 21.2 | 22.8 | 29.1 | 24.36 | 0.481 | 30.8 | 17.9 | 19.89 | 0.107 | -6.5 | +9.8 | 22.36 | 0.088 |
| | Percent interior clusters | 30.8 | 33.3 | 35.8 | 42.63 | 0.886 | 41.4 | 25.2 | 34.81 | 0.183 | -11.2 | +27.6 | 34.27 | **0.040** |
| Stem water potential ($\psi_s$) | $\psi_s$ Fruit-set | -0.25 | -0.27 | -0.31 | 0.15 | 0.403 | -0.29 | -0.27 | 0.12 | 0.529 | +0.02 | +0.04 | 0.16 | 0.560 |
| | $\psi_s$ veraison | -0.73 | -0.85 | -0.97 | 0.19 | 0.065 | -0.78 | -0.92 | 0.16 | 0.064 | +0.08 | +0.11 | 0.49 | 0.795 |
| | $\psi_s$ pre-harvest | -0.66 | -0.73 | -0.66 | 0.26 | 0.552 | -0.65 | -0.75 | 0.21 | 0.185 | +0.24 | +0.41 | 0.35 | 0.170 |
| Stomatal conductance ($g_s$) | $g_s$ veraison | 500.7 | 478.5 | 418.8 | 225.53 | 0.434 | 534.6 | 397.3 | 184.14 | 0.085 | +104.3 | +202.4 | 63.58 | **0.022** |
| | $g_s$ pre-harvest | 442.7 | 355.2 | 307.1 | 34.51 | **0.007** | 416.7 | 320.0 | 28.18 | **0.005** | +135.5 | +187.1 | 159.8 | 0.300 |
| Leaf osmotic potential ($\psi_\pi$) | $\psi_\pi$ | -1.42 | -1.55 | -1.45 | 0.20 | 0.208 | -1.43 | -1.52 | 0.16 | 0.129 | -0.21 | -0.15 | 0.20 | 0.349 |
| Estimated leaf chlorophyll content | Estimated leaf chlorophyll by SPAD meter | 32.2 | 30.5 | 30.6 | 2.92 | 0.206 | 32.8 | 29.3 | 2.38 | **0.024** | +0.9 | +1.1 | 0.97 | 0.435 |
| Leaf proline content | Leaf proline content | 1.11 | 1.07 | 1.08 | 0.01 | **0.012** | 1.09 | 1.09 | 0.01 | 0.340 | +0.01 | +0.02 | 0.04 | 0.490 |
| Leaf $\delta^{13}C$ | Leaf $\delta^{13}C$ | -29.16 | -28.83 | -28.42 | 0.64 | 0.076 | -28.79 | -28.81 | 0.52 | 0.897 | -0.17 | -0.37 | 2.92 | 0.801 |

# Appendix C

## Statistics Chapter 4

**Table 84** **Mean area under the curve, LSD 5%, and p values for berry weight, seed fresh weight, seed dry weight, seed water content, Brix, pH, TA, seed tannin concentration, seed total phenolics, skin tannin concentration, and skin total phenolics (Chapter 4).**

| Evaluation | Treatment | 2013-2014 | | | 2014-2015 | | |
|---|---|---|---|---|---|---|---|
| | | WH | GB5 | GB10 | WH | GB5 | GB10 |
| Berry weight (Figure 23) | CON | 1.32 | 1.35 | 1.16 | 1.60 | 1.40 | 1.36 |
| | RI | 1.24 | 1.27 | 1.05 | 1.36 | 1.08 | 1.08 |
| | LSD 5% | 0.09 | 0.08 | 0.09 | 0.13 | 0.31 | 0.29 |
| | P value | 0.081 | **0.040** | **0.021** | **0.003** | **0.044** | 0.057 |
| Seed fresh weight (Figure 24) | CON | 37.08 | 40.33 | 34.85 | 45.89 | 47.17 | 52.01 |
| | RI | 36.10 | 38.83 | 33.30 | 42.99 | 42.25 | 47.17 |
| | LSD 5% | 2.81 | 2.69 | 2.22 | 3.40 | 7.19 | 7.63 |
| | P value | 0.426 | 0.224 | 0.139 | 0.082 | 0.145 | 0.171 |
| Seed dry weight (Figure 25) | CON | 19.81 | 22.64 | 18.25 | 26.90 | 28.94 | 31.08 |
| | RI | 19.37 | 21.81 | 17.20 | 25.58 | 26.57 | 28.99 |
| | LSD 5% | 1.92 | 1.14 | 0.87 | 1.75 | 4.26 | 3.54 |
| | P value | 0.592 | 0.126 | **0.026** | 0.115 | 0.223 | 0.199 |
| | CON | 46.3 | 43.5 | 47.4 | 40.8 | 38.1 | 39.7 |

| Evaluation | Treatment | 2013-2014 | | | 2014-2015 | | |
|---|---|---|---|---|---|---|---|
| | | WH | GB5 | GB10 | WH | GB5 | GB10 |
| Seed water content (Figure 26) | RI | 46.1 | 43.3 | 47.8 | 40.0 | 36.6 | 37.5 |
| | LSD 5% | 2.3 | 1.8 | 2.0 | 2.0 | 1.2 | 4.7 |
| | P value | 0.830 | 0.885 | 0.636 | 0.315 | **0.017** | 0.286 |
| Brix (Figure 27) | CON | 15.0 | 15.8 | 15.1 | 14.8 | 17.4 | 16.6 |
| | RI | 16.1 | 16.2 | 15.3 | 16.5 | 18.8 | 17.8 |
| | LSD 5% | 0.9 | 1.5 | 1.4 | 0.9 | 1.5 | 1.8 |
| | P value | **0.025** | 0.056 | 0.736 | **0.005** | 0.056 | 0.141 |
| pH (Figure 28) | CON | 3.27 | 3.29 | 3.41 | 3.31 | 3.26 | 3.34 |
| | RI | 3.31 | 3.25 | 3.37 | 3.39 | 3.35 | 3.34 |
| | LSD 5% | 0.07 | 0.10 | 0.10 | 0.06 | 0.15 | 0.07 |
| | P value | 0.157 | 0.384 | 0.418 | **0.014** | 0.194 | 0.904 |
| Titratable acidity (TA) (Figure 29) | CON | 7.2 | 6.0 | 6.8 | 8.7 | 8.4 | 8.7 |
| | RI | 7.2 | 6.5 | 7.1 | 8.6 | 7.8 | 8.1 |
| | LSD 5% | 0.5 | 1.1 | 0.7 | 0.4 | 0.8 | 1.1 |
| | P value | 0.997 | 0.341 | 0.317 | 0.556 | 0.107 | 0.227 |
| Seed tannin content (Figure 30) | CON | 152.3 | 151.3 | 154.3 | 155.4 | 152.2 | 147.1 |
| | RI | 152.8 | 151.7 | 155.9 | 153.3 | 151.1 | 151.0 |
| | LSD 5% | 5.6 | 8.5 | 7.2 | 7.8 | 6.9 | 6.9 |
| | P value | 0.848 | 0.918 | 0.608 | 0.520 | 0.728 | 0.213 |
| | CON | 1.653 | 1.649 | 1.784 | 1.466 | 1.465 | 1.436 |

| Evaluation | Treatment | 2013-2014 | | | 2014-2015 | | |
|---|---|---|---|---|---|---|---|
| | | WH | GB5 | GB10 | WH | GB5 | GB10 |
| Seed total phenolics (Figure 31) | RI | 1.645 | 1.643 | 1.779 | 1.426 | 1.410 | 1.430 |
| | LSD 5% | 0.036 | 0.069 | 0.090 | 0.059 | 0.053 | 0.081 |
| | P value | 0.612 | 0.850 | 0.890 | 0.145 | 0.045 | 0.876 |
| Skin tannin content (Figure 32) | CON | 1.8 | 2.0 | 1.5 | 2.1 | 2.7 | 1.4 |
| | RI | 1.5 | 2.1 | 1.5 | 1.8 | 2.1 | 1.3 |
| | LSD 5% | 0.2 | 0.2 | 0.3 | 0.5 | 0.3 | 0.2 |
| | P value | **0.012** | 0.690 | 0.758 | 0.168 | **0.002** | 0.656 |
| Skin total phenolics (Figure 33) | CON | 0.531 | 0.564 | 0.465 | 0.499 | 0.571 | 0.398 |
| | RI | 0.510 | 0.571 | 0.467 | 0.434 | 0.502 | 0.416 |
| | LSD 5% | 0.042 | 0.064 | 0.093 | 0.105 | 0.079 | 0.073 |
| | P value | 0.266 | 0.791 | 0.964 | 0.183 | 0.078 | 0.567 |

**Table 85 Results of the two-way ANOVAs on the effect of "site" and "treatment" for each season in Chapter 4 (grapes). Numbers correspond to means, LSD 5%, and p values.**

| Section | Evaluation | Site | | | | | Treatment | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | WH | GB5 | GB10 | LSD 5% | p value | CON | RI | LSD 5% | p value |
| Berry parameters | Berry weight 2013-2014 | 1.28 | 1.31 | 1.11 | 0.05 | **0.006** | 1.28 | 1.19 | 0.04 | **0.012** |
| | Berry weight 2014-2015 | 1.48 | 1.24 | 1.22 | 0.12 | **0.019** | 1.45 | 1.17 | 0.10 | **0.007** |
| | Seed fresh weight 2013-2014 | 36.59 | 39.58 | 34.08 | 0.96 | **0.003** | 37.42 | 36.08 | 0.78 | **0.018** |
| | Seed fresh weight 2014-2015 | 44.44 | 44.71 | 49.59 | 3.49 | **0.038** | 48.36 | 44.14 | 2.85 | **0.024** |
| | Seed dry weight 2013-2014 | 19.59 | 22.22 | 17.72 | 0.93 | **0.005** | 20.23 | 19.46 | 0.76 | **0.048** |
| | Seed dry weight 2014-2015 | 26.24 | 27.75 | 30.03 | 1.65 | **0.020** | 28.97 | 27.05 | 1.35 | **0.026** |
| | Seed water content 2013-2014 | 46.2 | 43.4 | 47.6 | 1.01 | **0.006** | 45.7 | 45.7 | 0.83 | 0.884 |
| | Seed water content 2014-2015 | 40.4 | 37.4 | 38.6 | 2.05 | **0.047** | 39.6 | 38.0 | 1.68 | 0.057 |
| Brix, pH, and TA | Brix 2013-2014 | 15.55 | 16.00 | 15.20 | 1.44 | 0.258 | 15.30 | 15.87 | 1.17 | 0.173 |
| | Brix 2014-2015 | 15.65 | 18.10 | 17.20 | 0.77 | **0.010** | 16.27 | 17.70 | 0.63 | **0.010** |
| | pH 2013-2014 | 3.29 | 3.27 | 3.39 | 0.14 | 0.114 | 3.32 | 3.31 | 0.12 | 0.667 |
| | pH 2014-2015 | 3.35 | 3.31 | 3.34 | 0.15 | 0.521 | 3.30 | 3.36 | 0.12 | 0.185 |
| | TA 2013-2014 | 7.20 | 6.25 | 6.95 | 0.77 | 0.061 | 6.67 | 6.93 | 0.63 | 0.208 |
| | TA 2014-2015 | 8.65 | 8.10 | 8.40 | 0.88 | 0.216 | 8.60 | 8.17 | 0.72 | 0.122 |
| YAN | PAAN 2013-2014 | 142.50 | 130.25 | 208.25 | 117.9 | 0.176 | 156.50 | 164.17 | 96.3 | 0.765 |
| | PAAN 2014-2015 | 269.90. | 193.40 | 206.25 | 170.5 | 0.319 | 231.60 | 214.77 | 139.2 | 0.655 |
| | AN 2013-2014 | 44.35 | 47.00 | 54.10 | 8.09 | 0.065 | 48.97 | 48.00 | 6.60 | 0.593 |
| | AN 2014-2015 | 40.55 | 35.95 | 39.30 | 10.42 | 0.341 | 40.17 | 37.03 | 8.51 | 0.254 |

| Section | Evaluation | Site | | | | | Treatment | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | WH | GB5 | GB10 | LSD 5% | p value | CON | RI | LSD 5% | p value |
| | YAN 2013-2014 | 186.85 | 177.30 | 262.35 | 125.1 | 0.163 | 205.50 | 212.17 | 102.1 | 0.805 |
| | YAN 2014-2015 | 310.45 | 229.35 | 245.55 | 172.5 | 0.304 | 271.77 | 251.80 | 140.8 | 0.604 |
| MCP seeds | Seed tannin concentration 2013-2014 | 152.5 | 151.5 | 155.1 | 2.06 | **0.032** | 152.6 | 153.5 | 1.69 | 0.175 |
| | Seed tannin concentration 2014-2015 | 154.4 | 151.7 | 149.1 | 9.9 | 0.273 | 151.6 | 151.8 | 8.1 | 0.906 |
| | Seed total phenolics 2013-2014 | 1.649 | 1.646 | 1.781 | 0.004 | **<.001** | 1.695 | 1.689 | 0.004 | **0.017** |
| | Seed total phenolics 2014-2015 | 1.446 | 1.437 | 1.433 | 0.077 | 0.798 | 1.455 | 1.422 | 0.063 | 0.149 |
| MCP Skins | Skin tannin concentration 2013-2014 | 1.6 | 2.0 | 1.5 | 0.5 | 0.083 | 1.8 | 1.7 | 0.4 | 0.617 |
| | Skin tannin concentration 2014-2015 | 1.9 | 2.4 | 1.4 | 0.8 | 0.061 | 2.0 | 1.7 | 0.7 | 0.189 |
| | Skin total phenolics 2013-2014 | 0.520 | 0.567 | 0.466 | 0.046 | **0.021** | 0.520 | 0.516 | 0.037 | 0.691 |
| | Skin total phenolics 2014-2015 | 0.466 | 0.536 | 0.407 | 0.149 | 0.126 | 0.489 | 0.451 | 0.122 | 0.307 |
| $\delta^{13}$C juice | $\delta^{13}$C juice 2013-2014 | -27.35 | -26.72 | -26.38 | 1.26 | 0.151 | -27.04 | -26.59 | 1.03 | 0.203 |
| | $\delta^{13}$C juice 2014-2015 | -25.35 | -25.03 | -26.28 | 1.57 | 0.191 | -25.88 | -25.23 | 1.57 | 0.217 |
| Amino acids 2013-2014 | ASP | 247 | 155 | 298 | 175 | 0.136 | 232 | 234 | 143 | 0.949 |
| | GLU | 225 | 297 | 357 | 220 | 0.229 | 288 | 297 | 179 | 0.850 |
| | CYS | 0 | 10 | 9 | 48 | 0.673 | 7 | 6 | 39 | 0.933 |
| | ASN | 6 | 30 | 52 | 62 | 0.167 | 29 | 29 | 51 | 0.976 |
| | SER | 580 | 600 | 787 | 436 | 0.283 | 651 | 661 | 356 | 0.915 |
| | GLN | 592 | 754 | 1,089 | 1,037 | 0.311 | 894 | 730 | 847 | 0.491 |
| | HIS | 171 | 153 | 128 | 119 | 0.451 | 163 | 139 | 97 | 0.388 |
| | GLY | 28 | 41 | 46 | 34 | 0.257 | 38 | 39 | 28 | 0.815 |

| Section | Evaluation | Site | | | | | Treatment | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | WH | GB5 | GB10 | LSD 5% | p value | CON | RI | LSD 5% | p value |
| Amino acids 2014-2015 | THR | 1,046 | 1,180 | 1,420 | 986 | 0.422 | 1,219 | 1,212 | 805 | 0.975 |
| | ARG | 4,257 | 3,908 | 4,815 | 4,481 | 0.722 | 4,397 | 4,256 | 3,659 | 0.883 |
| | ALA | 1,764 | 1,831 | 2,322 | 1,529 | 0.405 | 2,025 | 1,919 | 1,248 | 0.750 |
| | TAU | 1,123 | 1,212 | 1,334 | 633 | 0.491 | 1,220 | 1,226 | 517 | 0.970 |
| | TYR | 8 | 35 | 11 | 23 | 0.061 | 14 | 22 | 19 | 0.198 |
| | VAL | 243 | 395 | 481 | 349 | 0.185 | 379 | 367 | 285 | 0.875 |
| | MET | 74 | 113 | 109 | 116 | 0.442 | 101 | 97 | 95 | 0.864 |
| | TRP | 73 | 82 | 64 | 81 | 0.671 | 78 | 68 | 66 | 0.572 |
| | PHE | 96 | 148 | 229 | 123 | 0.083 | 166 | 149 | 100 | 0.531 |
| | ILE | 167 | 291 | 385 | 298 | 0.167 | 289 | 273 | 244 | 0.813 |
| | LYS | 84 | 53 | 37 | 84 | 0.249 | 67 | 50 | 69 | 0.399 |
| | LEU | 207 | 354 | 464 | 389 | 0.197 | 353 | 330 | 317 | 0.788 |
| | PRO | 1,852 | 3,250 | 2,321 | 1,110 | 0.062 | 2,420 | 2,529 | 906 | 0.656 |
| | ASP | 164 | 108 | 164 | 243 | 0.605 | 157 | 135 | 198 | 0.677 |
| | GLU | 406 | 218 | 399 | 112 | **0.029** | 340 | 342 | 91 | 0.932 |
| | CYS | n.r | n.r | n.r | n.r | n.r | n.r | n.r | n.r | n.r |
| | ASN | 17 | 2 | 17 | 67 | 0.623 | 16 | 8 | 54 | 0.587 |
| | SER | 750 | 617 | 678 | 697 | 0.747 | 702 | 662 | 569 | 0.789 |
| | GLN | 1,088 | 901 | 1,064 | 2,276 | 0.931 | 1,218 | 817 | 1,858 | 0.451 |
| | HIS | 294 | 293 | 267 | 149 | 0.714 | 295 | 275 | 121 | 0.549 |

| Section | Evaluation | Site | | | | | Treatment | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | WH | GB5 | GB10 | LSD 5% | p value | CON | RI | LSD 5% | p value |
| | GLY | 53 | 39 | 35 | 49 | 0.440 | 45 | 40 | 40 | 0.681 |
| | THR | 1,682 | 1,402 | 1,467 | 1,604 | 0.764 | 1,559 | 1,475 | 1,310 | 0.809 |
| | ARG | 4,869 | 3,101 | 3,983 | 5,806 | 0.538 | 4,218 | 3,751 | 4,741 | 0.713 |
| | ALA | 2,721 | 1,846 | 2,060 | 2,493 | 0.447 | 2,423 | 1,994 | 2,035 | 0.460 |
| | TAU | 1,566 | 898 | 1,349 | 918 | 0.164 | 1,271 | 1,270 | 750 | 0.995 |
| | TYR | 20 | 11 | 11 | 38 | 0.562 | 18 | 10 | 31 | 0.366 |
| | VAL | 593 | 476 | 451 | 626 | 0.648 | 534 | 479 | 511 | 0.686 |
| | MET | 147 | 130 | 85 | 165 | 0.412 | 125 | 116 | 134 | 0.804 |
| | TRP | 155 | 172 | 92 | 143 | 0.237 | 149 | 131 | 116 | 0.587 |
| | PHE | 232 | 121 | 200 | 340 | 0.488 | 214 | 155 | 278 | 0.462 |
| | ILE | 436 | 301 | 303 | 523 | 0.552 | 370 | 323 | 427 | 0.682 |
| | LYS | 45 | 15 | 30 | 230 | 0.862 | 20 | 40 | 188 | 0.696 |
| | LEU | 552 | 382 | 378 | 663 | 0.547 | 462 | 413 | 542 | 0.732 |
| | PRO | 3,046 | 3,515 | 2,720 | 2,083 | 0.424 | 3,151 | 3,036 | 1,701 | 0.798 |

**Table 86 Results of the combined analysis in Chapter 4 (grapes). Numbers correspond to means, LSD 5%, and p values. Only results including the two seasons are presented.**

| Section | Evaluation | Site | | | | | Treatment | | | | Season | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | WH | GB5 | GB10 | LSD 5% | P value | CON | RI | LSD 5% | P value | CON | RI | LSD 5% | P value |
| Berry parameters | Berry weight | 1.38 | 1.28 | 1.16 | 0.07 | **0.010** | 1.37 | 1.18 | 0.05 | **0.005** | -0.18 | +0.01 | 0.11 | **0.017** |
| | Seed fresh weight | 40.52 | 42.14 | 41.83 | 2.22 | 0.151 | 42.89 | 40.11 | 1.81 | **0.022** | -10.94 | -8.06 | 2.08 | **0.027** |
| | Seed dry weight | 22.91 | 24.99 | 23.88 | 1.23 | **0.037** | 24.60 | 23.25 | 1.01 | **0.029** | -8.74 | -7.59 | 0.85 | **0.028** |
| | Seed water content | 43.3 | 40.4 | 43.1 | 0.59 | **0.004** | 42.6 | 41.9 | 0.48 | **0.021** | +6.1 | +7.7 | 2.46 | 0.108 |
| Brix, pH, and TA | Brix | 15.60 | 17.05 | 16.20 | 1.10 | 0.058 | 15.78 | 16.78 | 0.90 | **0.041** | -0.79 | -1.83 | 0.57 | **0.023** |
| | pH | 3.32 | 3.29 | 3.37 | 0.12 | 0.210 | 3.31 | 3.34 | 0.10 | 0.448 | +0.02 | -0.05 | 0.13 | 0.145 |
| | TA | 7.93 | 7.18 | 7.68 | 0.18 | **0.006** | 7.63 | 7.55 | 0.14 | 0.130 | -1.93 | -1.23 | 1.31 | 0.149 |
| YAN | PAAN | 206.20 | 161.82 | 207.25 | 140.5 | 0.442 | 194.05 | 189.47 | 114.7 | 0.879 | -75.1 | -50.6 | 68.41 | 0.263 |
| | AN | 42.45 | 41.47 | 46.70 | 9.03 | 0.222 | 44.57 | 42.52 | 7.37 | 0.354 | +8.80 | -10.97 | 3.80 | 0.134 |
| | YAN | 248.65 | 203.32 | 253.95 | 145.3 | 0.424 | 238.63 | 231.98 | 118.7 | 0.832 | -66.3 | -39.6 | 64.82 | 0.219 |
| MCP seeds | Seed tannin concentration | 153.5 | 151.6 | 152.1 | 5.96 | 0.502 | 152.1 | 152.6 | 4.86 | 0.686 | +1.1 | +1.6 | 6.45 | 0.746 |
| | Seed total phenolics | 1.547 | 1.542 | 1.607 | 0.039 | **0.030** | 1.575 | 1.556 | 0.032 | 0.117 | +0.240 | +0.267 | 0.062 | 0.199 |
| MCP skins | Skin tannin concentration | 1.8 | 2.2 | 1.4 | 0.48 | **0.039** | 1.9 | 1.7 | 0.39 | 0.192 | -0.3 | 0.0 | 0.75 | 0.303 |
| | Seed total phenolics | 0.493 | 0.552 | 0.436 | 0.084 | 0.054 | 0.504 | 0.483 | 0.069 | 0.315 | +0.031 | +0.065 | 0.116 | 0.330 |
| $\delta^{13}C$ juice | $\delta^{13}C$ juice | -26.35 | -25.88 | -26.33 | 1.35 | 0.410 | -26.46 | -25.91 | 1.11 | 0.167 | -1.16 | -1.36 | 1.46 | 0.608 |
| Amino acids | ASP | 206 | 132 | 231 | 200 | 0.288 | 194 | 184 | 163 | 0.818 | +75 | +100 | 113 | 0.448 |
| | GLU | 315 | 258 | 378 | 163 | 0.166 | 314 | 320 | 133 | 0.876 | -52 | -45 | 100 | 0.795 |
| | CYS | 0 | 5 | 4 | 24 | 0.673 | 3 | 3 | 20 | 0.933 | +7 | +6 | 39 | 0.933 |

| Section | Evaluation | Site | | | | | Treatment | | | | Season | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | WH | GB5 | GB10 | LSD 5% | P value | CON | RI | LSD 5% | P value | CON | RI | LSD 5% | P value |
| | ASN | 11 | 16 | 34 | 64 | 0.439 | 23 | 18 | 53 | 0.761 | +13 | +21 | 4 | **0.015** |
| | SER | 665 | 609 | 732 | 510 | 0.647 | 676 | 661 | 416 | 0.889 | -51 | -1 | 456 | 0.682 |
| | GLN | 840 | 827 | 1,076 | 1,587 | 0.776 | 1,056 | 773 | 1,296 | 0.447 | -324 | -87 | 1,274 | 0.509 |
| | HIS | 233 | 223 | 198 | 109 | 0.491 | 229 | 207 | 89 | 0.391 | -131 | -136 | 129 | 0.893 |
| | GLY | 40 | 40 | 41 | 41 | 0.999 | 41 | 40 | 34 | 0.878 | -7 | -1 | 15 | 0.215 |
| | THR | 1,364 | 1,291 | 1,444 | 1,189 | 0.868 | 1,389 | 1,344 | 971 | 0.860 | -340 | -263 | 978 | 0.767 |
| | ARG | 4,563 | 3,504 | 4,399 | 4,767 | 0.654 | 4,308 | 4,003 | 3,892 | 0.769 | +719 | +505 | 3,336 | 0.715 |
| | ALA | 2,242 | 1,838 | 2,191 | 1,880 | 0.664 | 2,224 | 1,957 | 1,535 | 0.532 | -398 | -75 | 1,406 | 0.427 |
| | TAU | 1,344 | 1,055 | 1,341 | 624 | 0.276 | 1,246 | 1,248 | 510 | 0.988 | -51 | -45 | 786 | 0.976 |
| | TYR | 14 | 23 | 11 | 30 | 0.397 | 16 | 16 | 25 | 0.995 | -4 | +12 | 12 | **0.028** |
| | VAL | 418 | 436 | 466 | 466 | 0.909 | 457 | 423 | 381 | 0.740 | -155 | -122 | 324 | 0.621 |
| | MET | 111 | 121 | 97 | 134 | 0.762 | 113 | 106 | 109 | 0.820 | -24 | -19 | 80 | 0.828 |
| | TRP | 114 | 127 | 78 | 79 | 0.205 | 113 | 100 | 64 | 0.453 | -70 | -63 | 139 | 0.848 |
| | PHE | 164 | 134 | 215 | 221 | 0.443 | 190 | 152 | 180 | 0.462 | -47 | -6 | 211 | 0.492 |
| | ILE | 302 | 296 | 344 | 391 | 0.897 | 330 | 298 | 319 | 0.715 | -82 | -50 | 273 | 0.665 |
| | LYS | 65 | 34 | 34 | 133 | 0.601 | 43 | 45 | 109 | 0.963 | +46 | +10 | 180 | 0.474 |
| | LEU | 379 | 368 | 421 | 487 | 0.892 | 408 | 372 | 398 | 0.734 | -109 | -82 | 395 | 0.796 |
| | PRO | 2,449 | 3,382 | 2,521 | 1,577 | 0.199 | 2,785 | 2,782 | 1,288 | 0.992 | -732 | -507 | 893 | 0.393 |

# Appendix D

# Statistics Chapter 5

**Table 87 Results of the two-way ANOVAs on the effect of "site" and "treatment" in Chapter 5 (wine). Numbers correspond to means, LSD 5%, and p values.**

| Section | Evaluation | Site | | | | | Treatment | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | WH | GB5 | GB10 | LSD 5% | p value | CON | RI | LSD 5% | p value |
| Wine pH | Wine pH | 4.03 | 3.78 | 4.16 | 0.304 | 0.063 | 3.98 | 4.00 | 0.248 | 0.725 |
| Wine TA | Wine TA | 4.09 | 4.17 | 3.51 | 0.814 | 0.121 | 3.98 | 3.87 | 0.664 | 0.558 |
| MCP | Wine tannin concentration | 226.5 | 388.8 | 361.2 | 51.31 | **0.009** | 313.1 | 337.9 | 41.89 | 0.126 |
| | Wine total phenolics | 0.592 | 0.546 | 0.551 | 0.171 | 0.550 | 0.543 | 0.584 | 0.139 | 0.338 |
| Wine colour by Somers' method | Wine colour density (a.u.) | 3.108 | 3.105 | 2.704 | 1.153 | 0.400 | 2.803 | 3.142 | 0.941 | 0.261 |
| | Wine colour hue (a.u.) | 0.896 | 0.754 | 1.076 | 0.077 | **0.006** | 0.915 | 0.903 | 0.063 | 0.499 |
| | Degree of red pigment colouration (%) | 13.67 | 16.34 | 14.76 | 4.01 | 0.193 | 14.70 | 15.14 | 3.27 | 0.620 |
| | Estimate of SO2 resistant pigments (a.u.) | 0.597 | 0.510 | 0.534 | 0.275 | 0.498 | 0.519 | 0.575 | 0.224 | 0.396 |
| | Total red pigments (a.u.) | 12.057 | 10.857 | 8.774 | 6.393 | 0.286 | 10.058 | 11.068 | 5.220 | 0.493 |
| | Total phenolics (a.u.) | 22.260 | 20.808 | 20.998 | 8.540 | 0.760 | 20.703 | 22.008 | 6.973 | 0.505 |
| | Total anthocyanins (mg/L) | 221.221 | 200.150 | 157.696 | 118.799 | 0.267 | 183.853 | 202.192 | 96.999 | 0.501 |
| Wine colour by CIELab method | Luminosity (L) | 40.73 | 42.48 | 48.19 | 14.04 | 0.259 | 45.60 | 41.99 | 11.46 | 0.308 |
| | Chroma (C) | 53.39 | 58.31 | 46.18 | 9.43 | 0.061 | 51.96 | 53.30 | 7.70 | 0.531 |
| | Hue angle (h) | 20.69 | 14.57 | 24.93 | 8.11 | 0.061 | 19.54 | 20.58 | 6.62 | 0.567 |
| | Redness (a) | 49.86 | 56.34 | 41.82 | 6.40 | **0.021** | 48.91 | 49.77 | 5.23 | 0.550 |

| Section | Evaluation | Site | | | | | Treatment | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | WH | GB5 | GB10 | LSD 5% | p value | CON | RI | LSD 5% | p value |
| | Blueness (b) | 18.93 | 14.80 | 19.48 | 10.48 | 0.312 | 17.09 | 18.38 | 8.56 | 0.581 |
| | Colour intensity | 3.099 | 2.946 | 2.534 | 1.331 | 0.359 | 2.688 | 3.031 | 1.087 | 0.307 |
| | Tonality | 0.760 | 0.595 | 0.908 | 0.092 | **0.009** | 0.756 | 0.753 | 0.075 | 0.866 |
| Wine fatty acids | 2-Methylbutanoic acid | 703.57 | 717.10 | 899.41 | 243.24 | 0.118 | 774.38 | 772.33 | 198.60 | 0.969 |
| | Acetic acid | 492,060 | 542,502 | 540,221 | 24,720 | **0.020** | 515,684 | 534,171 | 20,184 | 0.059 |
| | Butanoic acid | 971.92 | 1,429.28 | 1,569.58 | 402.20 | **0.043** | 1,323.19 | 1,324.00 | 328.40 | 0.992 |
| | Hexanoic acid | 831.33 | 847.06 | 845.76 | 139.90 | 0.874 | 863.27 | 819.50 | 114.23 | 0.241 |
| | Isobutyric acid | 6,493.43 | 7,279.55 | 9,325.44 | 2,104.56 | 0.053 | 7,642.23 | 7,756.71 | 1,718.37 | 0.801 |
| | Isovaleric acid | 768.03 | 730.92 | 813.99 | 306.19 | 0.594 | 778.95 | 763.01 | 250.01 | 0.810 |
| | Octanoic acid | 445.99 | 578.37 | 529.21 | 163.46 | 0.139 | 541.85 | 493.86 | 133.47 | 0.262 |
| Alcohols | 1-Hepatanol | 52.62 | 44.33 | 45.28 | 5.73 | **0.041** | 47.11 | 47.70 | 6.68 | 0.640 |
| | *cis*-3-Hexen-1-ol | 50.30 | 41.63 | 56.47 | 8.75 | **0.036** | 49.66 | 49.27 | 7.14 | 0.838 |
| | Hexanol | 3438.85 | 2417.56 | 3438.85 | 245.36 | **0.006** | 2841.72 | 2945.65 | 200.33 | 0.155 |
| | Isoamyl alcohol | 222006.9 | 192345.6 | 203079.1 | 49706.00 | 0.228 | 207555.9 | 204065.1 | 40584.78 | 0.747 |
| | Phenylethyl alcohol | 38036.03 | 29519.20 | 27577.48 | 16581.76 | 0.194 | 32650.10 | 30771.70 | 13538.95 | 0.611 |
| | *trans*-3-Hexen-1-ol | 132.20 | 98.21 | 89.09 | 17.56 | **0.016** | 104.90 | 108.10 | 14.34 | 0.439 |
| Esters | 2-Phenylethyl acetate | 24.17 | 56.54 | 24.98 | 16.06 | **0.020** | 35.17 | 35.29 | 13.11 | 0.973 |
| | Ethyl acetate | 69801.24 | 97059.99 | 93975.30 | 25653.21 | 0.074 | 86096.38 | 87749.64 | 20945.76 | 0.761 |
| | Ethyl butanoate | 262.93 | 414.45 | 262.93 | 186.47 | 0.096 | 372.28 | 396.37 | 152.25 | 0.942 |
| | Ethyl cinnamate | 1.11 | 0.90 | 2.08 | 0.19 | **0.003** | 1.43 | 1.30 | 0.15 | 0.072 |

| Section | Evaluation | Site | | | | | Treatment | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | WH | GB5 | GB10 | LSD 5% | p value | CON | RI | LSD 5% | p value |
| Monoterpenes, norisoprenoids and aldehydes | Ethyl decanoate | 138.62 | 186.90 | 164.99 | 147.31 | 0.501 | 165.03 | 161.98 | 120.28 | 0.923 |
| | Ethyl heptanoate | 3.89 | 4.25 | 3.54 | 1.50 | 0.327 | 3.95 | 3.83 | 1.23 | 0.707 |
| | Ethyl hexanoate | 388.45 | 487.87 | 388.45 | 56.52 | **0.032** | 453.07 | 434.96 | 46.15 | 0.233 |
| | Ethyl hydrocinnamate | 0.58 | 0.77 | 1.50 | 0.086 | **<.001** | 0.96 | 0.94 | 0.071 | 0.545 |
| | Ethyl isobutyrate | 106.80 | 145.45 | 142.45 | 47.29 | 0.115 | 132.48 | 130.71 | 38.62 | 0.862 |
| | Ethyl isovalerate | 8.94 | 11.95 | 10.15 | 3.95 | 0.155 | 10.35 | 10.34 | 3.22 | 0.988 |
| | Ethyl lactate | 46945.49 | 57311.99 | 58014.78 | 4239.60 | **0.012** | 54416.68 | 53764.83 | 3461.62 | 0.503 |
| | Ethyl octanoate | 538.88 | 720.44 | 634.41 | 188.76 | 0.104 | 638.86 | 623.63 | 154.12 | 0.712 |
| | Ethyl pentanoate | 1.41 | 1.39 | 1.64 | 0.21 | 0.059 | 1.48 | 1.48 | 0.17 | 0.889 |
| | Hexyl acetate | 16.56 | 54.57 | 9.40 | 9.12 | **0.004** | 26.71 | 26.98 | 7.45 | 0.890 |
| | Isoamyl acetate | 453.48 | 1272.67 | 394.22 | 516.99 | **0.029** | 742.73 | 670.85 | 422.12 | 0.540 |
| | Citronellol | 9.40 | 7.18 | 9.41 | 0.41 | **0.003** | 8.57 | 8.75 | 0.33 | 0.144 |
| | Geraniol | 5.79 | 5.14 | 5.79 | 0.40 | **0.030** | 5.54 | 5.61 | 0.33 | 0.428 |
| | Linalool | 35.81 | 27.89 | 34.39 | 0.41 | **<.001** | 32.39 | 33.01 | 0.34 | **0.016** |
| | β-Damascenone | 9.43 | 7.90 | 11.86 | 2.74 | **0.048** | 9.59 | 9.87 | 2.23 | 0.649 |
| | β-Ionone | 0.92 | 0.79 | 0.75 | 0.29 | 0.220 | 0.82 | 0.82 | 0.24 | 0.976 |
| | Benzaldehyde | 47.97 | 29.13 | 124.69 | 83.22 | 0.068 | 67.82 | 66.71 | 67.95 | 0.950 |

### 6.1.3 Canonical variate analyses results

**Acids**

**Table 88 Between group distances for volatile acids**

| | GB10C | GB10RI | GB5C | GB5RI | WHC | WHRI |
|---|---|---|---|---|---|---|
| GB1OC | 0.000 | | | | | |
| GB10RI | **1.902** | 0.000 | | | | |
| GB5C | 1.362 | 1.958 | 0.000 | | | |
| GB5RI | 1.958 | 1.362 | **2.785** | 0.000 | | |
| WHC | 1.458 | 2.135 | 2.313 | 1.845 | 0.000 | |
| WHRI | 2.135 | 1.458 | 1.845 | 2.313 | **3.122** | 0.000 |

**Table 89 Between group separations for alcohols**

| | GB10C | GB10RI | GB5C | GB5RI | WHC | WHRI |
|---|---|---|---|---|---|---|
| GB1OC | 0.000 | | | | | |
| GB10RI | **1.519** | 0.000 | | | | |
| GB5C | 1.163 | 1.155 | 0.000 | | | |
| GB5RI | 1.155 | 1.163 | **1.750** | 0.000 | | |
| WHC | 1.769 | 1.013 | 1.419 | 1.587 | 0.000 | |
| WHRI | 1.013 | 1.769 | 1.587 | 1.419 | **2.450** | 0.000 |

**Table 90 Between group separations for Esters**

| | GB10C | GB10RI | GB5C | GB5RI | WHC | WHRI |
|---|---|---|---|---|---|---|
| GB1OC | 0.000 | | | | | |
| GB10RI | **4.419** | 0.000 | | | | |
| GB5C | 6.669 | 4.031 | 0.000 | | | |
| GB5RI | 4.031 | 6.669 | **10.095** | 0.000 | | |
| WHC | 3.344 | 5.035 | 7.697 | 3.776 | 0.000 | |
| WHRI | 5.035 | 3.344 | 4.776 | 7.967 | **7.318** | 0.000 |

**Table 91 Between group separations for monoterpenes, norisoprenoids and aldehydes**

| | GB10C | GB10RI | GB5C | GB5RI | WHC | WHRI |
|---|---|---|---|---|---|---|
| GB1OC | 0.000 | | | | | |
| GB10RI | **0.9626** | 0.000 | | | | |
| GB5C | 0.8157 | 1.0501 | 0.000 | | | |
| GB5RI | 1.0501 | 0.8157 | **1.6154** | 0.000 | | |
| WHC | 1.5770 | 0.9789 | 1.8688 | 0.8912 | 0.000 | |
| WHRI | 0.9789 | 1.5770 | 0.8912 | 1.8688 | **2.4420** | 0.000 |

# Appendix E

## GC-MS quantification parameters

**Table 92 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] | $R^2$ [f] | Purity of Standards (%) | CAS No | Supplier |
|---|---|---|---|---|---|---|---|---|---|
| Methyl acetate | (1) | 5.18 | 43 | 74 (19), 42 (10) | - | - | 99.8% | 79-20-9 | Sigma-Aldrich |
| $d_5$-Ethyl butanoate | (2) | 11.51 | 93 | 34 (95), 106 (15) | - | - | 100 A% | - | Lincoln |
| $d_3$-Isoamyl acetate | (3) | 14.63 | 46 | 90 (13), 76 (8) | - | - | 100 A% | 1219804-75-7 | Lincoln |
| $d_5$-Ethyl hexanoate | (4) | 19.10 | 93 | 74 (35), 34 (30) | - | - | 100 A% | - | Lincoln |
| $d_3$-Hexyl acetate | (5) | 20.36 | 46 | 64 (29) | - | - | 99 A% | 1219805-39-6 | Lincoln |
| $d_{13}$-Hexan-1-ol | (6) | 21.93 | 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) | 28.02 | 82 | 112 (98), 110 (91) | - | - | 98 A% | 17901-93-8 | Sigma-Aldrich |
| $d_5$-Ethyl decanoate | (9) | 33.29 | 93 | 106 (37), 120 (7) | - | - | 97 A% | - | Lincoln |
| $d_5$-1-Phenyl ethanol | (10) | 36.30 | 112 | 84 (89 ), 127 (28 ) | - | - | 98 A% | 90162-45-1 | Isotech |
| Ethyl acetate | 1 | 6.58 | 61 | 70 (95), 73 (33), 88 (33) | 0 – 17,912[c] | 0.9996 | 99.5% | 141-78-6 | Fisher |
| Ethyl Isobutyrate | 2 | 9.40 | 71 | 88 (36), 116 (28) | 0 – 59.6[d] | 0.9987 | 99% | 97-62-1 | Sigma-Aldrich |
| Ethyl butanoate | 2 | 11.63 | 88 | 101 (16), 60 (34) | 0 – 65.0[c] | 0.9995 | 99% | 105-54-4 | Sigma-Aldrich |
| Ethyl isovalerate | 2 | 12.97 | 88 | 57 (76), 85 (71) | 0 – 6.6[d] | 0.9994 | 98% | 108-64-5 | Sigma-Aldrich |
| Isoamyl acetate | 3 | 14.77 | 43 | 87 (23), 73 (18) | 0 – 182[b] | 0.9973 | 99% | 123-92-2 | Sigma-Aldrich |

| Analyte | ISTD[a] | RT (mins) | Target Ion m/z | Confirming Ions m/z (% to Target Ion) | Calibration Range µg/L (1/10 dilution)[b] | $R^2$ [f] | Purity of Standards (%) | CAS No | Supplier |
|---|---|---|---|---|---|---|---|---|---|
| Ethyl pentanoate | 4 | 15.30 | 88 | 85 (90), 101 (26) | 0 – 0.82[d] | 0.9996 | 99% | 539-82-2 | Sigma-Aldrich |
| Isoamyl alcohol | 6 | 17.35 | 42 | 70 (89), 41 (82) | 0 – 58,886[c] | 0.9998 | 99% | 123-51-3 | Sigma-Aldrich |
| Ethyl hexanoate | 4 | 19.27 | 88 | 60 (33), 101 (25) | 0 – 113.2[d] | 0.9997 | 99% | 123-66-0 | Sigma-Aldrich |
| Hexyl acetate | 5 | 20.48 | 43 | 61 (25), 84 (19) | 0 – 8.1[b] | 0.9983 | 99% | 142-92-7 | Sigma-Aldrich |
| Ethyl lactate | 6 | 21.82 | 45 | 75 (7), 47 (2) | 0 – 12,172[d] | 0.9997 | 98% | 687-47-8 | Sigma-Aldrich |
| Hexan-1-ol | 6 | 22.41 | 56 | 55 (48), 84 (5), 41 (36) | 0 – 905[c] | 0.9999 | 99% | 111-27-3 | Sigma-Aldrich |
| *trans*-3-Hexen-1-ol | 6 | 22.65 | 67 | 82 (64), 100 (5) | 0 – 28.5[c] | 0.9999 | 98% | 928-97-2 | Sigma-Aldrich |
| Ethyl heptanoate | 4 | 22.96 | 88 | 60 (33), 113 (33) | 0 – 1.17[d] | 0.9998 | 99% | 106-30-9 | Sigma-Aldrich |
| *cis*-3-Hexen-1-ol | 6 | 23.34 | 41 | 67 (90), 82 (43) | 0 – 38.1[d] | 0.9972 | 98% | 928-96-1 | Sigma-Aldrich |
| 1-Heptanol | 6 | 25.97 | 70 | 56 (88), 41 (78) | 0 – 51.5[d] | 0.9998 | 99% | 111-70-6 | Sigma-Aldrich |
| Ethyl octanoate | 7 | 26.84 | 101 | 70 (79), 129 (29) | 0 – 167[d] | 0.9998 | 99% | 106-32-1 | Sigma-Aldrich |
| Benzaldehyde | 8 | 27.93 | 77 | 106 (97), 51(44) | 0 – 127[d] | 0.9999 | 99% | 100-52-7 | Sigma-Aldrich |
| Ethyl decanoate | 9 | 33.48 | 88 | 101 (37), 115 (8) | 0 – 185[d] | 0.9999 | 99% | 110-38-3 | Sigma-Aldrich |
| 2-Phenyl ethanol | 10 | 39.15 | 91 | 92 (62), 122 (31) | 0 – 12,812[d] | 0.9977 | 99% | 60-12-8 | Sigma-Aldrich |

[a] Internal Standards used are in brackets. [b] Eight standards were used to create the calibration range however less standards were used where appropriate; [c] six standards; [d] five standards. [f] All fitted standard (calibration) curves were Quadratic functions

**Table 93 Quantification parameters for the 8 compounds in the Trace profile.**

| 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 |
|---|---|---|---|---|---|---|---|---|---|
| $d_3$-Linalool | (1) | 39.65 | 74 | 124 (19) | - | - | 99 A% | 1216673-02-7 | CDN isotopes |
| $d_3$-β-Ionone | (2) | 69.15 | 180 | 46 (88), 138 (11), 181 (13) | - | - | 100 A% | - | Lincoln |
| $d_5$-Ethyl trans-cinnamate | (3) | 76.95 | 136 | 108 (64), 181 (25) | - | - | 99.4 A% | 856765-68-9 | CDN isotopes |
| Linalool | 1 | 39.79 | 121 | 136 (30) | 0 – 8.82[d] | 0.9999 | 97 | 78-70-6 | Sigma-Aldrich |
| Citronellol | 1 | 55.27 | 82 | 95 (79), 109 (26), 138 (16) | 0 – 1.95[c] | 0.9989 | 99 | 7540-51-4 | Sigma-Aldrich |
| 2-phenyl ethyl acetate | 3 | 57.91 | 104 | 91 (17), 105 (11) | 0 – 9.76[d] | 0.9999 | 99 | 103-45-7 | Sigma-Aldrich |
| β-Damascenone | 2 | 59.86 | 190 | 91 (61), 105 (43) | 0 – 1.30[b] | 0.9998 | 1.3% wt[f] | 107-92-6 | Sigma-Aldrich |
| Geraniol | 1 | 61.08 | 123 | 93 (157) | 0 – 0.98[c] | 0.9988 | 98 | 106-24-1 | Sigma-Aldrich |
| Ethyl hydrocinnamate | 3 | 64.26 | 104 | 107 (40), 178 (19) | 0 – 0.33[d] | 0.9999 | 99 | 2021-28-5 | Sigma-Aldrich |
| β-Ionone | 2 | 69.32 | 177 | 135 (16), 178 (9) | 0 – 0.33[d] | 0.9998 | 96 | 14901-07-6 | Sigma-Aldrich |
| Ethyl cinnamate | 3 | 76.98 | 131 | 103 (60), 77 (41), 176 (21) | 0 – 0.98[d] | 0.9999 | 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 94 Quantification parameters for the 7 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) | 12.04 | 46 | 63 (72) | - | - | 99.5 A% | 1186-52-3 | Sigma-Aldrich |
| $d_7$-Butyric acid | (2) | 14.42 | 63 | 46 (27), 58 (7) | - | - | 99.5 A% | 73607-83-7 | CDN isotopes |
| $d_{11}$-Hexanoic acid | (3) | 17.00 | 63 | 77 (43), 93 (12) | - | - | 98 A% | 95348-44-0 | Sigma-Aldrich |
| $d_2$-Octanoic acid | (4) | 19.47 | 62 | 74 (33) 102 (12) | - | - | 98 A% | 64118-36-1 | CDN isotopes |
| Acetic acid | 1 | 12.10 | 43 | 60 (82), 45 (84) | 0 – 205,479[c] | 0.9994 | 99.7 | 64-19-7 | Sigma-Aldrich |
| Isobutyric acid | 2 | 13.77 | 88 | 42 (26) | 0 – 4,802[b] | 0.9995 | 99 | 79-31-2 | Sigma-Aldrich |
| Butanoic acid | 2 | 14.54 | 60 | 43 (22), 55 (9) | 0 – 781[c] | 0.9997 | 99 | 107-92-6 | Sigma-Aldrich |
| Isovaleric acid | 2 | 15.08 | 60 | 87 (18) | 0 – 298[b] | 0.9992 | 99 | 503-74-2 | Sigma-Aldrich |
| 2-Methyl-butanoic acid | 2 | 15.10 | 74 | 57 (66) | 0 – 199[c] | 0.9995 | 98 | 116-53-0 | Sigma-Aldrich |
| Hexanoic acid | 3 | 17.16 | 60 | 73 (41), 87 (12) | 0 – 1280[c] | 0.9985 | 99.5 | 142-62-1 | Sigma-Aldrich |
| Octanoic acid | 4 | 19.52 | 60 | 73 (56), 101 (20) | 0 – 800[d] | 0.9955 | 99 | 124-07-2 | 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

# References


Acevedo-Opazo, C., S. Ortega-Farias, and S. Fuentes. 2010. Effects of grapevine (*Vitis vinifera* L.) water status on water consumption, vegetative growth and grape quality: An irrigation scheduling application to achieve regulated deficit irrigation. Agricultural Water Management 97:956-964 doi: 10.1016/j.agwat.2010.01.025.

Adams, D.O. 2006. Phenolics and Ripening in Grape Berries. American Journal of Enology and Viticulture 57:249-256.

Allen, R.G., L.S. Pereira, D. Raes, and M. Smith. 1998. Crop evapotranspiration-Guidelines for computing crop water requirements. FAO Irrigation and drainage 56:1-15.

Allen, S.E., H.M. Grimshaw, J.A. Parkinson, and C. Quarmby. 1974. Chemical analysis of ecological materials. Blackwell scientific publications, Oxford.

Alsina, M.M., F. de Herralde, X. Aranda, R. Savé, and C. Biel. 2007. Water relations and vulnerability to embolism are not related: Experiments with eight grapevine cultivars Vitis 46:1-6.

Anderson, J.D., G.V. Jones, A. Tait, A. Hall, and M.C.T. Trought. 2012. Analysis of viticulture region climate structure and suitability in New Zealand. Journal International des Sciences de la Vigne et du Vin 46:149-165.

Andrews, J.T., H. Heymann, and M. Ellersieck. 1990. Sensory and Chemical Analyses of Missouri Seyval blanc Wines. American Journal of Enology and Viticulture 41:116-120.

Ayala, F., J.F. Echávarri, and A.I. Nagueruela. 2012. MSCV®. Grupo de color. Universidad de La Rioja-Universidad de Zaragoza, La Rioja-Zaragoza. Spain.

Ayala, F., J.F. Echávarri, and A.I. Negueruela. 1997. A New Simplified Method for Measuring the Color of Wines. I. Red and Rosé Wines. American Journal of Enology and Viticulture 48:357-363.

Becker, T., and M. Knoche. 2011. Water Movement through the Surfaces of the Grape Berry and Its Stem. American Journal of Enology and Viticulture 62:340-350 doi: 10.5344/ajev.2011.10056.

Bell, S.-J., and P.A. Henschke. 2005. Implications of nitrogen nutrition for grapes, fermentation and wine. Australian Journal of Grape and Wine Research 11:242-295 doi: 10.1111/j.1755-0238.2005.tb00028.x.

Bellvert, J., P.J. Zarco-Tejada, J. Girona, and E. Fereres. 2013. Mapping crop water stress index in a 'Pinot-noir' vineyard: comparing ground measurements with thermal remote sensing imagery from an unmanned aerial vehicle. Precision Agriculture 1-16 doi: 10.1007/s11119-013-9334-5.

Bennett, J.S. 2002. Relatioships between carbohydrate supply and reserves and the reproductive growth of grapevines (*Vitis vinifera* L.). Thesis, Lincoln University.

Berdeja, M., G. Hilbert, Z.W. Dai, M. Lafontaine, M. Stoll, H.R. Schultz, and S. Delrot. 2014. Effect of water stress and rootstock genotype on Pinot Noir berry composition. Australian Journal of Grape and Wine Research 20:409-421 doi: 10.1111/ajgw.12091.

Bertamini, M., L. Zulini, K. Muthuchelian, and N. Nedunchezhian. 2006. Effect of water deficit on photosynthetic and other physiological responses in grapevine (*Vitis vinifera* L. cv. Riesling) plants. Photosynthetica 44:151-154 doi: 10.1007/s11099-005-0173-0.

Beslic, Z., S. Todic, and D. Tesic. 2010. Validation of non-destructive methodology of grapevine leaf area estimation on cv. Blaufränkisch (*Vitis vinifera* L.). South African Journal for Enology and Viticulture 31:22-25.

Bohmrich, R. 1996. Terroir:competing perspectives on the roles of soil, climate and people. Journal of Wine Research 7:33-46 doi: 10.1080/09571269608718059.

Bondada, B., and J. Shutthanandan. 2012. Understanding Differential Responses of Grapevine (*Vitis vinifera* L.) Leaf and Fruit to Water Stress and Recovery Following Re-Watering. American Journal of Plant Sciences 3:1232-1240.

Bota, J., O. Stasyk, J. Flexas, and H. Medrano. 2004. Effect of water stress on partitioning of 14C-labelled photosynthates in Vitis vinifera. Functional Plant Biology 31:697-708 doi: 10.1071/FP03262.

Bota, J., M. Tomás, J. Flexas, H. Medrano, and J.M. Escalona. 2015. Differences among grapevine cultivars in their stomatal behavior and water use efficiency under progressive water stress. Agricultural Water Management doi: 10.1016/j.agwat.2015.07.016.

Botelho, M., A. Cruz, and R.d. Castro. 2012. Densidade do coberto vegetal na casta Alfrocheiro (*Vitis vinifera* L.) I. Efeitos na estrutura e microclima do coberto vegetal, vigor e expressão vegetativa. Ciência e Técnica Vitivinícola 27:103-114.

Boulton, R. 1980. The General Relationship Between Potassium, Sodium and pH in Grape Juice and Wine. American Journal of Enology and Viticulture 31:182-186.

Bramley, R.G.V. 2005. Understanding variability in winegrape production systems 2. Within vineyard variation in quality over several vintages. Australian Journal of Grape and Wine Research 11:33-42 doi: 10.1111/j.1755-0238.2005.tb00277.x.

Bramley, R.G.V., and R.P. Hamilton. 2004. Understanding variability in winegrape production systems. Australian Journal of Grape and Wine Research 10:32-45 doi: 10.1111/j.1755-0238.2004.tb00006.x.

Bramley, R.G.V., M.C.T. Trought, and J.P. Praat. 2011. Vineyard variability in Marlborough, New Zealand: characterising variation in vineyard performance and options for the implementation of Precision Viticulture. Australian Journal of Grape and Wine Research 17:72-78 doi: 10.1111/j.1755-0238.2010.00119.x.

Bray, E.A. 1997. Plant responses to water deficit. Trends in Plant Science 2:48-54 doi: 10.1016/S1360-1385(97)82562-9.

Brunetto, G., G. Trentin, C.A. Ceretta, E. Girotto, F. Lorensini, A. Miotto, G.R. Zaferi Moser, and G. Wellington de Melo. 2012. Use of the SPAD-502 in estimating nitrogen content in leaves and grape yield in grapevines in soils with different texture. American Journal of Plant Sciences 3:1546-1561.

Burns, S. 2012. The importance of soil and geology in tasting terroir with a case history from the Willamette Valley, Oregon. . *In* The geography of wine: regions, terroir and techniques. Dougherty, P. H. (Ed.), pp. 95-108. Springer, Dordrecht; London.

Carew, A.L., P. Smith, D.C. Close, C. Curtin, and R.G. Dambergs. 2013. Yeast Effects on Pinot noir Wine Phenolics, Color, and Tannin Composition. Journal of Agricultural and Food Chemistry 61:9892-9898 doi: 10.1021/jf4018806.

Chacón, J.L., E. García, J. Martínez, R. Romero, and S. Gómez. 2009. Impact of the vine water status on the berry and seed phenolic composition of 'Merlot' (*Vitis vinifera* L.) cultivated in a warm climate: Consequences for the style of wine. Vitis 48:7-9.

Chaves, M.M., T.P. Santos, C.R. Souza, M.F. Ortuño, M.L. Rodrigues, C.M. Lopes, J.P. Maroco, and J.S. Pereira. 2007. Deficit irrigation in grapevine improves water-use efficiency while controlling vigour and production quality. Annals of Applied Biology 150:237-252 doi: 10.1111/j.1744-7348.2006.00123.x.

Chaves, M.M., O. Zarrouk, R. Francisco, J.M. Costa, T. Santos, A.P. Regalado, M.L. Rodrigues, and C.M. Lopes. 2010. Grapevine under deficit irrigation: hints from physiological and molecular data. Annals of Botany 105:661-676 doi: 10.1093/aob/mcq030.

Chen, T., Q. Gui, J.J. Shi, X.Y. Zhang, and F.S. Chen. 2013. Analysis of variation of main components during aging process of Shanxi aged vinegar. Acetic acid bacteria 2:31-38 doi: 10.4081/aab.2013.s1.e6.

Choné, X., C. Van Leeuwen, D. Dubourdieu, and J.P. Gaudillère. 2001. Stem Water Potential is a Sensitive Indicator of Grapevine Water Status. Annals of Botany 87:477-483 doi: 10.1006/anbo.2000.1361.

Cifre, J., J. Bota, J.M. Escalona, H. Medrano, and J. Flexas. 2005. Physiological tools for irrigation scheduling in grapevine (*Vitis vinifera* L.): An open gate to improve water-use efficiency? Agriculture, Ecosystems & Environment 106:159-170 doi: 10.1016/j.agee.2004.10.005.

Cliff, M.A., and M.C. Dever. 1996. Sensory and compositional profiles of British Columbia Chardonnay and Pinot noir wines. Food Research International 29:317-323 doi: 10.1016/0963-9969(96)83271-6.

Conde, C., P. Silva, N. Fontes, A.C.P. Dias, R.M. Tavares, M.J. Sousa, A. Agasse, S. Delrot, and H. Geros. 2007. Biochemical changes throughout grape berry development and fruit and wine quality Food 1:1-22.

Constantini, E.A.C., F. Campostrini, P.G. Arcara, P. Cherubini, P. Storchi, and M. Pierucci. 1996. Soil and climate functional characters for grape ripening and wine quality of Vino Nobile di Montepulciano. Acta Horticulturae (ISHS) 427:45-57.

Coombe, B.G. 1995. Adoption of a system for identifying grapevine growth stages. Australian Journal of Grape and Wine Research 1:104-110 doi: 10.1111/j.1755-0238.1995.tb00086.x.

Cortell, J.M., M. Halbleib, A.V. Gallagher, T.L. Righetti, and J.A. Kennedy. 2007. Influence of Vine Vigor on Grape (*Vitis vinifera* L. cv. Pinot noir) Anthocyanins. 2. Anthocyanins and Pigmented Polymers in Wine. Journal of Agricultural and Food Chemistry 55:6585-6595 doi: 10.1021/jf070196n.

Cortell, J.M., H.K. Sivertsen, J.A. Kennedy, and H. Heymann. 2008. Influence of Vine Vigor on Pinot noir Fruit Composition, Wine Chemical Analysis, and Wine Sensory Attributes. American Journal of Enology and Viticulture 59:1-10.

Costa, J.M., M.F. Ortuño, C.M. Lopes, and M.M. Chaves. 2012. Grapevine varieties exhibiting differences in stomatal response to water deficit. Functional Plant Biology 39:179-189 doi: 10.1071/FP11156.

Costantini, E.A.C., P. Bucelli, and S. Priori. 2012. Quaternary landscape history determines the soil functional characters of terroir. Quaternary International 265:63-73 doi: 10.1016/j.quaint.2011.08.021.

Covarrubias, J.I., and A. Rombolà. 2013. Physiological and biochemical responses of the iron chlorosis tolerant grapevine rootstock 140 Ruggeri to iron deficiency and bicarbonate. Plant and Soil 370:305-315 doi: 10.1007/s11104-013-1623-2.

Creasy, G.L., and P.B. Lombard. 1993. Vine Water Stress and Peduncle Girdling Effects on Pre- and Post-Veraison Grape Berry Growth and Deformability. American Journal of Enology and Viticulture 44:193-197.

Creasy, G.L., S.F. Price, and P.B. Lombard. 1993. Evidence for Xylem Discontinuity in Pinot noir and Merlot Grapes: Dye Uptake and Mineral Composition During Berry Maturation. American Journal of Enology and Viticulture 44:187-192.

Cruz, A., M. Botelho, J. Silvestre, and R.d. Castro. 2012. Soil management: introduction of tillage in a vineyard with a long-term natural cover. Ciência e Técnica Vitivinícola 27:27-38.

De Andrés-de Prado, R., M. Yuste-Rojas, X. Sort, C. Andrés-Lacueva, M. Torres, and R.M. Lamuela-Raventós. 2007. Effect of Soil Type on Wines Produced from *Vitis vinifera* L. Cv. Grenache in Commercial Vineyards. Journal of Agricultural and Food Chemistry 55:779-786 doi: 10.1021/jf062446q.

De Herralde, F., R. Savé, X. Aranda, and C. Biel. 2010. Grapevine Roots and Soil Environment: Growth, Distribution and Function. *In* Methodologies and Results in Grapevine Research. Delrot, S. et al. (Eds.), pp. 1-20. Springer Netherlands

De la Hera, M.L., P. Romero, E. Gómez-Plaza, and A. Martinez. 2007. Is partial root-zone drying an effective irrigation technique to improve water use efficiency and fruit quality in field-grown wine grapes under semiarid conditions? Agricultural Water Management 87:261-274 doi: 10.1016/j.agwat.2006.08.001.

De Royer Dupré, N., R. Schneider, J.C. Payan, E. Salançon, and A. Razungles. 2014. Effects of Vine Water Status on Dimethyl Sulfur Potential, Ammonium, and Amino Acid Contents in Grenache Noir Grapes (*Vitis vinifera*). Journal of Agricultural and Food Chemistry 62:2760-2766 doi: 10.1021/jf404758g.

De Souza, C.R., J.P. Maroco, T.P. dos Santos, M.L. Rodrigues, C. Lopes, J.S. Pereira, and M.M. Chaves. 2005a. Control of stomatal aperture and carbon uptake by deficit irrigation in two grapevine cultivars. Agriculture, Ecosystems & Environment 106:261-274 doi: 10.1016/j.agee.2004.10.014.

De Souza, C.R., J.P. Maroco, T.P. dos Santos, M.L. Rodrigues, C.M. Lopes, J.S. Pereira, and M.M. Chaves. 2003. Partial rootzone drying: regulation of stomatal aperture and carbon assimilation in field-grown grapevines (*Vitis vinifera* cv. Moscatel). Functional Plant Biology 30:653-662 doi: 10.1071/FP02115.

De Souza, C.R., J.P. Maroco, T.P. dos Santos, M.L. Rodrigues, C.M. Lopes, J.S. Pereira, and M.M. Chaves. 2005b. Impact of deficit irrigation on water use efficiency and carbon isotope composition ($\delta^{13}C$) of field-grown grapevines under Mediterranean climate. Journal of Experimental Botany 56:2163-2172 doi: 10.1093/jxb/eri216.

Deloire, A., A. Carbonneau, Z. Wang, and H. Ojeda. 2004. Vine and water. A short review. Journal International des Sciences de la Vigne et du Vin 38:1-13.

Deluc, L., D. Quilici, A. Decendit, J. Grimplet, M. Wheatley, K. Schlauch, J.-M. Merillon, J. Cushman, and G. Cramer. 2009. Water deficit alters differentially metabolic pathways affecting important flavor and quality traits in grape berries of Cabernet Sauvignon and Chardonnay. BMC Genomics 10:212.

Dougherty, P.H. 2012. Introduction to the geographical study of viticulture and wine production. *In* The goegraphy of wine: regions, terroir and techniques Dougherty, P. H. (Ed.), pp. 3-36. Springer, Dordrecht; London.

Douglas, D., M.A. Cliff, and A.G. Reynolds. 2001. Canadian terroir: characterization of Riesling wines from the Niagara Peninsula. Food Research International 34:559-563.

Doupis, G., K. Chartzoulakis, A. Beis, and A. Patakas. 2011. Allometric and biochemical responses of grapevines subjected to drought and enhanced ultraviolet-B radiation. Australian Journal of Grape and Wine Research 17:36-42 doi: 10.1111/j.1755-0238.2010.00114.x.

Dumas, V., E. Lebon, and R. Morlat. 1997. Differentiation of local climate in the alsatian vineyard. Journal International des Sciences de la Vigne et du Vin 31:1-9.

Dunlevy, J.D., C.M. Kalua, R.A. Keyzers, and P.K. Boss. 2009. The Production of Flavour & Aroma Compounds in Grape Berries. *In* Grapevine Molecular Physiology & Biotechnology. Roubelakis-Angelakis, K. (Ed.), pp. 293-340. Springer Netherlands

Düring, H. 1987. Stomatal response to alteration of soil and air humidity in grapevines. Vitis 26:9-18.

Durner, D., F. Weber, J. Neddermeyer, K. Koopmann, P. Winterhalter, and U. Fischer. 2010. Sensory and Color Changes Induced by Microoxygenation Treatments of Pinot noir before and after Malolactic Fermentation. American Journal of Enology and Viticulture 61:474-485 doi: 10.5344/ajev.2010.09122.

Edwards, E.J., and P.R. Clingeleffer. 2013. Interseasonal effects of regulated deficit irrigation on growth, yield, water use, berry composition and wine attributes of Cabernet Sauvignon grapevines. Australian Journal of Grape and Wine Research 19:261-276 doi: 10.1111/ajgw.12027.

Eichhorn, K.W., and D.H. Lorenz. 1977. Phanologische entwicklungsstadien der rebe. Nachrichtenblatt des Deutschen Pflanzenschutzdienstes 29:119-120.

Escudero, A., E. Campo, L. Fariña, J. Cacho, and V. Ferreira. 2007. Analytical Characterization of the Aroma of Five Premium Red Wines. Insights into the Role of Odor Families and the Concept of Fruitiness of Wines. Journal of Agricultural and Food Chemistry 55:4501-4510 doi: 10.1021/jf0636418.

Etchebarne, F., H. Ojeda, and J.J. Hunter. 2010. Leaf:Fruit ratio and vine water status effects on Grenache noir (*Vitis vinifera* L.) berry composition: water, sugar, organic acids and cations. South African Journal for Enology and Viticulture 31:106-115.

Falcão, L.D., V.M. Burin, E.S. Chaves, H.J. Vieira, E. Brighenti, J.P. Rosier, and M.T. Bordignon-Luiz. 2010. Vineyard altitude and mesoclimate influences on the phenology and maturation of Cabernet Sauvignon grapes from Santa Catarina State. Journal International des Sciences de la Vigne et du Vin 44:135-150.

Falcetti, M., and F. Iacono. 1996. Ecophysiological description of sites and wine sensory properties as a tool for zoning in viticulture. Acta Horticulturae (ISHS) 427:395-404.

Farquhar, G.D., and R.A. Richards. 1984. Isotopic Composition of Plant Carbon Correlates With Water-Use Efficiency of Wheat Genotypes. Functional Plant Biology 11:539-552 doi: 10.1071/PP9840539.

Feng, H., F. Yuan, P.A. Skinkis, and M.C. Qian. 2015. Influence of cluster zone leaf removal on Pinot noir grape chemical and volatile composition. Food Chemistry 173:414-423 doi: 10.1016/j.foodchem.2014.09.149.

Fernandes De Oliveira, A., M.G. Mameli, L. De Pau, D. Satta, and G. Nieddu. 2013a. Deficit irrigation strategies in Vermentino for improving groundwater use in clay soils. *In* 18th International Symposium of the Group of *International* Experts of vitivinicultural System for CoOperation (G*i*ESCO 2013). pp. 134-139. Ciência e Técnica Vitivinícola, Porto, Portugal.

Fernandes De Oliveira, A., M.G. Mameli, L. De Pau, D. Satta, and G. Nieddu. 2013b. Deficit irrigation strategies in *Vitis vinifera* L. cv. Cannonau under mediterranean climate. Part I - Physiological responses, growth, yield and berry composition South African Journal for Enology and Viticulture 34:170-183.

Fernández-López, J., L. Almela, J. Obón, and R. Castellar. 2010. Determination of Antioxidant Constituents in Cactus Pear Fruits. Plant Foods for Human Nutrition 65:253-259 doi: 10.1007/s11130-010-0189-x.

Ferrandino, A., A. Carra, L. Rolle, A. Schneider, and A. Schubert. 2012. Profiling of Hydroxycinnamoyl Tartrates and Acylated Anthocyanins in the Skin of 34 *Vitis vinifera* Genotypes. Journal of Agricultural and Food Chemistry 60:4931-4945 doi: 10.1021/jf2045608.

Ferrandino, A., and C. Lovisolo. 2014. Abiotic stress effects on grapevine (*Vitis vinifera* L.): Focus on abscisic acid-mediated consequences on secondary metabolism and berry quality. Environmental and Experimental Botany 103:138-147 doi: 10.1016/j.envexpbot.2013.10.012.

Ferreira, V., R. López, and J.F. Cacho. 2000. Quantitative determination of the odorants of young red wines from different grape varieties. Journal of the Science of Food and Agriculture 80:1659-1667 doi: 10.1002/1097-0010(20000901)80:11<1659::AID-JSFA693>3.0.CO;2-6.

Findlay, N., K.J. Oliver, N. Nil, and B.G. Coombe. 1987. Solute Accumulation by Grape Pericarp Cells: IV. Perfusion of pericarp apoplast via the pedicel and evidence for xylem malfunction in ripening berries. Journal of Experimental Botany 38:668-679 doi: 10.1093/jxb/38.4.668.

Fitzharris, B.B., and W. Enducher. 1996. Climatic Conditions for Wine Grape Growing. New Zealand Geographer 52:1-11 doi: 10.1111/j.1745-7939.1996.tb00458.x.

Flexas, J., J. Bota, J.M. Escalona, B. Sampol, and H. Medrano. 2002. Effects of drought on photosynthesis in grapevines under field conditions: an evaluation of stomatal and mesophyll limitations. Functional Plant Biology 29:461-471 doi: 10.1071/PP01119.

Galmés, J., A. Pou, M.M. Alsina, M. Tomàs, H. Medrano, and J. Flexas. 2007. Aquaporin expression in response to different water stress intensities and recovery in Richter-110 (*Vitis* sp.): relationship with ecophysiological status. Planta 226:671-681 doi: 10.1007/s00425-007-0515-1.

Gaudillère, J.P., C. van Leeuwen, and N. Ollat. 2002. Carbon isotope composition of sugars in grapevine, an integrated indicator of vineyard water status. Journal of Experimental Botany 53:757-763 doi: 10.1093/jexbot/53.369.757.

Genovese, A., M.T. Lisanti, A. Gambuti, P. Piombino, and L. Moio. 2007. Relationship between sensory perception and aroma compounds of monovarietal red wines. *In* pp. 549-556. International Society for Horticultural Science (ISHS), Leuven, Belgium

Ginestar, C., J. Eastham, S. Gray, and P. Iland. 1998. Use of Sap-Flow Sensors to Schedule Vineyard Irrigation. II. Effects of Post-Veraison Water Deficits on Composition of Shiraz Grapes. American Journal of Enology and Viticulture 49:421-428.

Girard, B., D. Yuksel, M.A. Cliff, P. Delaquis, and A.G. Reynolds. 2001. Vinification effects on the sensory, colour and GC profiles of Pinot noir wines from British Columbia. Food Research International 34:483-499 doi: 10.1016/S0963-9969(00)00177-0.

Gladstones, J. 2011. Wine, Terroir and climate change. Wakefield Press, Adelaide.

Gomez-Alonso, S., and E. Garcia-Romero. 2010. Effect of irrigation and variety on oxygen (δ18O) and carbon (δ13C) stable isotope composition of grapes cultivated in a warm climate. Australian Journal of Grape and Wine Research 16:283-289 doi: 10.1111/j.1755-0238.2009.00089.x.

González-Barreiro, C., R. Rial-Otero, B. Cancho-Grande, and J. Simal-Gándara. 2015. Wine Aroma Compounds in Grapes: A Critical Review. Critical Reviews in Food Science and Nutrition 55:202-218 doi: 10.1080/10408398.2011.650336.

Gouthu, S., P.A. Skinkis, J. Morre, C.S. Maier, and L.G. Deluc. 2012. Berry nitrogen status altered by cover cropping: Effects on berry hormone dynamics, growth and amino acid composition of Pinot Noir. Food Chemistry 135:1-8 doi: 10.1016/j.foodchem.2012.04.019.

Greenspan, M.D., K.A. Shackel, and M.A. Matthews. 1994. Developmental changes in the diurnal water budget of the grape berry exposed to water deficits. Plant, Cell & Environment 17:811-820.

Gregan, S.M., J.J. Wargent, L. Liu, J. Shinkle, R. Hofmann, C. Winefield, M. Trought, and B. Jordan. 2012. Effects of solar ultraviolet radiation and canopy manipulation on the biochemical composition of Sauvignon Blanc grapes. Australian Journal of Grape and Wine Research 18:227-238 doi: 10.1111/j.1755-0238.2012.00192.x.

Guinard, J.-X., and M. Cliff. 1987. Descriptive Analysis of Pinot noir Wines from Carneros, Napa, and Sonoma. American Journal of Enology and Viticulture 38:211-215.

Gunes, A., G. Soylemezoglu, A. Inal, E.G. Bagci, S. Coban, and O. Sahin. 2006. Antioxidant and stomatal responses of grapevine (*Vitis vinifera* L.) to boron toxicity. Scientia Horticulturae 110:279-284 doi: 10.1016/j.scienta.2006.07.014.

Guth, H. 1997. Quantitation and Sensory Studies of Character Impact Odorants of Different White Wine Varieties. Journal of Agricultural and Food Chemistry 45:3027-3032 doi: 10.1021/jf970280a.

Hakimi-Rezaei, J., and A.G. Reynolds. 2010. Characterization of Niagara Peninsula Cabernet franc Wines by Sensory Analysis. American Journal of Enology and Viticulture 61:1-14.

Hale, C.R., and R.J. Weaver. 1962. The effect of developmental stage on direction of translocation of photosynthate in *Vitis vinifera*. Hilgardia 33:89-131 doi: 10.3733/hilg.v33n03p039.

Hanlin, R.L., M. Hrmova, J.F. Harbertson, and M.O. Downey. 2010. Review: Condensed tannin and grape cell wall interactions and their impact on tannin extractability into wine. Australian Journal of Grape and Wine Research 16:173-188 doi: 10.1111/j.1755-0238.2009.00068.x.

Harbertson, J.F., R.E. Hodgins, L.N. Thurston, L.J. Schaffer, M.S. Reid, J.L. Landon, C.F. Ross, and D.O. Adams. 2008. Variability of Tannin Concentration in Red Wines. American Journal of Enology and Viticulture 59:210-214.

Harbertson, J.F., J.A. Kennedy, and D.O. Adams. 2002. Tannin in Skins and Seeds of Cabernet Sauvignon, Syrah, and Pinot noir Berries during Ripening. American Journal of Enology and Viticulture 53:54-59.

Hendry, G.A.F., J.D. Houghton, and S.B. Brown. 1987. Tansley Review No. 11. The Degradation of Chlorophyll-A Biological Enigma. New Phytologist 107:255-302 doi: 10.2307/2433054.

Herrero-Langreo, A., B. Tisseyre, J.-P. Goutouly, T. Scholasch, and C. van Leeuwen. 2013. Mapping Grapevine (*Vitis vinifera* L.) Water Status during the Season Using Carbon Isotope Ratio (δ13C) as Ancillary Data. American Journal of Enology and Viticulture 64:307-315 doi: 10.5344/ajev.2013.12125.

Hochberg, U., A. Degu, A. Fait, and S. Rachmilevitch. 2013a. Near isohydric grapevine cultivar displays higher photosynthetic efficiency and photorespiration rates under drought stress as compared with near anisohydric grapevine cultivar. Physiologia Plantarum 147:443-452 doi: 10.1111/j.1399-3054.2012.01671.x.

Hochberg, U., A. Degu, D. Toubiana, T. Gendler, Z. Nikoloski, S. Rachmilevitch, and A. Fait. 2013b. Metabolite profiling and network analysis reveal coordinated changes in grapevine water stress response. BMC Plant Biology 13:184.

Hofmann, R.W., B.D. Campbell, S.J. Bloor, E.E. Swinny, K.R. Markham, K.G. Ryan, and D.W. Fountain. 2003. Responses to UV-B radiation in *Trifolium repens* L. – physiological links to plant productivity and water availability. Plant, Cell & Environment 26:603-612 doi: 10.1046/j.1365-3040.2003.00996.x.

Holzapfel, B.P., and J.P. Smith. 2012. Developmental Stage and Climatic Factors Impact More on Carbohydrate Reserve Dynamics of Shiraz than Cultural Practice. American Journal of Enology and Viticulture doi: 10.5344/ajev.2012.11071.

Holzapfel, B.P., J.P. Smith, S.K. Field, and W.J. Hardie. 2010. Dynamics of carbohydrate reserves in cultivated grapevines. Horticultural reviews 37:143-211.

Iland, P.G., N. Bruer, G. Edwards, S. Weeks, and E. Wilkes. 2004. Chemical analysis of grapes and wine: techniques and concepts. . Patrick Iland Wine Promotions PTY Ltd., Campbelltown, South Australia, Australia.

Imre, S.P. 2011. A Multi-Disciplinary Study to Quantify Terroir in Central Otago and Waipara Pinot Noir Vineyards. Thesis, The University of Auckland.

Imre, S.P., P.A. Kilmartin, T. Rutan, J.L. Mauk, and L. Nicolau. 2012. Influence of soil geochemistry on the chemical and aroma profiles of Pinot noir wines Journal of food, agriculture & environment 10:280-288.

Imre, S.P., and J.L. Mauk. 2009. Geology and Wine 12. New Zealand Terroir. Geocience Canada 36:145-159.

International Organisation of Vine and Wine. 2014. Determination of chromatic characteristics according to CIELab. *In* Compendium of international methods of wine and must analysis. Vol. 1. OIV, Paris.

Intrigliolo, D.S., and J.R. Castel. 2006. Vine and soil-based measures of water status in a Tempranillo vineyard Vitis 45:157-163.

Intrigliolo, D.S., and J.R. Castel. 2009. Response of *Vitis vinifera* cv. 'Tempranillo' to partial rootzone drying in the field: Water relations, growth, yield and fruit and wine quality. Agricultural Water Management 96:282-292 doi: 10.1016/j.agwat.2008.08.001.

Jackson, D. 2001. Climate. Vol. 2. Monographs in cool climate viticulture. Daphne Brasell Associates Ltd. with Gypsum Press Wellington, New Zealand.

Jacobsen, J.G., and L.H. Smith. 1968. Biochemistry and physiology of taurine and taurine derivatives. Physiological Reviews 48:424-511.

Jones, G. 2003. Winegrape Phenology. *In* Phenology: An Integrative Environmental Science. Vol. 39. Schwartz, M. (Ed.), pp. 523-539. Tasks for Vegetation Science. Springer Netherlands

Jones, G. 2006. Climate and terroir: Impacts of climate variability and change on wine. *In* Fine wine and terroir: the geocience perspective. Macqueen, R. W. and L. D. Meinert (Eds.), pp. 203-216. Geological Association of Canada, St. John's, N.L.

Jones, G., M. Moriondo, B. Bois, A. Hall, and A. Duff. 2009. Analysis of the spatial climate structure in viticulture regions worldwide. Bulletin de l'OIV 82:507-517.

Jones, G.V., and R.E. Davis. 2000. Climate Influences on Grapevine Phenology, Grape Composition, and Wine Production and Quality for Bordeaux, France. American Journal of Enology and Viticulture 51:249-261.

Jones, G.V., M.A. White, O.R. Cooper, and K. Storchmann. 2005. Climate Change and Global Wine Quality. Climatic Change 73:319-343 doi: 10.1007/s10584-005-4704-2.

Jones, J.E., S.J. Wilson, G. Lee, and A.M. Smith. 2010. Effect of frost damage and pruning on current crop and return crop of Pinot Noir. New Zealand Journal of Crop and Horticultural Science 38:209-216 doi: 10.1080/01140671.2010.498402.

Kataoka, H., and N. Ohnishi. 1986. Ocurrence of taurine in plants. Agricultural and biological chemistry 50:1887-1888.

Kavi Kishor, P.B., and N. Sreenivasulu. 2014. Is proline accumulation per se correlated with stress tolerance or is proline homeostasis a more critical issue? Plant, Cell & Environment 37:300-311 doi: 10.1111/pce.12157.

Keller, M., Y.U.N. Zhang, P.M. Shrestha, M. Biondi, and B.R. Bondada. 2015. Sugar demand of ripening grape berries leads to recycling of surplus phloem water via the xylem. Plant, Cell & Environment 38:1048-1059 doi: 10.1111/pce.12465.

Kemp, B. 2010. The Effect of the Timing of Leaf Removal on Berry Ripening, Flavour and Aroma Compounds in Pinot Noir Wine. Thesis, Lincoln University.

Kemp, B.S., R. Harrison, and G.L. Creasy. 2011. Effect of mechanical leaf removal and its timing on flavan-3-ol composition and concentrations in Vitis vinifera L. cv. Pinot Noir wine. Australian Journal of Grape and Wine Research 17:270-279 doi: 10.1111/j.1755-0238.2011.00150.x.

Kennedy, J.A., M.A. Matthews, and A.L. Waterhouse. 2000a. Changes in grape seed polyphenols during fruit ripening. Phytochemistry 55:77-85 doi: 10.1016/S0031-9422(00)00196-5.

Kennedy, J.A., G.J. Troup, J.R. Pilbrow, D.R. Hutton, D. Hewitt, C.R. Hunter, R. Ristic, P.G. Iland, and G.P. Jones. 2000b. Development of seed polyphenols in berries from *Vitis vinifera* L. cv. Shiraz. Australian Journal of Grape and Wine Research 6:244-254 doi: 10.1111/j.1755-0238.2000.tb00185.x.

Kliewer, W.M. 1977. Influence of Temperature, Solar Radiation and Nitrogen on Coloration and Composition of Emperor Grapes. American Journal of Enology and Viticulture 28:96-103.

Kontkanen, D., A.G. Reynolds, M.A. Cliff, and M. King. 2005. Canadian terroir: sensory characterization of Bordeaux-style red wine varieties in the Niagara Peninsula. Food Research International 38:417-425.

Lähdesmäki, P. 1986. Determination of taurine and other acidic amino acids in plants. Phytochemistry 25:2409-2411 doi: 10.1016/S0031-9422(00)81707-0.

Landcare Research New Zealand. 2015. S-map online. Landcare research informatic team, New Zealand.

Leathwick, J., F. Morgan, G. Wilson, D. Rutledge, M. McLeod, and K. Johnston. 2002. Land environments of New Zealand: a technical guide. Ministry for the environment, Auckland.

Ledderhof, D., A.G. Reynolds, L. Manin, and R. Brown. 2014. Influence of water status on sensory profiles of Ontario Pinot noir wines. LWT - Food Science and Technology 57:65-82 doi: 10.1016/j.lwt.2013.12.010.

Lee, D.-H. 2015. *In vitro* analysis of taurine as anti-stress agent in tomato (*Solanum lycopersicum*) - Preliminary study. *In* Taurine 9. Marcinkiewicz, J. and S. W. Schaffer (Eds.), pp. 75-85. Advances in experimental medicine and biology 803. Springer International Publishing, Switzerland.

Lee, J., and R.P. Schreiner. 2010. Free amino acid profiles from 'Pinot noir' grapes are influenced by vine N-status and sample preparation method. Food Chemistry 119:484-489 doi: 10.1016/j.foodchem.2009.06.045.

Li, H., Y.-S. Tao, H. Wang, and L. Zhang. 2008. Impact odorants of Chardonnay dry white wine from Changli County (China). European Food Research and Technology 227:287-292 doi: 10.1007/s00217-007-0722-9.

Lincoln University soil resources class. 2014. A discovery and discussion of the soils of Greystone vineyard. Lincoln University, New Zealand.

Lopes, C., and P.A. Pinto. 2005. Easy and accurate estimation of grapevine leaf area with simple mathematical models. Vitis 44:55-61.

Lopes, C.M., A. Monteiro, J.P. Machado, N. Fernandes, and A. Araújo. 2008. Cover cropping in a slopping non-irrigated vineyard: II - effects on vegetative growth, yield, berry and wine quality of "Cabernet Sauvignon" grapevines. Ciência e Técnica Vitivinícola 23:37-43.

Lopes, C.M., T.P. Santos, A. Monteiro, M.L. Rodrigues, J.M. Costa, and M.M. Chaves. 2011. Combining cover cropping with deficit irrigation in a Mediterranean low vigor vineyard. Scientia Horticulturae 129:603-612 doi: 10.1016/j.scienta.2011.04.033.

Lopes, C.M.A., and P.A. Pinto. 2000. Estimation de la surface foliaire principale et secondaire d'un sarment de vigne. . Progre`s Agricole et Viticole 117:160-166.

Lovisolo, C., I. Perrone, A. Carra, A. Ferrandino, J. Flexas, H. Medrano, and A. Schubert. 2010. Drought-induced changes in development and function of grapevine (*Vitis* spp.) organs and in their hydraulic and non-hydraulic interactions at the whole-plant level: a physiological and molecular update. Functional Plant Biology 37:98-116 doi: 10.1071/FP09191.

Magné, C., and F. Larher. 1992. High sugar content of extracts interferes with colorimetric determination of amino acids and free proline Analytical Biochemistry 200:115-118.

Medrano, H., J.M. Escalona, J. Bota, J. Gulías, and J. Flexas. 2002. Regulation of Photosynthesis of C3 Plants in Response to Progressive Drought: Stomatal Conductance as a Reference Parameter. Annals of Botany 89:895-905 doi: 10.1093/aob/mcf079.

Medrano, H., M. Tomás, S. Martorell, J.-M. Escalona, A. Pou, S. Fuentes, J. Flexas, and J. Bota. 2015. Improving water use efficiency of vineyards in semi-arid regions. A review. Agronomy for Sustainable Development 35:499-517 doi: 10.1007/s13593-014-0280-z.

Mercurio, M.D., R.G. Dambergs, D. Cozzolino, M.J. Herderich, and P.A. Smith. 2010. Relationship between Red Wine Grades and Phenolics. 1. Tannin and Total Phenolics Concentrations. Journal of Agricultural and Food Chemistry 58:12313-12319 doi: 10.1021/jf103230b.

Mercurio, M.D., R.G. Dambergs, M.J. Herderich, and P.A. Smith. 2007. High Throughput Analysis of Red Wine and Grape Phenolics Adaptation and Validation of Methyl Cellulose Precipitable Tannin Assay and Modified Somers Color Assay to a Rapid 96 Well Plate Format. Journal of Agricultural and Food Chemistry 55:4651-4657 doi: 10.1021/jf063674n.

National Institute of Water and Atmospheric Research (NIWA) Ltd. 2013. Regional climatologies. https://www.niwa.co.nz/regional%20climatologies.

National Institute of Water and Atmospheric Research (NIWA) Ltd. 2014. CliFlo: NIWA's National Climate Database on the Web. http://cliflo.niwa.co.nz/.

Nepi, M. 2014. Beyond nectar sweetness: the hidden ecological role of non-protein amino acids in nectar. Journal of Ecology 102:108-115 doi: 10.1111/1365-2745.12170.

Nepi, M., C. Soligo, D. Nocentini, M. Abate, M. Guarnieri, G. Cai, L. Bini, M. Puglia, L. Bianchi, and E. Pacini. 2012. Amino acids and protein profile in floral nectar: Much more than a simple reward. Flora - Morphology, Distribution, Functional Ecology of Plants 207:475-481 doi: 10.1016/j.flora.2012.06.002.

New Zealand Winegrowers. 2014. Vineyard register report 2014. New Zealand wine

New Zealand Winegrowers. 2015. Annual report 2015. New Zealand wine

Ojeda, H., C. Andary, E. Kraeva, A. Carbonneau, and A. Deloire. 2002. Influence of Pre- and Postveraison Water Deficit on Synthesis and Concentration of Skin Phenolic Compounds during Berry Growth of *Vitis vinifera* cv. Shiraz. American Journal of Enology and Viticulture 53:261-267.

Ojeda, H., A. Deloire, and A. Carbonneau. 2001. Influence of water deficits on grape berry growth. Vitis 40:141-145.

Oliveira, C., A.C. Silva Ferreira, M. Mendes Pinto, T. Hogg, F. Alves, and P. Guedes de Pinho. 2003. Carotenoid Compounds in Grapes and Their Relationship to Plant Water Status. Journal of Agricultural and Food Chemistry 51:5967-5971 doi: 10.1021/jf034275k.

Ozden, M., U. Demirel, and A. Kahraman. 2009. Effects of proline on antioxidant system in leaves of grapevine (Vitis vinifera L.) exposed to oxidative stress by H2O2. Scientia Horticulturae 119:163-168 doi: 10.1016/j.scienta.2008.07.031.

Padgett-Johnson, M., L.E. Williams, and M.A. Walker. 2000. The Influence of Vitis riparia Rootstock on Water Relations and Gas Exchange of Vitis vinifera cv. Carignane Scion Under Non-Irrigated Conditions. American Journal of Enology and Viticulture 51:137-143.

Padgett-Johnson, M., L.E. Williams, and M.A. Walker. 2003. Vine Water Relations, Gas Exchange, and Vegetative Growth of Seventeen Vitis Species Grown under Irrigated and Nonirrigated Conditions in California. Journal of the American Society for Horticultural Science 128:269-276.

Palliotti, A., A. Cartechini, and F. Ferranti. 2000. Morpho-anatomical and Physiological Characteristics of Primary and Lateral Shoot Leaves of Cabernet Franc and Trebbiano Toscano Grapevines Under Two Irradiance Regimes. American Journal of Enology and Viticulture 51:122-130.

Pasantes-Morales, H., and R. Flores. 1991. Taurine content of Mexican beans. Journal of Food Composition and Analysis 4:322-328 doi: 10.1016/0889-1575(91)90018-2.

Pastor del Rio, J.L., and J.A. Kennedy. 2006. Development of Proanthocyanidins in *Vitis vinifera* L. cv. Pinot noir Grapes and Extraction into Wine. American Journal of Enology and Viticulture 57:125-132.

Patakas, A., N. Nikolaou, E. Zioziou, K. Radoglou, and B. Noitsakis. 2002. The role of organic solute and ion accumulation in osmotic adjustment in drought-stressed grapevines. Plant Science 163:361-367 doi: 10.1016/S0168-9452(02)00140-1.

Pedreira dos Santos, T., C.M. Lopes, M.L. Rodrigues, C.R. de Souza, J.M. Ricardo-da-Silva, J.P. Maroco, J.S. Pereira, and M.M. Chaves. 2007. Effects of deficit irrigation strategies on cluster microclimate for improving fruit composition of Moscatel field-grown grapevines. Scientia Horticulturae 112:321-330 doi: 10.1016/j.scienta.2007.01.006.

Pellegrino, A., P. Clingeleffer, N. Cooley, and R. Walker. 2014. Management practices impact vine carbohydrate status to a greater extent than vine productivity. Frontiers in Plant Science 5:283 doi: 10.3389/fpls.2014.00283.

Peyrot des Gachons, C., C. Van Leeuwen, T. Tominaga, J.-P. Soyer, J.-P. Gaudillère, and D. Dubourdieu. 2005. Influence of water and nitrogen deficit on fruit ripening and aroma potential of *Vitis vinifera* L cv Sauvignon blanc in field conditions. Journal of the Science of Food and Agriculture 85:73-85 doi: 10.1002/jsfa.1919.

Poni, S., M. Galbignani, E. Magnanini, F. Bernizzoni, A. Vercesi, M. Gatti, and M.C. Merli. 2014. The isohydric cv. Montepulciano (Vitis vinifera L.) does not improve its whole-plant water use efficiency when subjected to pre-veraison water stress. Scientia Horticulturae 179:103-111 doi: 10.1016/j.scienta.2014.09.021.

Porro, D., C. Dorigatti, M. Stefanini, and A. Ceschini. 2001. Use of SPAD meter in diagnosis of nutritional status in apple and grapevine. Acta Horticulturae (ISHS) 564:243-252.

Qian, M.C., Y. Fang, and K. Shellie. 2009. Volatile Composition of Merlot Wine from Different Vine Water Status. Journal of Agricultural and Food Chemistry 57:7459-7463 doi: 10.1021/jf9009558.

Reynard, J.-S., V. Zufferey, G.-C. Nicol, and F. Murisier. 2011. Soil parameters impact the vine-fruit-wine continuum by altering vine nitrogen status Journal International des Sciences de la Vigne et du Vin 45:211-221.

Reynolds, A.G., and J.E. Vanden Heuvel. 2009. Influence of Grapevine Training Systems on Vine Growth and Fruit Composition: A Review. American Journal of Enology and Viticulture 60:251-268.

Ristic, R., and P.G. Iland. 2005. Relationships between seed and berry development of *Vitis vinifera* L. cv Shiraz: Developmental changes in seed morphology and phenolic composition. Australian Journal of Grape and Wine Research 11:43-58 doi: 10.1111/j.1755-0238.2005.tb00278.x.

Robinson, A.L., P.K. Boss, P.S. Solomon, R.D. Trengove, H. Heymann, and S.E. Ebeler. 2014. Origins of Grape and Wine Aroma. Part 1. Chemical Components and Viticultural Impacts. American Journal of Enology and Viticulture 65:1-24 doi: 10.5344/ajev.2013.12070.

Roby, G., and M.A. Matthews. 2004. Relative proportions of seed, skin and flesh, in ripe berries from Cabernet Sauvignon grapevines grown in a vineyard either well irrigated or under water deficit. Australian Journal of Grape and Wine Research 10:74-82 doi: 10.1111/j.1755-0238.2004.tb00009.x.

Rogiers, S.Y., J.M. Hatfield, and M. Keller. 2004. Irrigation, nitrogen, and rootstock effects on volume loss of berries from potted Shiraz vines. Vitis 43:1-6.

Rogiers, S.Y., B.P. Holzapfel, and J.P. Smith. 2011. Sugar accumulation in roots of two grape varieties with contrasting response to water stress. Annals of Applied Biology 159:399-413 doi: 10.1111/j.1744-7348.2011.00505.x.

Romero, P., I.C. Dodd, and A. Martinez-Cutillas. 2012. Contrasting physiological effects of partial root zone drying in field-grown grapevine (*Vitis vinifera* L. cv. Monastrell) according to total soil water availability. Journal of Experimental Botany 63:4071-4083 doi: 10.1093/jxb/ers088.

Romero, P., J.I. Fernández-Fernández, and A. Martinez-Cutillas. 2010. Physiological Thresholds for Efficient Regulated Deficit-Irrigation Management in Winegrapes Grown under Semiarid Conditions. American Journal of Enology and Viticulture 61:300-312.

Romero, P., R. Gil-Muñoz, F.M. del Amor, E. Valdés, J.I. Fernández, and A. Martinez-Cutillas. 2013. Regulated Deficit Irrigation based upon optimum water status improves phenolic composition in Monastrell grapes and wines. Agricultural Water Management 121:85-101 doi: 10.1016/j.agwat.2013.01.007.

Romero, P., and A. Martinez-Cutillas. 2012. The effects of partial root-zone irrigation and regulated deficit irrigation on the vegetative and reproductive development of field-grown Monastrell grapevines. Irrigation Science 30:377-396 doi: 10.1007/s00271-012-0347-z.

Rose, R., C.L. Rose, S.K. Omi, K.R. Forry, D.M. Durall, and W.L. Bigg. 1991. Starch determination by perchloric acid vs enzymes: evaluating the accuracy and precision of six colorimetric methods. Journal of Agricultural and Food Chemistry 39:2-11 doi: 10.1021/jf00001a001.

Rozan, P., Y.-H. Kuo, and F. Lambein. 2001. Amino acids in seeds and seedlings of the genus Lens. Phytochemistry 58:281-289 doi: 10.1016/S0031-9422(01)00200-X.

Rusjan, D. 2010. Aromas in Grape and Wine. *In* Methodologies and Results in Grapevine Research. Delrot, S. et al. (Eds.), pp. 411-442. Springer Netherlands

Rustioni, L., M. Rossini, M. Calatroni, and O. Failla. 2011. Influence of bunch exposure on anthocyanins extractability from grapes skins (*Vitis vinifera* L.). Vitis 50:137-143.

Rutan, T., M. Herbst-Johnstone, B. Pineau, and P.A. Kilmartin. 2014. Characterization of the Aroma of Central Otago Pinot noir Wines Using Sensory Reconstitution Studies. American Journal of Enology and Viticulture doi: 10.5344/ajev.2014.14003.

Sabon, I., G. de Revel, Y. Kotseridis, and A. Bertrand. 2002. Determination of Volatile Compounds in Grenache Wines in Relation with Different Terroirs in the Rhone Valley. Journal of Agricultural and Food Chemistry 50:6341-6345 doi: 10.1021/jf025611k.

Sanchez-de-Miguel, P., P. Junquera, M. de la Fuente, L. Jimenez, R. Linares, P. Baeza, and J.R. Lissarrague. 2011. Estimation of vineyard leaf area by linear regression. Spanish Journal of Agricultural Research 9:202-212.

Santesteban, L.G., C. Miranda, I. Barbarin, and J.B. Royo. 2015. Application of the measurement of the natural abundance of stable isotopes in viticulture: a review. Australian Journal of Grape and Wine Research 21:157-167 doi: 10.1111/ajgw.12124.

Santesteban, L.G., C. Miranda, I. Urretavizcaya, and J.B. Royo. 2012. Carbon isotope ratio of whole berries as an estimator of plant water status in grapevine (*Vitis vinifera* L.) cv. 'Tempranillo'. Scientia Horticulturae 146:7-13 doi: 10.1016/j.scienta.2012.08.006.

Santesteban, L.G., and J.B. Royo. 2006. Water status, leaf area and fruit load influence on berry weight and sugar accumulation of cv. 'Tempranillo' under semiarid conditions. Scientia Horticulturae 109:60-65 doi: dx.doi.org/10.1016/j.scienta.2006.03.003.

Schlosser, J., A.G. Reynolds, M. King, and M. Cliff. 2005. Canadian terroir: sensory characterization of Chardonnay in the Niagara Peninsula. Food Research International 38:11-18.

Schreiner, R.P., J. Lee, and P.A. Skinkis. 2013. N, P, and K Supply to Pinot noir Grapevines: Impact on Vine Nutrient Status, Growth, Physiology, and Yield. American Journal of Enology and Viticulture 64:26-38 doi: 10.5344/ajev.2012.12064.

Schreiner, R.P., C.F. Scagel, and J. Lee. 2014. N, P, and K Supply to Pinot noir Grapevines: Impact on Berry Phenolics and Free Amino Acids. American Journal of Enology and Viticulture 65:43-49 doi: 10.5344/ajev.2013.13037.

Schultz, H.R. 2003. Differences in hydraulic architecture account for near-isohydric and anisohydric behaviour of two field-grown Vitis vinifera L. cultivars during drought. Plant, Cell & Environment 26:1393-1405 doi: 10.1046/j.1365-3040.2003.01064.x.

Schuster, D., D. Jackson, and R. Tipples. 2002. Canterbury grapes & wines. Shoal Bay Press Ltd., Christchurch, New Zealand.

Shaw, T.B. 2012. A climatic analysis of wine regions growing pinot noir. Journal of Wine Research 23:203-228 doi: 10.1080/09571264.2012.697356.

Sipiora, M.J., M.M. Anderson, and M.A. Matthews. 2005. Composition of *Vitis vinifera* L. cv. Pinot noir Fruit and Wines from Carneros Appellation in Response to Potassium Fertlization and Supplemental Irrigation. *In* Soil Environment and Vine Mineral Nutrition Symposium. pp. 185-192, San Diego, CA.

Smart, R., and M. Robinson. 1991. Sunlight into wine: A handbook of winegrape canopy management. Winetitles, Adelaide.

Smith, F.A., and J.A. Raven. 1979. Intracellular pH and its Regulation. Annual Review of Plant Physiology 30:289-311 doi: 10.1146/annurev.pp.30.060179.001445.

Somers, C.T., and M.E. Evans. 1977. Spectral evaluation of young red wines: Anthocyanin equilibria, total phenolics, free and molecular $SO_2$, “chemical age”. Journal of the Science of Food and Agriculture 28:279-287 doi: 10.1002/jsfa.2740280311.

Song, J., R. Smart, H. Wang, B. Dambergs, A. Sparrow, and M.C. Qian. 2015a. Effect of grape bunch sunlight exposure and UV radiation on phenolics and volatile composition of *Vitis vinifera* L. cv. Pinot noir wine. Food Chemistry 173:424-431 doi: 10.1016/j.foodchem.2014.09.150.

Song, J., R.E. Smart, R.G. Dambergs, A.M. Sparrow, R.B. Wells, H. Wang, and M.C. Qian. 2014. Pinot Noir wine composition from different vine vigour zones classified by remote imaging technology. Food Chemistry 153:52-59 doi: 10.1016/j.foodchem.2013.12.037.

Song, M., Y. Xia, and E. Tomasino. 2015b. Investigation of a Quantitative Method for the Analysis of Chiral Monoterpenes in White Wine by HS-SPME-MDGC-MS of Different Wine Matrices. Molecules 20:7359.

Spayd, S.E., J.M. Tarara, D.L. Mee, and J.C. Ferguson. 2002. Separation of Sunlight and Temperature Effects on the Composition of *Vitis vinifera* cv. Merlot Berries. American Journal of Enology and Viticulture 53:171-182.

Steele, M.R., A.A. Gitelson, and D.C. Rundquist. 2008. A Comparison of Two Techniques for Nondestructive Measurement of Chlorophyll Content in Grapevine Leaves. Agron. J. 100:779-782 doi: 10.2134/agronj2007.0254N.

Sternad Lemut, M., P. Sivilotti, P. Franceschi, R. Wehrens, and U. Vrhovsek. 2013a. Use of Metabolic Profiling To Study Grape Skin Polyphenol Behavior as a Result of Canopy Microclimate Manipulation in a 'Pinot noir' Vineyard. Journal of Agricultural and Food Chemistry 61:8976-8986 doi: 10.1021/jf4030757.

Sternad Lemut, M., K. Trost, P. Sivilotti, P. Arapitsas, and U. Vrhovsek. 2013b. Early versus late leaf removal strategies for Pinot Noir (*Vitis vinifera* L.): effect on colour-related phenolics in young wines following alcoholic fermentation. Journal of the Science of Food and Agriculture 93:3670-3681 doi: 10.1002/jsfa.6193.

Stines, A.P., J. Grubb, H. Gockowiak, P.A. Henschke, P.B. HØJ, and R. van Heeswijck. 2000. Proline and arginine accumulation in developing berries of *Vitis vinifera* L. in Australian vineyards: Influence of vine cultivar, berry maturity and tissue type. Australian Journal of Grape and Wine Research 6:150-158 doi: 10.1111/j.1755-0238.2000.tb00174.x.

Tesic, D., D.J. Woolley, E.W. Hewett, and D.J. Martin. 2002a. Environmental effects on cv Cabernet Sauvignon (*Vitis vinifera* L.) grown in Hawke's Bay, New Zealand.: 1. Phenology and characterisation of viticultural environments. Australian Journal of Grape and Wine Research 8:15-26 doi: 10.1111/j.1755-0238.2002.tb00207.x.

Tesic, D., D.J. Woolley, E.W. Hewett, and D.J. Martin. 2002b. Environmental effects on cv Cabernet Sauvignon (*Vitis vinifera* L.) grown in Hawke's Bay, New Zealand.: 2. Development of a site index. Australian Journal of Grape and Wine Research 8:27-35 doi: 10.1111/j.1755-0238.2002.tb00208.x.

Tevatia, R., J. Allen, D. Rudrappa, D. White, T.E. Clemente, H. Cerutti, Y. Demirel, and P. Blum. 2015. The taurine biosynthetic pathway of microalgae. Algal Research 9:21-26 doi: 10.1016/j.algal.2015.02.012.

Thomas, H. 2013. Senescence, ageing and death of the whole plant. New Phytologist 197:696-711 doi: 10.1111/nph.12047.

Tomás, M., H. Medrano, J.M. Escalona, S. Martorell, A. Pou, M. Ribas-Carbó, and J. Flexas. 2014. Variability of water use efficiency in grapevines. Environmental and Experimental Botany 103:148-157 doi: 10.1016/j.envexpbot.2013.09.003.

Tomás, M., H. Medrano, A. Pou, J.M. Escalona, S. Martorell, M. Ribas-Carbó, and J. Flexas. 2012. Water-use efficiency in grapevine cultivars grown under controlled conditions: effects of water stress at the leaf and whole-plant level. Australian Journal of Grape and Wine Research 18:164-172 doi: 10.1111/j.1755-0238.2012.00184.x.

Tomasino, E. 2011. Characterization of regional examples of New Zealand Pinot noir by means of sensory and chemical analysis. Thesis, Lincoln University.

Tomasino, E., R. Harrison, J. Breitmeyer, R. Sedcole, R. Sherlock, and A. Frost. 2015. Aroma composition of 2-year-old New Zealand Pinot Noir wine and its relationship to sensory characteristics using canonical correlation analysis and addition/omission tests. Australian Journal of Grape and Wine Research 21:376-388 doi: 10.1111/ajgw.12149.

Tomasino, E., R. Harrison, R. Sedcole, and A. Frost. 2013. Regional Differentiation of New Zealand Pinot noir Wine by Wine Professionals Using Canonical Variate Analysis. American Journal of Enology and Viticulture 64:357-363 doi: 10.5344/ajev.2013.12126.

Tonkin, P., T. Webb, P. Almond, L. Hassall, R. Harrison, and G.L. Creasy. 2014. The soils and landscapes associated with North Canterbury wines. Draft report. Lincoln University, New Zealand.

Tramontini, S., J. Döring, M. Vitali, A. Ferrandino, M. Stoll, and C. Lovisolo. 2014. Soil water-holding capacity mediates hydraulic and hormonal signals of near-isohydric and near-anisohydric *Vitis* cultivars in potted grapevines. Functional Plant Biology 41:1119-1128 doi: 10.1071/FP13263.

Trought, M.C.T., and R.G.V. Bramley. 2011. Vineyard variability in Marlborough, New Zealand: characterising spatial and temporal changes in fruit composition and juice quality in the vineyard. Australian Journal of Grape and Wine Research 17:79-89 doi: 10.1111/j.1755-0238.2010.00120.x.

Turner, N.C. 1988. Measurement of plant water status by the pressure chamber technique. Irrigation Science 9:289-308 doi: 10.1007/BF00296704.

Ubalde, J.M., X. Sort, R.M. Poch, and M. Porta. 2007. Influence of edapho-climatic factors on grape quality in Conca de Barbera vineyards (Catalonia, Spain). Journal International des Sciences de la Vigne et du Vin 41:33-41.

Ugliano, M., and P.A. Henschke. 2009. Yeasts and Wine Flavour. *In* Wine Chemistry and Biochemistry. Moreno-Arribas, M. V. and M. C. Polo (Eds.), pp. 313-392. Springer New York, New York, NY.

Van Heeswijck, R., A.P. Stines, J. Grubb, I.S. Møller, and P.B. Høj. 2001. Molecular Biology and Biochemistry of Proline Accumulation in Developing Grape Berries. *In* Molecular Biology & Biotechnology of the Grapevine. Roubelakis-Angelakis, K. (Ed.), pp. 87-108. Springer Netherlands

Van Leeuwen, C. 2009. Soils and Terroir Expression in Wines. *In* Soil and Culture. Landa, E. R. and C. Feller (Eds.), pp. 453-465. Springer Netherlands

Van Leeuwen, C., P. Friant, X. Choné, O. Tregoat, S. Koundouras, and D. Dubourdieu. 2004. Influence of Climate, Soil, and Cultivar on Terroir. American Journal of Enology and Viticulture 55:207-217.

Van Leeuwen, C., P. Pieri, and P. Vivin. 2010. Comparison of Three Operational Tools for the Assessment of VineWater Status: Stem Water Potential, Carbon Isotope Discrimination Measured on Grape Sugar and Water Balance. *In* Methodologies and Results in Grapevine Research. Delrot, S. et al. (Eds.), pp. 87-106. Springer Netherlands

Van Leeuwen, C., and G. Seguin. 2006. The concept of terroir in viticulture. Journal of Wine Research 17:1-10 doi: 10.1080/09571260600633135.

Van Leeuwen, C., O. Tregoat, X. Chone, B. Bois, D. Pernet, and J.P. Gaudillère. 2009. Vine water status is a key factor in grape ripening and vintage quality for red Bordeaux wine. How can it be assessed for vineyard management purposes? Journal International des Sciences de la Vigne et du Vin 43:121-134.

Vranova, V., K. Rejsek, K. Skene, and P. Formanek. 2011. Non-protein amino acids: plant, soil and ecosystem interactions. Plant and Soil 342:31-48 doi: 10.1007/s11104-010-0673-y.

Williams, L.E. 1996. Grape. *In* Photoassimilate Distribution in Plants and Crops: Source-Sink Relationships. Zamski, E. and A. Schaffer (Eds.). Dekker, New York, NY.

Winkler, A.J., J.A. Cook, W.M. Kliewer, and L.A. Lider. 1974. Climate and soils. *In* General Viticulture pp. 58-76. University of California Press, Berkeley and Los Angeles, California.

Zapata, C., E. Deléens, S. Chaillou, and C. Magné. 2004. Partitioning and mobilization of starch and N reserves in grapevine (*Vitis vinifera* L.). Journal of Plant Physiology 161:1031-1040 doi: 10.1016/j.jplph.2003.11.009.

Zea, L., L. Moyano, J. Moreno, B. Cortes, and M. Medina. 2001. Discrimination of the aroma fraction of Sherry wines obtained by oxidative and biological ageing. Food Chemistry 75:79-84 doi: 10.1016/S0308-8146(01)00190-X.

Zhu, J.-K. 2002. Salt and drought stress signal transduction in plants. Annual Review of Plant Biology 53:247-273 doi: 10.1146/annurev.arplant.53.091401.143329.

Zsófi, Z., L. Gál, Z. Szilágyi, E. Szűcs, M. Marschall, Z. Nagy, and B. Bálo. 2009. Use of stomatal conductance and pre-dawn water potential to classify terroir for the grape variety Kékfrankos. Australian Journal of Grape and Wine Research 15:36-47 doi: 10.1111/j.1755-0238.2008.00036.x.

Zyakun, A.M., L.A. Oganesyants, A.L. Panasyuk, E.I. Kuz'mina, A.A. Shilkin, B.P. Baskunov, V.N. Zakharchenko, and V.P. Peshenko. 2013. Mass spectrometric analysis of the 13C/12C abundance ratios in vine plants and wines depending on regional climate factors (Krasnodar krai and Rostov oblast, Russia). Journal of Analytical Chemistry 68:1136-1141 doi: 10.1134/S106193481313011X.