The effect of within-vineyard variability in vigour and water status on carbon discrimination in *Vitis vinifera* L. cv Merlot

by

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Declaration

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Summary

Within-vineyard variability in vigour and water status commonly occurs in South African vineyards. Different soil types found over short distances are probably the main cause of vigour variability, while differences in grapevine water status are commonly induced by lateral water flow in the vineyard, blocked irrigation emitters and differences in soil water-holding capacity. These factors can cause heterogeneous ripening and differences in fruit quality between different parts of the vineyard, an aspect that needs to be avoided as far as possible in order to produce quality wines. Measurements of carbon isotope discrimination (CID) have proved to be a tool to assess grapevine physiology in order to study the effects of environmental parameters on leaf carbon dioxide (CO_2) gas exchange and stomatal conductance (gs). Grapevine water deficit stress/strain in reaction to these environmental conditions can then be determined by observing the amount of ^{13}C absorbed by plant material after discrimination of ^{13}C has taken place, and this is influenced by the grapevine stress condition and can indicate water-use efficiency.

In this study, the variability of grapevine water status and vigour was determined in order to quantify these parameters in different parts of the vineyard. Two separate trials were conducted, the first at Wellington, South Africa, where different irrigation regimes resulted in variability in grapevine water status between plots. The second trial was at Stellenbosch, South Africa, where plots were divided among different vigour classes and irrigation was applied in different quantities for different irrigation treatments. Within-vineyard variability in water status (Wellington and Stellenbosch) and vigour (Stellenbosch) were then quantified and the effects on some grapevine physiological parameters and berry composition were measured.

The treatments in the Wellington trial led to differences in grapevine water status, which could be quantified by measurements of stem water potential (SWP) and leaf water potential (LWP). Soil variability also led to differences in grapevine vigour, which were quantified by measurements of pruning mass, leaf area and shoot length. The effect of the variability in grapevine water status on grapevine physiology was assessed by measuring CID, which was the main focus of the study. Other physiological measurements, such as gs and leaf and canopy temperature, were also conducted. The effect of these conditions on grape berry composition was also studied.

In the Stellenbosch trial, soil water content, plant water status measurements (SWP, pre-dawn LWP and LWP), physiological measurements (CID and gs) and berry size measurements were used to classify plots into water status treatments (“wet” and “dry” treatments). The effect of vigour differences was analysed separately from these treatments by using pruning mass as a covariate in the statistical analyses. The effect of vigour variability on the measurements was studied by looking at the effect of the covariate on the measurements, while shoot growth rate, shoot length and leaf area measurements were conducted as vegetative growth measurements. Differences in measurements were then studied between the treatments and between the vigour levels of the different plots.

In the Wellington trial, plant water status was determined by irrigation, showing increased stress for treatments that received less irrigation. The differences in plant water status then caused differences in grapevine physiology between the treatments, leading to increased gs for

increased irrigation. This of course influenced leaf internal CO_2 and therefore CID, although CID was also clearly influenced by berry development. Berry size was influenced by irrigation, with larger berries found in wetter treatments, while berry chemical composition was influenced by the irrigation regime, with increased irrigation leading to increased pH and leading to trends showing increased total soluble solids and malic acid, and reduced total and tartaric acid and colour intensity.

In the Stellenbosch trial, plots with higher vigour had increased shoot growth rate, longer shoots and increased leaf area, although topping influenced this. Wet treatment vines also showed slightly longer shoots and larger leaf areas. There were differences in soil water content between the wet and dry treatments, and this led to differences in plant water status. Vigour also influenced pre-dawn LWP, especially in the 2007 season, as higher-vigour vines struggled more to rehydrate through the night.

Differences in plant water potential led to differences in grapevine physiology, with increased gs for vines from the wet treatment, while higher-vigour vines had slightly increased gs. The differences in gs led to gas exchange differences and therefore differences in CID, meaning that water status and vigour influenced CID. CID measurements illustrated the long term effect of water status on plant physiology, while measurements such as SWP illustrated the short term effects. CID measurements therefore proved to be accumulative over the season, in contrast to SWP measurements that were much more dependent on the current state of grapevine water status. Other physiological measurements showed that wet-treatment vines had higher photosynthetic rates and evapotranspiration and lower leaf temperatures, while higher-vigour vines had slightly increased evapotranspiration and decreased leaf temperatures. Wet-treatment vines had larger berries, while a higher vigour also led to slightly larger berries. Berry composition was influenced by treatment, where wet-treatment vines had increased pH and total soluble solids, while higher-vigour vines had increased juice pH and, in the 2008 season, decreased total soluble solids.

Extremely stressed conditions did not show significant effects on plant water potential, but SWP measurements indicated slightly higher stress for the extremely stressed vines and LWP showed slightly less stressed conditions for these vines. Measurements of gs showed slightly lower values for the extremely stressed vines, while measurements of CID showed large significant differences, with the extremely stressed vines having measurements showing high stress. The measurement therefore indicated highly stressed conditions accurately, while other physiological measurements, such as photosynthetic rate, evapotranspiration and leaf temperatures, only showed trends and no significant differences. Measurements of stomatal conductance reacted to plant water status measurements throughout the diurnal measurement days, while CID only reacted slightly with gs changes during these days and was perhaps influenced more by berry chemical composition and development at this early stage of the season.

Vigour and water status therefore influenced grapevine physiology, with a more direct effect by water status and an indirect effect by vigour due to microclimatic differences. This also influenced berry composition and therefore quality.

In future studies, CID measurements should be done on juice from which organic acids have been removed in order to eliminate the effect of seasonal berry composition on the measurement.

Measurements of CID proved to be an integrative, but sensitive, indicator of grapevine stress, especially at the end of the season. It might at best be useful as a post-harvest management tool for producers or grape buyers, especially for irrigation control, as has also been stated by Van Leeuwen *et al*. (2007).

Opsomming

Binne-wingerd variasie in groeikrag en waterstatus is algemeen in Suid-Afrikaanse wingerde. Verskillende grondsoorte wat na aan mekaar voorkom, is seker een van die vernaamste oorsake van variasie in groeikrag, terwyl verskille in wingerdwaterstatus algemeen deur laterale watervloei in die wingerd, verstopte besproeiingspuite en verskille in grond waterhouvermoë geïnduseer word. Hierdie faktore kan aanleiding gee tot heterogene rypwording en verskille in vrugkwaliteit tussen verskillende dele van die wingerd, ‘n aspek wat so ver moontlik vermy moet word om kwaliteitwyne te kan produseer. Die meting van koolstof-isotoopdiskriminasie (KID) is bewys om as gereedskap te kan dien vir die assessering van wingerdfisiologie om die effekte van omgewingsparameters op blaar koolstofdioksied (CO_2) - gasuitruiling en stomatale geleiding (gs) te bestudeer. Die stres/stremming as gevolg van ‘n watertekort in die wingerd in reaksie op hierdie omgewingstoestande kan dan bepaal word deur te kyk na hoeveel ^{13}C deur die plantmateriaal geabsorbeer word ná ^{13}C-diskriminasie plaasgevind het, en dít word deur die wingerdstrestoestande beïnvloed en kan ‘n aanduiding verskaf van die doeltreffendheid van waterverbruik.

In hierdie studie is die variasie in wingerdwaterstatus en groeikrag bepaal om hierdie parameters in verskillende dele van die wingerd te kwantifiseer. Twee afsonderlike proewe is uitgevoer, die eerste by Wellington, Suid-Afrika, waar verskillende besproeiingsregimes gelei het tot verskille in die wingerdwaterstatus tussen persele. Die tweede proef was by Stellenbosch, Suid-Afrika, waar persele tussen verskillende groeikragklasse verdeel is en besproeiing in verskillende hoeveelhede vir verskillende besproeiingsbehandelings toegepas is. Binne-wingerd variasie in waterstatus (Wellington en Stellenbosch) en groeikrag (Stellenbosch) is toe gekwantifiseer en die effekte op sekere wingerd-fisiologiese parameters en korrelsamestelling is gemeet.

Die behandelings in die Wellington-proef het gelei tot verskille in wingerdwaterstatus, wat deur metings van stamwaterpotensiaal (SWP) en blaarwaterpotensiaal (BWP) gekwantifiseer kon word. Grondverskille het ook gelei tot verskille in wingerdgroeikrag, wat deur metings van snoeimassa, blaaroppervlak en lootlengte gekwantifiseer is. Die effek van die variasie in wingerdwaterstatus op wingerdfisiologie is deur metings van KID bepaal wat die hooffokus van hierdie studie was. Ander fisiologiese metings, soos gs en blaar- en lowertemperatuur, is ook gedoen. Die effekte van hierdie toestande op die samestelling van die druiwekorrels is ook bestudeer.

In die Stellenbosch-proef is grondwaterinhoud, metings van plantwaterstatus (SWP, voor-sonopgang SWP en BWP), fisiologiese metings (KID en gs) en metings van korrelgrootte gebruik om die persele in waterstatusbehandelings (“nat” en “droë” behandelings) te verdeel. Die effek van verskille in groeikrag is apart van hierdie behandelings geanaliseer deur snoeimassa as ‘n kovariaat in die statistiese analises te gebruik. Die effek van groeikragvariasie op die metings is bestudeer deur ondersoek in te stel na die effek van die kovariaat op die metings, terwyl lootgroeitempo-, lootlengte- en blaaroppervlakmetings as metings van vegetatiewe groei uitgevoer is. Verskille in metings tussen die behandelings en tussen die groeikragvlakke van die verskillende persele is toe bestudeer.

In die Wellington-proef is plantwaterstatus deur besproeiing bepaal, met verhoogde stres in behandelings waar daar minder besproeiing toegedien is. Die verskille in plantwaterstatus het dan verskille in wingerdfisiologie tussen die behandelings veroorsaak, wat gelei het tot ‘n verhoogde gs in die geval van verhoogde besproeiing. Dit het natuurlik ‘n effek op die interne CO_2 van die blaar en dus op KID gehad, hoewel KID ook duidelik deur korrelontwikkeling beïnvloed is. Korrelgrootte is deur besproeiing beïnvloed, met groter korrels in die natter behandelings, terwyl die chemiese samestelling van die korrel deur besproeiingsregime beïnvloed is. Verhoogde besproeiing het pH verhoog en gelei na tendense wat verhoogde totale oplosbare vaste stowwe en appelsuur, en verminderde totale suur, wynsteensuur en kleurintensiteit getoon het.

In die Stellenbosch-proef het persele met hoër groeikrag ook verhoogde lootgroeitempo, langer lote en verhoogde blaaroppervlak getoon, hoewel dit deur top beïnvloed is. Wingerdstokke van die nat behandeling het ook effe langer lote en groter blaaroppervlakke getoon. Daar was verskille in grondwaterinhoud tussen die nat en droë behandelings en dit het verskille in plantwaterstatus veroorsaak. Groeikrag is ook deur voor-sonopgang BWP beïnvloed, veral in die 2007-seisoen, aangesien stokke met hoër groeikrag meer gesukkel het om in die nag te rehidreer.

Verskille in plantwaterpotensiaal het gelei tot verskille in wingerdfisiologie, met ‘n verhoogde gs vir stokke in die nat behandeling, terwyl stokke met hoër groeikrag ‘n effens verhoogde gs getoon het. Die verskille in gs het gelei tot verskille in gasuitruiling en dus verskille in KID, wat beteken dat waterstatus en groeikrag ‘n invloed op KID het. KID was meer verteenwoordigend van die langtermyneffekte van water status op plantfisiologie, terwyl metings soos SWP die korttermyneffekte weerspieël het. KID metings was dus akkumalatief oor die seisoen, terwyl SWP metings meer ‘n weerspieëling was van die huidige toestand van plantwaterpotensiaal. Ander fisiologiese metings het getoon dat stokke in die nat behandeling ‘n hoër fotosintese-tempo en evapotranspirasie sowel as laer blaartemperature ondervind het, terwyl die stokke met hoër groeikrag effe verhoogde evapotranspirasie en verminderde blaartemperature getoon het. Stokke in die nat behandeling het groter korrels gehad, terwyl hoër groeikrag ook effens groter korrels veroorsaak het. Korrelsamestelling is deur die behandelings beïnvloed, met stokke in die nat behandeling wat verhoogde pH en totale oplosbare vaste stowwe getoon het, terwyl stokke met hoër groeikrag verhoogde pH van die sap en verminderde totale oplosbare vaste stowwe (laasgenoemde in die 2008-seisoen) gehad het.

Uitermate toestande van stres het geen beduidende effekte op plantwaterpotensiaal getoon nie, hoewel SWP-metings effens hoër stres vir die uitermate gestresde wingerde getoon het en BWP effens minder gestresde toestande vir hierdie stokke getoon het. Metings van gs het effens laer waardes vir die uitermate gestresde stokke getoon, terwyl metings van KID groot noemenswaardige verskille getoon het, met die metings vir die uitermate gestresde wingerde wat hoër stres aangedui het. Dié meting het dus hoogs gestresde toestande akkuraat aangedui, terwyl ander fisiologiese metings, soos tempo van fotosintese, evapotranspirasie en blaartemperature net tendense en nie beduidende verskille aangedui het nie. Metings van stomatale geleiding het dwarsdeur die dae waarop daaglikse metings gedoen is op plantwaterstatusmetings gereageer, terwyl KID net effens met gs-veranderinge op hierdie dae

gereageer het en moontlik meer deur die chemiese samestelling en ontwikkeling van die korrel in hierdie vroeë stadium van die seisoen beïnvloed is.

Groeikrag en waterstatus het dus wingerdfisiologie beïnvloed, met ‘n meer direkte effek deur waterstatus en ‘n indirekte effek deur groeikrag as gevolg van mikroklimaatsverskille. Dit het ook korrelsamestelling en dus kwaliteit beïnvloed.

In toekomstige studies moet KID-metings gedoen word op sap waarvan die organiese sure verwyder is om die effek van seisoenale korrelsamestelling op die meting uit te sluit.

Metings van KID is getoon om ‘n integrerende, maar gevoelige, aanduider van wingerdstres te wees, veral aan die einde van die seisoen. Dit is ten beste miskien bruikbaar as naoes-bestuursgereedskap vir produsente of druiwekopers, veral vir besproeiingsbeheer, soos ook reeds deur Van Leeuwen *et al*. (2007) aangedui is.

This thesis is dedicated to
my parents for making my studies possible and to the Lord for giving me the strength

Biographical sketch

Gerhard Rossouw was born in Paarl on 4 January 1984. He grew up in Vredendal and matriculated at Vredendal High School in 2002. He obtained his BScAgric degree in viticulture and oenology in 2006 and enrolled for his MScAgric degree in viticulture in 2007.

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Preface

This thesis is presented as a compilation of five chapters. .

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Chapter 1

Introduction and project aims

INTRODUCTION AND PROJECT AIMS

1.1 INTRODUCTION

Stress or strain can develop by various and complex means in grapevines, causing alterations in grapevine performance, which in turn leads to reactions affecting grape development and plant growth. This will ultimately affect fruit composition and quality, and therefore will determine wine quality to a certain degree. This stress/strain is caused by environmental and management inputs, which include climate (rainfall, temperature, light intensity, etc.), vineyard soil composition and the terroir of the vineyard, while irrigation and canopy management also influence the stress development of a vineyard. Grapevine water status and water balance is influenced by different factors, like available soil water, rain and irrigation, intercepted sunlight and potential evapotranspiration (PET), changes in these factors will therefore influence grapevine water status. Soil water content and rain/irrigation will influence the amount of water uptake by the roots, while sunlight interception influences stomatal conductance (De Souza *et al*., 2003) and therefore plant water loss, while PET also indicates plant water loss through transpiration.

Water stress can be manipulated by means of irrigation to induce mild water deficits, increasing grapevine water-use efficiency and potentially improving grape composition by keeping grapevine vigour at optimal levels, increasing the likelihood of the production of more fruit of good quality, and increasing the potential of the grapevine to reach its yield potential (Dry and Loveys, 1998). Within vineyards, variability in vigour and water deficit is a common phenomenon, especially in South Africa and particularly in the Western Cape region because of highly variable soils and terroirs in the region. The resulting variability in vineyards can cause some problems for the grapevine grower when it leads to heterogeneous ripening and quality for different regions within the vineyard, making precision viticulture necessary to promote homogeneity.

To understand the impact of within-vineyard variability in vigour and water deficit on grapevine performance, it is necessary to measure this variability and quantify it in order to see its integrated effect on the development of quality in the fruit. Carbon isotope discrimination measured on grape carbohydrates ($^{13}C/^{12}C$) is a technologically advanced method to determine grapevine water status by using a sample of grape juice for analysis. This ratio can give an overview of the leaf photosynthetic discrimination as sugar translocation takes place from the leaves to the berries (Davies and Robinson, 1996), and therefore the impact of factors affecting photosynthesis can be studied. Variability in grapevine vigour and water status will influence photosynthesis, and thus the photosynthetic rate in different areas in a vineyard. Measuring carbon isotopic discrimination could therefore indicate the potential effect on grapevine performance and development when assessed along with different levels of water deficit and differing grapevine vigour.

In many studies, grapevine water deficit status is only expressed by measurement of stem water potential, pre-dawn leaf water potential and midday leaf water potential as plant-based water status measurements, while carbon discrimination research develops internationally as a

tool for measuring grapevine water status. More consideration therefore needs to be given to it, as an accurate stress status determinant, especially in South Africa, as it is an integrative parameter, carrying more complex information than water potential measurements (Gaudillere *et al*., 1999).

In this study, plant-based water status determinants were used to assess grapevine water status in plots of differing vigour within a vineyard, while vegetative growth was also measured to ultimately determine the variability of vigour and water status before evaluating the effect of these parameters on carbon isotopic discrimination. Grape composition was also evaluated for the different treatments. Treatments included differences in irrigation regimes in a vineyard in the Wellington area, and different irrigation regimes within different vigour areas in a vineyard in the Stellenbosch area. In summary an attempt was made to assess grapevine vigour variability and water status differences on an intra-vineyard scale (between vines within the vineyard blocks of the studies) and possible advantages of using carbon isotope discrimination to quantify variability through the season.

1.2 PROJECT AIMS

In previous studies of carbon discrimination in viticulture, a lot of research was done on the effect of water deficits on carbon discrimination, but almost no studies evaluated the effects of vigour variability or the integrated effects of vigour and water stratus variability on carbon isotopic discrimination.

The main aims of this study were to measure the effects of vigour variability and different watering regimes on berry carbohydrates carbon discrimination.

AIM 1: Measuring the effects of different irrigation regimes, including a partial rootzone drying (PRD) treatment (in total 24 plots within the vineyard), on carbon discrimination in a warm summer area, with semi-arid conditions and cool winters (Bonnardot, 2005) (Wellington area, Western Cape, South Africa).

AIM 2: Measuring the effect of variability in vigour and water status within a vineyard on carbon discrimination in a warm summer area (cooler than the Wellington area) with cool spring temperatures and cool, wet winters (Bonnardot, 2005) in the Stellenbosch area of the Western Cape, South Africa.

The secondary aims were:

A. Measuring the effects of variability in vigour levels and water deficits on grapevine physiology.
B. Measuring the effects of variability in vigour levels and water deficits on grapevine reproductive development.

From this study, producers may potentially gain information on how carbon discrimination could be used to optimise irrigation scheduling to manipulate water status levels within a vineyard so

as to achieve optimal balance in the vegetative and reproductive growth for optimum grape quality.

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Chapter 2

Literature review

The effect of within-vineyard variability in vigour and water status on carbon discrimination in *Vitis vinifera* L. cv Merlot

Literature Review

2.1. INTRODUCTION

Vigour variability is a reality in almost all vineyards. Topographical and soil differences are possible within small or especially within larger vineyard blocks, while microclimate and even mesoclimate differences may also exist between different parts of a vineyard. These differences, especially differences in soil water holding capacity, will cause differences in the growth pattern of vines within the different parts of the vineyard, leading to differences in vigour. The water use efficiency can also be affected by differences in soil water content and water-holding capacity. Vigour differences lead to differences in the water use of vines, as vines with a higher vigour and larger leaf areas may have increased evapotranspiration compared to vines with smaller leaf areas (Williams *et al*., 2003). This will lead to lower (more negative) water potentials in the grapevine due to increased water loss through transpiration and evaporation. Variability in water status between vines in different parts of a vineyard block can therefore occur easily, and mainly originates from soil differences.

The long-term effects of water status variability should also not be overlooked, as grapevine vigour adapts on long term to soil water availability (JP Gaudillere, personal communication 2009), therefore limiting water use in long-term water deficit conditions, which is for instance found on poor fertility, sandy soil patches within vineyards.

It should thus be clear that variability may also affect grapevine physiology and grape composition (Silvilotti *et al*., 2005). Photosynthesis may be influenced due to changes in stomatal conductance and gas exchange. This will alter the carbon fixation during photosynthesis, leading to changes in the carbon isotope discrimination (CID) because of changes in the intercellular CO_2 level. The depletion of ^{13}C will therefore differ between vines in the areas of differing vigour in a vineyard. Differences in grapevine physiology due to these variations may also affect grape composition and therefore, potentially, wine quality (Esteban *et al*., 2001). It is therefore necessary to understand these variations and the effect they may have on grapevine physiology, as well as how berry quality can be altered by this, so that the importance of the management of this variability can be understood.

2.2. CARBON ISOTOPE DISCRIMINATION

Carbon is an essential element for plants to grow and develop, as carbon dioxide from the atmosphere is fixed by plants to form carbohydrates and other organic compounds. These compounds are then used as building materials for essential plant products, such as lipids and amino acids, and are also used for cellular respiration. The carbon found in carbon dioxide includes three naturally occurring isotopes, the two stable isotopes, ^{12}C and ^{13}C, and the radioactive ^{14}C, which is only found in trace amounts in the atmosphere. These isotopes are incorporated into plant biomass, and the amount of ^{12}C and ^{13}C that is incorporated can be an indicator of the stress level the plant was exposed to at the time when certain plant materials

are sampled. The incorporated ^{14}C can be used for radiocarbon dating, where the level of ^{14}C found in plant material equals the ^{14}C in the atmosphere at the time the material was formed.

2.2.1 CARBON DISCRIMINATION AND PHOTOSYNTHESIS IN PLANTS

During the process of photosynthesis by plants, CO_2 is assimilated. During this assimilation, discrimination takes place between two naturally occurring stable isotopes of carbon, ^{12}C (98,9%) and ^{13}C (1,1%) (Farquhar *et al.,* 1980; Brugnoli *et al.,* 1988; Farquhar *et al*., 1989; Gaudillere *et al.,* 2002). CID will therefore also take place when CO_2 is incorporated into plant biomass (Farquhar *et al*., 1989). Plants show a positive discrimination against ^{13}C via the photosynthetic process by preferentially taking up ^{12}C, as photosynthesis is faster with ^{12}C compared to the heavier ^{13}C (Farquhar *et al*., 1989). The positive discrimination against ^{13}C is instigated by ribulose-1,5-bisphosphate (RuP_2) carboxylase-oxygenase (Rubisco) during carboxylation reactions because of the intrinsically lower reactivity of ^{13}C in comparison to that of ^{12}C (Farquhar *et al*., 1982; Brugnoli and Farquhar, 2000; De Souza *et al.,* 2005). Carbon dioxide uptake during photosynthesis is facilitated by diffusion from the atmosphere through the leaf boundary layer and the stomata. This diffusion will therefore affect CID, as the rate of diffusion influences the gradient of the partial pressure of CO_2 across the stomata (Farquhar and Sharkey, 1982; Evans *et al.,* 1986). The lower reactivity of ^{13}C can be caused by a slower diffusion rate of ^{13}C in comparison with ^{12}C, making photosynthesis easier with ^{12}C, as mentioned. Discrimination therefore leads to lower levels of ^{13}C in the carbon fixed during photosynthesis (Evans *et al*., 1986).

The discrimination against ^{13}C by Rubisco, for example, will decline due to the low internal CO_2 in the leaves, leading to a higher assimilation of ^{13}C into C_3 plant leaves and subsequently $\delta^{13}C$ (carbon isotope composition) increases, showing the effect of the CO_2 ratio across the stomata on discrimination. Mild and severe water deficits cause a decreased supply of CO_2 to Rubisco due to decreased gas exchange, because water deficit promotes stomatal closure, which is primarily responsible for a decrease in CO_2 fixation (Lal *et al*., 1996).

Further discrimination takes place when CO_2 progresses through the leaf intercellular spaces to the chloroplasts' sites of carboxylation. Discrimination is therefore instituted by diffusion and carboxylation, and therefore also related to the intercellular and atmospheric pressures of CO_2 (Farquhar and Sharkey, 1982; Brugnoli *et al.,* 1988).

The leaf carbon isotope composition ($\delta^{13}C$) from assimilation and diffusion into the leaves dominates the whole plant $\delta^{13}C$ and the internal partitioning, and the metabolism of primary assimilates may produce differences in $\delta^{13}C$ in the plant organs (Leavitt and Long, 1985; Gleixner *et al.,* 1993; Brugnoli and Farquhar, 2000; Le Roux-Swarthout *et al.,* 2001; Ghashghaie *et al.,* 2001; De Souza *et al.*, 2005). Further differences in $\delta^{13}C$ between plant parts can be due to differences in lipid composition, fractionation processes during transport (will be discussed) and/or synthesis of metabolites, contributing to changes in the ^{13}C signature of different metabolites and organs (Brugnoli and Farquhar, 2000).

The fractionation of carbon in plant material takes place when isotopes are broken down into fractions during CO_2 fixation in photosynthesis, in the process when CO_2 is converted into organic material (O'Leary, 1981; Evans *et al.,* 1986). According to Macko *et al*. (1998),

carbohydrates isolated from plants and animals have carbon isotope compositions that likely result from the isotope fractionations during the incorporation and metabolism of carbon. Fractionation is also influenced by Rubisco activity, which takes part during the fractionation of carbon isotopes, also forming part of carboxylation fractionation (Farquhar *et al*., 1989). Fractionation is also affected by various environmental factors, which will be discussed.

By measuring the sugar $\delta^{13}C$ in grape berries, estimations could therefore be made of the leaf photosynthetic CID. This is possible because sucrose translocated from the leaves to the berries is converted to glucose and fructose by hydrolysis through invertase, and the fixed carbon isotopes should therefore also be incorporated into these sugars, reflecting the discrimination at leaf level (Davies and Robinson, 1996). Plants in which more ^{13}C is incorporated into the sugars indicate a better water use efficiency (Farquhar *et al*., 1982). This happens because plants with water stress (reduced discrimination against ^{13}C) will have better water use efficiency due to reduced transpiration as stomatal aperture declines.

Delta ^{13}C reflects the effect of plant water status on photosynthesis throughout the growing season (Farquhar and Richards, 1984). Values of $\delta^{13}C$ less negative than -21.5 can be seen as severe water deficits, while values more negative than -26 may indicate no water deficits (Table 2.1) (Van Leeuwen *et al*., 2007).

2.2.2 DETERMINING CARBON ISOTOPES

Isotope ratio mass spectrometry is a common way to determine CID. This method allows the measurement of the relative abundance of isotopes in a given sample (Paul *et al*., 2007). The isotope ratio measured by this method is compared to a measured standard (international standard for CO_2 from belemnite found in the PeeDee limestone formation) to determine the accurate carbon isotope composition of the sample. This measurement must be very precise, with high sensitivity, because variations in ^{13}C between materials are one to 10 parts per thousand, thus favouring the use of mass spectrometry (Boutton, 1991). For the measurement of CID, samples are analysed in gaseous form as CO_2, and then compared to the standard. The mass spectrometer contains an ion source, which ionises the CO_2 molecules. The molecules are then neutralised, causing electrical currents to form, and these are amplified and used to compute the carbon isotope ratios (Boutton, 1991).

As mentioned earlier, the ratio between intercellular and atmospheric CO_2 pressures determines discrimination. This ratio is then used to determine the ratio of $^{12}C/^{13}C$ (Farquhar *et al*., 1989; Gaudillere *et al*., 2002). A robust model was developed to determine this ratio, and thus CID (Brugnoli and Farquhar, 2000). Stable light isotope mass spectrometry is used for this. The deviation of the isotope compounds (R) of the material is measured by this from a standard:

$$R = \frac{^{13}CO_2}{^{12}CO_2} \qquad \text{(Equation 2.2.2.1)}$$

Delta ^{13}C for C_3 plants is determined by the following formula:

$$\delta^{13}C\,‰ = \left(\frac{R_{sample}}{R_{standard}} - 1\right) \times 1000 \qquad \text{(Equation 2.2.2.2)}$$

(Farquhar and Richards, 1984; Bettarini *et al*., 1995)

Where R_{sample} and $R_{standard}$ are the ratios of ^{13}C and ^{12}C of the CO_2 and the reference standard, Pee Dee Belemenite (PBD), respectively. This is used to report the natural abundance of ^{13}C in the samples.

2.2.3 FACTORS AFFECTING CARBON DISCRIMINATION

Any factor that affects the ratio between intercellular and atmospheric CO_2 concentrations will affect CID. Dark respiration, photosynthesis and environmental factors, such as temperature, drought and light intensity, will be amongst the factors having an influence (Farquhar *et al*., 1982). Dark respiration will affect intercellular CO_2 and might cause a non-uniform distribution of ^{13}C within hexose molecules (Rossmann *et al*., 1991), and isotope effects might take place during the decarboxylation of pyruvate (Jordan *et al*., 1978). According to a model described by authors such as Warren and Adams (2006), CID is a function of CO_2 concentrations: a) in the air (which is affected by altitude and therefore vineyard terroir), b) at the leaf surface, c) in the intercellular air spaces and d) in the chloroplast. According to the model, fractionation is due to: a) diffusion through the boundary layer, b) diffusion through the stomata, c) diffusion and dissolution of CO_2 into water, d) net fractionation by Rubisco and phosphoenolpyruvate carboxylase, e) due to mitochondrial respiration and f) due to photorespiration.

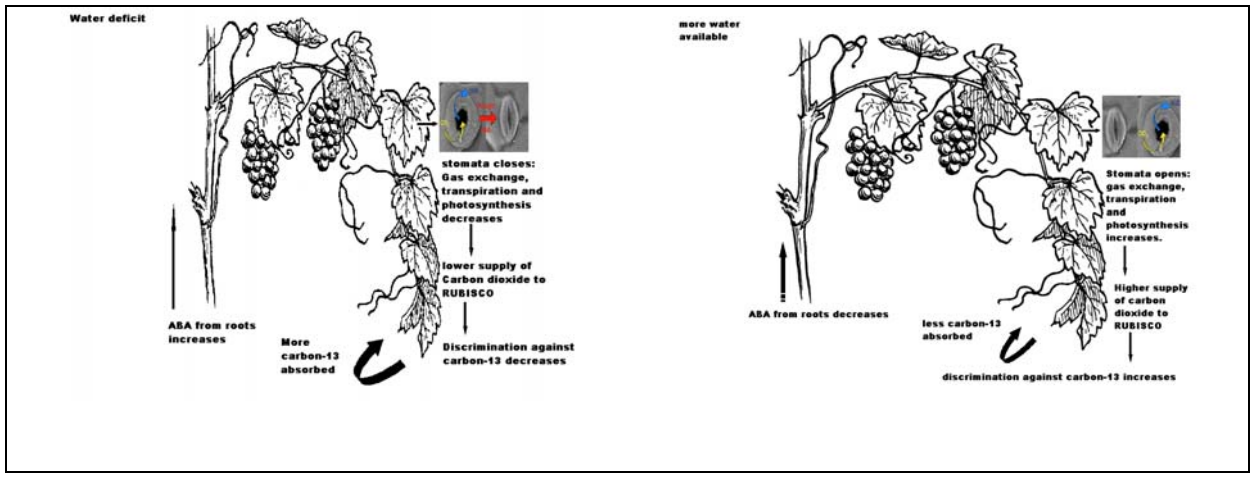

Figure 2.1 Discrimination against ^{13}C in water deficit conditions (left) and well-watered conditions (right).

A water deficit (Figure 2.1) is the main factor affecting $\delta^{13}C$, as it causes plants to reduce their stomatal conductance and intercellular CO_2, and thereby reduce transpiration and photosynthesis (Lajtha and Marshall 1994; Stamatiadis *et al*., 2007). This will happen because water deficit conditions cause closure of the stomata, decreasing gas exchange rates (lowering intercellular CO_2) and weakening the discrimination capacities of plant enzymes (Rubisco) against ^{13}C. This leads to more ^{13}C absorption than under less limiting conditions (Bodin and Morlat, 2006), causing a higher ratio of $^{12}C/^{13}C$. Plants exposed to less water deficits (Figure 2.1) will again show more discrimination and a lower ratio of $^{12}C/^{13}C$ because of increased stomatal aperture (De Souza *et al*., 2005).

If vines are treated by deficit irrigation and partial rootzone drying (PRD), they experience an increase in plant water use, because the increased stomatal closure leads to reduced water loss through transpiration, which is accompanied by an increase in $\delta^{13}C$ values in the berries. It can therefore be stated that stomatal control massively impacts the discrimination against ^{13}C,

and due to grapevine varietal differences in stomatal control, differences in CID can be expected between cultivars (Gaudillere *et al*., 2002). Carbon assimilation will also be affected by the change in stomatal control due to water deficits, which could potentially lead to a reduction in the translocation of assimilates from the leaves to other plant organs through the phloem in the grapevine, as reported for other C_3 plants (Huber *et al.*, 1984; Deng *et al.,* 1989). As mild water deficits increase water-use efficiency, water-use efficiency will correlate negatively with intercellular CO_2, which reduces under conditions of water stress (Farquhar *et al*., 1982; Stamatiadis *et al*., 2007). A negative relation can therefore exist between transpiration efficiency and CID, as found under glasshouse conditions (Gibberd *et* al., 2001), because CID decreases with decreased intercellular CO_2 conditions during water deficits (increased transpiration efficiency). Transpiration efficiency is affected by stomatal conductance and photosynthetic capacity, and these will then affect CID. Environmental and genotypic factors which affect transpiration efficiency through stomatal conductance and photosynthetic capacity of the leaves will then influence the ratio of intercellular and atmospheric CO_2 pressures, and therefore affect CID (Gibberd *et al*., 2001).

As with water deficits, nitrogen (N) availability (also influencing grapevine vigour) (Spayd *et al*., 1991; Spayd *et al*., 1993) is a large determinant of limitations to photosynthesis and CO_2 uptake, which will therefore also affect CID (Chapin *et al*., 1987; Hungate *et al*., 2003; Warren and Adams, 2006). Limited availability of soil nitrogen, which influences leaf nitrogen content, can also lead to increased CID as a result of reduced carbon uptake by the mesophyll (Bettarini *et al*., 1995). Increased salinity or decreased relative humidity will also influence intercellular CO_2 levels because of the decreasing effect they have on stomatal conductance (Evans *et al*., 1986). Metal ion concentrations and pH in plant vascular bundles may also affect enzymes (Rubisco) that are responsible for CID (O'Leary, 1981).

2.2.4 USE OF CARBON DISCRIMINATION IN VITICULTURE

When studying the distribution of the carbon isotopes, information can be gathered about the physical, chemical and metabolic processes involved in carbon transformations (Farquhar *et al*., 1989). Delta ^{13}C can be a long-term indicator of water-use efficiency (WUE) in a specific vineyard, as seasonal transpiration (ratio of dry mass produced and water loss) is reflected by $\delta^{13}C$ of plant material (Farquhar and Richards, 1984; De Souza *et al*., 2005). An increase in $\delta^{13}C$ accompanies a gain in water usage (Chaves *et al*., 2007). Transpiration efficiency (dry matter produced/water lost) may also reliably be predicted by CID (Gibberd *et* al., 2001). It can also be used to estimate stomatal closure over time to study differences in stomatal conductance and/or sink/source balances between treatments (De Souza *et al*., 2005). The measurement of CID is evidently a very integrating indicator, reflects the long-term effect of plant water status and depends on photosynthetic regulation characteristics (Gaudillere *et al*., 1999). Leaf $\delta^{13}C$ represents the ambient and intercellular CO_2 ratio, as organic compounds are the dominant source for leaf growth in the early spring and leaf $\delta^{13}C$ also reflects the previous year's carbon allocation and assimilation, as stored organic compounds in deciduous plants (such as the grapevine) are also used for early spring leaf growth (De Souza *et al*., 2005). The leaf $\delta^{13}C$ also is incorporated into berry sugars, as mentioned, and this sugar $\delta^{13}C$ can be used

as a sensitive detection method for plant water status under natural conditions. This is possible because berry sugar $\delta^{13}C$ correlates well with summer pre-dawn leaf water potential and can be used to characterise the vineyard soil's structural capacity to hold and provide water to the vines (Gaudillere *et al*., 2002). It therefore reflects plant water status and photosynthesis throughout the season (Farquhar and Richards, 1984).

When looking at CID in the sense of carbon assimilation, photo-assimilate exportation out of the producing leaves and photo-assimilate transport and partitioning within the plant, CID can likely be used to link photosynthesis with yield and fruit quality (Bota *et al*., 2004). Delta ^{13}C measured from harvest samples can be used to compare conditions that would help to provoke mild water deficits and thus be helpful to produce good quality grapes (Gaudillere *et al*., 2002).

Measuring CID is the only available tool capable to access water uptake conditions from véraison to harvest at a low cost without having to install heavy equipment in the vineyard (Van Leeuwen *et al*., 2001).

2.3. GRAPEVINE VIGOUR VARIABILITY

Variability in within-vineyard vigour can cause differences in the development of fruit quality and in fruit ripening and therefore affect wine quality (Bramley, 2005; Skinner, 2006), as high-vigour vines with big leaf areas may result in shaded leaves and clusters, causing slower fruit ripening, less fruit colour and flavour and lower bud fertility (White, 2003). The lower bud fertility will then lead to negative effects on bud burst, fruit set, berry growth and fruit quality. It is therefore important to be able to quantify this variability to manage it correctly for more uniformity in a vineyard. Nowadays, remote sensing is a common method to determine vineyard vigour variability and can often be used with other measured vigour parameters, such as pruning masses and shoot lengths, to show the spatial distribution of these conventional measurement techniques (Strever, 2003). Variability in vigour is caused by a number of factors, which will be discussed, and these factors must be managed correctly for a producer to induce growth uniformity in a vineyard. Irrigation, soil management, disease management and the planting of quality material are management inputs that can improve uniformity in a vineyard (Bramley, 2001). Management, together with the correct trellis system and pruning, can therefore be used to increase the ratio of leaves to fruit and to ensure exposure of the fruit and leaves to sunlight to promote quality and uniformity (Possingham, 2002). Controlling grapevine variability can help achieve flavour and aroma concentrations in wines (Long, 1997).

2.3.1 CAUSES OF VIGOUR VARIABILITY

As mentioned, there are a number of factors contributing to vigour variability within a vineyard. These factors can be of an environmental nature, from plants or can be induced through management practices (Taylor, 2001). The most common causes for grapevine variability in a vineyard are soil type variation, irrigation differences (e.g. due to blocked emitters), plant diseases, irregular pruning and differences in plant material (e.g. varied grapevine age) (Long, 1997), with soil water status variability probably being the most influential..

In South Africa it is common for soil types, and therefore soil water holding capacity to differ over short distances. Apart from affecting plant water status, soil type can also affect root development due to mechanical limitations and therefore indirectly water and nutrient availability. Root growth will then affect above-ground growth, as there is a balance between above-ground and subterranean growth. Different soil types imply differences in effective depth, texture, structure, nutrient content, water holding capacity, colour (influencing soil temperature and air temperature close to the ground) and soil chemical composition. These factors will influence root development and the way water and nutrients are absorbed by the grapevine. Therefore, each of these factors has the potential to influence grapevine growth vigour. Within a vineyard block it can therefore easily occur that one section has a deeper soil with a clay texture, leading to better water-holding capacity and more nutrients, compared to another part that may, for example, have shallow soil with sandy topsoil, fewer nutrients and a lower water-holding capacity. Vines growing on the first example would therefore tend to have higher vigour than those on the other soil, even though co-existing in the same vineyard.

Through all this it is therefore clear that water availability significantly affects grapevine vigour (see 2.4.3.1), and therefore also long-term vigour through potential effects on reserve nutrient accumulation (Bramley, 2001).

The site where a vineyard is planted will determine sunlight interception and wind exposure, which may also differ within a vineyard, especially where slope and aspect vary a lot, causing some vines to intercept more sunlight or wind than others (Carey, 2001). Grapevine mesoclimate, topography and soil will also affect growth and could lead to differences in grapevine vigour, as these factors may also differ within a single vineyard.

Management inputs can also induce within-vineyard variability. Incorrect long- or short-term practices can lead to heterogeneous canopies in the vineyard and, while soil preparation and the correct planting of vines should help to prevent this problem, it cannot always be successfully and cost effectively addressed through canopy management. Other management inputs, such as irrigation, fertilisation and pruning, must be optimised to avoid variability. Tipping and topping of shoots can lead to reduced shoot growth and therefore reduced grapevine vigour (Pisciotta *et al*., 2007). Increased tipping and topping actions in more vigorous areas of a vineyard can therefore be implemented, while less such actions can be implemented in less vigorous areas.

The differences in the occurrence of pests, diseases and weeds in different parts of a vineyard can also lead to differences in vigour. Weeds can reduce grapevine vigour, as they cause competition for water and nutrients, while diseases such as viruses and those caused by bacteria have the potential to drastically reduce vigour.

2.3.2 DETERMINATION OF GRAPEVINE VIGOUR

2.3.2.1 PRUNING MASS MEASUREMENTS

Vigour measured in pruning mass has been shown to have a negative correlation with $\delta^{13}C$ and $\delta^{13}C$ and might be a predictor of pruning mass (Stamatiadis *et al*., 2007). Pruning mass can be used to indicate if vines are well balanced in terms of growth (Kliewer and Dokoozlian, 2005).

By measuring pruning mass, one can establish the potential of a soil to support vigorous growth (Bodin and Morlat, 2006).

2.3.2.2 LEAF AREA MEASUREMENTS

Severe water deficits can cause a restriction on leaf area development, reducing the available leaf area for the interception of solar radiation and photosynthesis, which influences crop productivity (Menteith and Moss, 1977). The rate of leaf area development therefore decreases as the transpiration rate decreases because of hormonal influences (ABA) from the roots following increased water deficits (Bindi *et al*., 2005). Leaf area development when there are soil water deficits is a function of soil water content (Lecoeur and Sinclair, 1996; Sadras and Milroy, 1996; Bindi *et al*., 2005), and a decrease in leaf area development follows due to a substantial decrease in the fraction of transpirable soil water (Bindi *et al*., 2005). Leaf area development will therefore be more for vines that receive more water; but it can largely be due to increased leaf development on the lateral shoots (De Souza *et al*., 2005; Dos Santos *et al*., 2007). Leaf area can therefore be used to assess the impact of irrigation on grapevine vigour (Greven *et al*., 2005). The total leaf area of a grapevine and the total leaf area exposed to sunlight largely determine the fruiting capacity, if other factors do not limit growth and fruit primordia initiation (Kliewer and Dokoozlian, 2005).

2.3.2.3 SHOOT LENGTH

As mentioned, grapevine vigour is influenced by root development. When root development is stimulated, it will result in faster and longer shoot growth (later cessation of shoot growth) (Wang *et al*., 2001). This will lead to higher vigour than when root growth is more limited. Any factor (water status of soil, nutrients in soil, soil structure and soils texture) will therefore affect shoot growth. Lateral shoot development will increase with higher vigour and it is usually a function of the impact of irrigation on vigour (Greven *et al*., 2005; Dos Santos *et al*., 2007). The reason for the increase in lateral shoot growth might be due to an increase in cytokinin synthesis in the roots, which can stimulate lateral shoot growth (Dry *et al*., 2001).

2.3.3 EFFECTS OF VIGOUR VARIABILITY

Vigour variability causes non-uniformity in the canopies of vineyards, an aspect that makes precision viticulture necessary. Uniformity in the vineyard will result in improved grape quality, uniformity in fruit maturity and, consequently, higher quality wine (Morris, 2001). An overall reduction in wine quality and volume will be the result if such a vineyard is not managed correctly (Hall *et al*., 2002). Yield differences will also exist between different vigour areas (Dry, 2000), with vigorous vines normally having bigger yields than less vigorous vines. Vigour variability in a vineyard will not only be negative for fruit quality, but will also increase the need for extra managerial inputs to perform precision viticulture and must therefore be kept to a minimum. Vigour variability might also affect grape sugar content, as Van Leeuwen *et al*. (2007) found that must nitrogen content and therefore vigour were negatively correlated with grape sugar content. Vigour variability might also cause differences in the phenolic compounds in the berries of red grapes (high vigour reduces phenolics).

2.4. GRAPEVINE WATER STATUS

In a dry country like South Africa, water is not always as accessible as agricultural producers would like it to be. In viticulture it is a known fact that it is very important for grapevine water status to be optimal in order to produce quality fruit. According to Carbonneau (1988) and Deloire *et al*. (2004), water deficit effects on grapevines can be quantified as:

1. **Absent**: normal vegetative and berry growth, normal photosynthesis and berry ripening.
2. Mild **water deficits**: reduced vegetative growth, normal to reduced berry growth and photosynthesis and normal to stimulated berry ripening.
3. Moderate to severe **water deficits**: reduced to inhibited vegetative and berry growth, photosynthesis and berry ripening.
4. **Water stress**: Inhibited vegetative and berry growth, partial or total inhibition of photosynthesis and berry ripening.

According to Van Leeuwen *et al*. (2007), mild water deficit stress in grapevines has one negative effect (the reduction in photosynthesis), while it has many positive effects, like shoot growth cessation, reduction of berry size and stimulation of the synthesis of phenolic compounds. The optimal water status that should be obtained in a vineyard is mild water deficit, under which conditions berry quality potential increases despite the possible reduction in photosynthesis. This increase can be ascribed to reduced competition for sugars between shoot growth and fruit ripening and the reduced berry size (Van Leeuwen *et al*., 2007).

The monitoring of grapevine water status is therefore very important for the induction of mild water deficit, which would lead to better fruit quality parameters (Gaudillere *et al*., 2002). Irrigation can then be used to manipulate the grapevine water status in order to keep it at a level suitable for quality berry development throughout the season. According to Van Leeuwen and Seguin (2006), grapevine water status depends on the climate (rainfall and potential evapotranspiration), soil (mostly water-holding capacity) and the training system (canopy architecture and leaf area). Differences in microclimate, soil type and canopy size in a vineyard will therefore contribute to variability in grapevine water status.

2.4.1 CAUSES OF WATER DEFICITS

Water deficits in vineyards can be manipulated through irrigation, provided that water is available. In areas where irrigation water is more readily available to producers, vineyard water status can more easily be manipulated by altering irrigation frequencies. However, in areas where irrigation water is not available, water deficits may potentially occur more frequently and may be more severe.

The area where viticulture is practised therefore has a big impact on water deficits, because climate (rainfall differences and evapotranspiration) will be a determinant of the occurrence of water deficits (Van Leeuwen and Seguin, 2006). The soil found in the area will also be a factor, as a vineyard planted on a sandy soil will potentially be more prone to water deficits than one planted on a clayey soil. Soil texture, depth and pebble content (soil type) can therefore be seen

as important factors influencing water deficits, as they influence water-holding capacity (Fernandez-Illescas *et al*., 2001; Van Leeuwen and Seguin, 2006).

The topography of an area also determines soil water deficits, as steeper slopes drain more. Vines planted on such slopes could therefore be more prone to water deficits.

Management inputs other than irrigation can also influence water deficits, as soil preparation, for instance, influences the soil water-holding capacity. The canopy structure and therefore the training system of a vineyard will also influence water deficits, as vines on bigger trellis systems have a larger effective leaf area (grapevine vigour), which will increase transpiration and thus the potential for water deficits.

2.4.2 QUANTIFICATION OF GRAPEVINE WATER STATUS

2.4.2.1. LEAF AND CANOPY TEMPERATURES

These measurements can be used as indicators of stomatal closure as a result of water deficits; this is possible because water deficits cause stomatal closure, leading to higher leaf temperatures (Grant *et al*., 2007). This increase in leaf temperature is found because a lower rate of transpiration reduces evaporative cooling of the leaf, and the increase in leaf temperature then increases the driving force for transpiration (Gibberd *et al*., 2001). It can therefore be used to determine grapevine water deficits and stomatal aperture (Grant *et al*., 2007).

These measurements may also be used to distinguish between non-irrigated, irrigated and even deficit irrigates vines (Grant *et al*., 2007). Negative aspects of these measurements might be that they can be difficult to perform and the equipment may be expensive, while measurements may also be influenced by weather conditions like wind and radiation (Lebon *et al*., 2003).

2.4.2.2. GRAPEVINE WATER POTENTIAL

Pressure chamber measurements can provide values of pre-dawn, leaf (LWP) and stem water potential (SWP), as they describe the tension existing in certain plant parts when the measurement is taken. The size of these readings reduces due to water deficits (Bota *et al*., 2004) and can be highly variable and sensitive to environmental factors (Bindi *et al*., 2005).

Pre-dawn leaf water potential measurements indicate the plant water status at zero plant water flux and provide information on the root zone soil water potential, as the water potential in the leaves would largely be equilibrated with the water potential in the soil by dawn (Garnier and Berger, 1987; Choné *et al*., 2001) and equilibrated with the most humid soil layer explored by the roots (Van Leeuwen *et al*., 2007). Leaves are not transpiring at this stage and because microclimate conditions are similar between the leaves, each single leaf of a grapevine should have similar water potentials at this stage of the day (Van Leeuwen *et al*., 2007). Pre-dawn leaf water potential might be insensitive to variation in soil water content and might therefore be weakly correlated when soil moisture is heterogeneous and dry or uniform and wet (Pellegrino *et al*., 2006). Pre-dawn leaf water potential sometimes also fails to correlate well with stomatal conductance (Escalona *et al*., 1999; Lopes, 1999; Silvestre *et al*., 1999; Bindi *et al*., 2005). Pre-

dawn LWP is not an accurate indicator of water status in irrigated vineyards, because the grapevine might rehydrate in the night (pre-dawn LWP indicates no stress), although not enough water might be available for the evaporative demand the following day (Ameglio *et al*., 1999).

LWP reflects a combination of factors, including local leaf water demand (vapour pressure deficit and leaf-intercepted radiation), soil water availability, internal plant hydraulic conductivity and stomatal regulation (Choné *et al*., 2001). It can be an indicator of water deficits and used for irrigation scheduling (Grant *et al*., 2007), but might sometimes fail to correlate well with stomatal conductance (Bindi *et al*., 2005) and might vary too much according to the microclimate (Van Leeuwen *et al*., 2007). Midday LWP might not be a good indicator of grapevine water status, as vines show isohydric behaviour (Schultz, 2003) and the water potential variation of leaves is limited through stomatal regulation (Van Leeuwen, *et al*., 2007).

SWP (measured on a non-transpiring leaf) can be regarded as a robust measurement of water status, as it indicates xylem water potential (McCutchan and Shackel, 1992). It is the result of whole-plant transpiration and soil and soil/root hydraulic conductivity and indicates the capacity of a grapevine to conduct water from the soil to the atmosphere (Choné *et al*., 2001), and thus represents the water potential of the whole grapevine (Van Leeuwen *et al*., 2007). SWP can be used as an indicator of water deficit and can be used for irrigation scheduling (Grant *et al*., 2007). It is widely found that, of all these measurements, SWP is the one that is the most discriminating and the first indicator of a water deficit (Choné *et al*., 2001). SWP is better related to grapevine transport than LWP, and a better indicator of grapevine water status in irrigated vineyards than pre-dawn LWP. However, it might also be influenced by the climate (Van Leeuwen *et al*., 2007).

The problem with these measurements is that they are time consuming and destructive (Grant *et al*., 2007). The measurements might also prove to be expensive and can be influenced by weather conditions (Lebon *et al*., 2003). The values in Table 2.1 might vary between different plots due to vigour, root distribution and the climate (Van Leeuwen *et al*., 2007).

Table 2.1 Grapevine water deficit threshold levels for water potential and CID (from Van Leeuwen *et al*., 2007).

Water deficit	Midday SWP (MPa)	Midday LWP	Pre-dawn LWP	$^{13}C/^{12}C$ ratio
Absent	>-0.6	>-0.9	>-0.2	<-26
Weak	-0.6 to -0.9	-0.9 to -1.1	-0.2 to -0.3	-24.5 to -26
Moderate to weak	-0.9 to -1.1	-1.1 to -1.3	-0.3 to -0.5	-23 to -24.5
Moderate to severe	-1.1 to -1.4	-1.3 to -1.4	-0.5 to -0.8	-21.5 to -23
Severe	<-1.4	<-1.4	<-0.8	>-21.5

2.4.2.3. STOMATAL CONDUCTANCE

Stomatal closure is an important control mechanism for a plant's response to soil water deficits and is the result of root signalling (increase in ABA production and xylem pH increase and a reduction in cytokinins) (Davies *et al*., 2000; Chaves *et al*., 2007). Stomatal conductance reduces because of soil water deficits and environmental limitations, like steep leaf-to-air vapour gradients, high light intensity and temperature due to increased ABA in the xylem, and a decrease in xylem conductivity (Lovisolo *et al.,* 2002; De Souza *et al.,* 2003; Schultz, 2003).

A reduction in stomatal conductance leads to reductions in photosynthesis (as there is a strong correlation between photosynthetic rate and leaf conductance), yield and growth (Bota *et al.,* 2001; Flexas *et al*., 2002; Maroco *et al.,* 2002; Medrano *et al.,* 2003). Hormonal signals from the roots therefore control stomatal conductance and the subsequent photosynthesis (Schultz, 2000). This can happen when hormones, especially abscisic acid from the roots, move through the transpiration stream to the leaves, accumulating at or near the guard cells and causing the closure of the stomata. Because they influence the turgidity of the guard cells, the cells becomes less turgid and stomatal closure takes place. Photosynthesis will then reduce because of the lower CO_2 flux from the atmosphere through the stomata, causing photosynthetic rates to decrease. These signals will therefore be very important for regulating and improving water-use efficiency by letting the plant keep more of the water to be used by the source organs (e.g. leaves for growth and photosynthetic usage), so that there will still be a good supply of carbohydrates to the sink organs like the bunches for the development of good quality.

The signals are also important for the regulation of leaf nitrogen, leaf expansion and the development of the leaves through the supply of available water and nutrients from the soil (Davies and Zhang, 1991).

Root signalling due to water deficits can cause the alkalinisation of xylem sap, causing an increased uptake of ABA by the leaves, thus promoting stomatal closure. As has been mentioned, the pH of the sap transferred to the xylem and through the transpiration stream increases when a rootzone water deficit is sensed. This causes an increase in the concentration of ABA in the leaf tissue, leading to stomatal closure and a reduction in leaf growth (Davies *et al.*, 2001). The cause of this increase in pH could be a change in the nitrate reductase activity that occurs when the soil is drying. This can cause the alkalinisation, although it can also be caused by changes in proton pumps (Davies *et al.*, 2001). This increase in the pH of the xylem sap can only take place when there is ABA flux from the roots to the leaves (Davies *et al.*, 2001). This change in pH can also cause an increased uptake of ABA by the xylem vessels in the roots.

Stomatal conductance measurements can therefore be used as an indication of plant water status and for irrigation scheduling, as it reduces under water deficit. The problem with these measurements is that they might be time consuming and labour intensive and that they might only give point measurements (Grant *et al*., 2006). The measurements might also be costly and might be influenced by weather conditions (Lebon *et al*., 2003).

2.4.3 EFFECTS OF WATER DEFICITS

According to Van Leeuwen and Seguin (2006), quality red wine is produced when environmental conditions induce moderate grapevine vigour through moderate water deficit or by low nitrogen supply, because berry size is then reduced and phenolic compound synthesis is enhanced. While regular but not excessive water and nitrogen supply are needed for quality white wine, a reduced nitrogen supply for white cultivars limits aroma precursor synthesis and therefore reduces the wine quality (Peyrot de Gachons *et al*., 2005; Lavigne-Cruège *et al*., 2006).

2.4.3.1. VEGETATIVE EFFECTS

Vigour variability in vineyards can occur due to variability in water status, as greater water deficit can cause stomatal closure, followed by a reduction in photosynthesis, which causes a reduction in vigour. Water deficits therefore lead to reduced vigour (Chaves *et al*., 2007), while increased soil water and chemical fertility lead to increased vigour.

Mild water deficits can therefore cause a reduction in vigour, which under optimum conditions can cause an increase in light interception in the bunch zone. The variability in vigour could be caused by the variability in water status within a vineyard, and its effect on shoot growth. It is optimal to induce a mild water deficit in a vineyard, as this would be advantageous for shoot growth, since it leads to a better canopy microclimate and improved berry compositions as a result of a reduction in sinks for carbohydrates (Smart *et al*., 1990). Deficit irrigation can therefore be implemented to modify the reproductive and vegetative growth of grapevines, leading to increased fruit quality. Adaptations due to the levels of sunlight (sun- and shade-exposed vines) can be caused by the signals produced by the roots during water deficits (Yordanov *et al.*, 2003). These adaptations may include shoot and leaf development, where hormones, e.g. cytokinin from the roots, may increase shoot development and may improve leaf growth to lead to better leaf positioning for sunlight interception, increasing the photosynthetic capacity of the canopy.

Excessive vegetative growth (increased pruning weight) caused by unrestricted water supply to vines therefore competes for assimilates with berries and causes negative effects on quality. This strong vegetative growth can cause canopy closure, restricting flower bud initiation and ripening, while also increasing the development of disease (Pellegrino *et al*., 2006).

Just as an excess water supply can be problematic for grapevine and fruit development, severe water deficits also cause problems, as they stimulate stomatal closure (Escalona *et al*., 1999), leading to reduced or no assimilative activity and shoot growth. The result is a reduction in leaf area due to a long-term mechanism of plant adaptation to slow developing stress; this might lead to excessive bunch exposure to light (Pellegrino *et al*., 2006).

2.4.3.2. REPRODUCTIVE EFFECTS

The optimal development of yield and berry composition can correspond with moderate water deficit, and soil water management is therefore extremely important (Pellegrino *et al*., 2006). Irrigation will therefore affect grapevine physiology, which affects grape composition and yield. Berry composition may improve due to a reduction in carbohydrates by growing tips and the

water deficits may also cause a shift in photo-assimilate partitioning towards secondary metabolites and reproductive tissue, leading to improved fruit quality (Chaves *et al*., 2007). Irrigation will also affect yield and wine quality (Medrano *et al.,* 2003). Mild water deficits reduce competition for assimilates between vegetative and reproductive sinks and therefore improve berry quality (Pellegrino *et al*., 2006). A mild water deficit also causes an increase in skin anthocyanins and total phenols (Chaves *et al*., 2007).

An excess water supply can negatively affect acidity and colour and can cause acid imbalances and a high pH (Bravdo *et al*., 1985; Matthews *et al*., 1990; Esteban *et al*., 2001; Chaves *et al*., 2007). When vineyards are exposed to high water availability, some important berry components become diluted (Esteban *et al*., 1999). It also causes restrictions in soluble sugar development (Bravdo *et al*., 1985) because of competition for carbohydrates between continued shoot growth and the berries and because of the dilution effect, while it reduced pH and also causes a decreased crop load (crop yield:pruning mass), which can be an indicator of grapevine balance (optimal range 5-10) (Kliewer and Dokoozlian, 2005). Excessive water will also cause increases in total acidity in the grape must, increased arginine content and increased berry mass. Excessive water would therefore be negative for wine quality, also causing weak wood maturation during winemaking (Möller *et al*., 2007).

Severe water deficits can also influence berry composition, as reduced vegetative growth under these conditions can lead to weak sugar contents in the berries and negatively affect wood maturation (Möller *et al*., 2007). These deficits can also cause a restriction in the development of soluble solids, total acidity, pH and arginine and decreased berry mass, while crop load also decreases (Bravdo *et al*., 1985). Water deficit also causes increases in mannitol (Yordanov *et al*., 2003; Merchant *et al*., 2006), ABA, proline and sorbitol (Yordanov *et al*., 2003).

Berry sugar, acids, pH and other quality compositions alter due to different water availability levels, as described. During the development and ripening of berries (double sigmoid growth pattern) throughout the season, this availability will affect berry composition. Organic acids (malic and tartaric) are usually at the highest at around pea size and start to decrease at véraison, with a greater decrease for malic acid than tartaric acid. After véraison, tartaric acid is normally found in higher concentrations than malic acid and the difference seems to increase as ripening progresses. Irrigation will also influence this development, with non-irrigated vines usually showing higher differences in the concentrations of these acids. Higher malic acid concentrations are found in the berries of irrigated vines, while tartaric acid is not really influenced by the irrigation treatment. Malic acid concentrations are very changeable on the basis of different irrigation levels, because they are influenced by canopy microclimate, vegetative growth and yield. Titratable acidity is usually higher in irrigated vines, especially at the end of ripening (Esteban *et al.,* 1999).

Throughout the season, glucose is the predominant sugar in the berries until véraison, while fructose is predominant at the end of ripening. The irrigation treatment also causes changes in fructose and glucose development and the concentration of total soluble solids, normally with lower values of total soluble solids in irrigated than in non-irrigated vines towards ripening. The sugar concentration can increase when irrigation takes place during the ripening stage, when sugar accumulation takes place, but yield is also a determinant of berry sugar

content under conditions of water deficit. Low-yield vines under water deficit result in the sugar content to be enhanced, while high-yield vines under water deficits result in the sugar content being depressed due to incomplete ripening (Tregoat *et al*., 2002). Irrigation in the early berry development stage can cause a decrease in sugar accumulation and increased yield (Rühl and Alleweldt, 1985).

Increased berry mass due to irrigation will influence the concentration of berry components during ripening; increased mass can result in a higher solvent to solute ratio (Esteban *et al*., 1999). Yield is also increased in vines for which irrigation was implemented. The increase in yield due to irrigation can mainly be due to an increase in berry mass (Salon *et al*., 2004). Berry composition can therefore be manipulated by implementing the correct amount of irrigation at the right time.

There also is a relationship between the effects of water status and nitrogen availability (vigour), as they influence grape aroma. Grape aroma potentials are high at mild water deficits and moderate nitrogen supply, while severe water deficits and nitrogen deficiencies will cause a limited grape aroma potential (Peyrot des Gachons *et al*., 2005).

2.4.3.3. PHYSIOLOGICAL EFFECTS

The production of a number of compounds becomes altered due to water deficits. This is usually due to changes in root signals and reactions. This leads to the production of proline, mannitol, sorbitol, ascorbate, glutathione and alpha-tocopherol and the formation of new proteins and mRJNAs (Yordanov *et al.*, 2003). It is especially the formation of proline that is notable during water stress, as it increases causing a decrease in the osmotic potential and increasing the water potential gradient between the soil and the root cells, allowing water uptake by the root cells.

The fixation of carbon by photosynthesis reactions also alters, as described under carbon discrimination, as intercellular CO_2 is reduced because of the lower stomatal conductance in vines with more water stress, and more ^{13}C is absorbed, as mentioned previously.

The grapevine physiological activities therefore change due to water deficit changes, leading to water potential changes in the plant. This changes photosynthesis, respiration and transpiration because of the affect of plant hormones such as cytokinins, abscisic acid and jasmonic acid.

During soil drying, cytokinin activity and transport from the root (apex), which is an important site of production, are reduced. Cytokinins play a role in stomatal functioning, and these reduced concentrations arriving at the leaves during water stress will lead to a restriction in stomatal aperture and therefore a reduction in transpiration. This reduction in transpiration will affect water use by the grapevine, while cytokinin also influences leaf growth, senescence, abscission and shoot growth. Under stressed conditions, the reduction in shoot growth in particular will also lead to less but more efficient water usage.

ABA is released by root stellar tissues to the xylem vessels as the soil dries and the hormone therefore moves from the root tip, where it is synthesised, into the transpiration stream and upwards to the aboveground plant parts. Here it is deposited and accumulates at or near the guard cells of the stomata. ABA then causes stomatal closure because it causes the guard

cells to loose water as a result of less negative osmotic potential due to a decrease in potassium flux into the cells. The closure then leads to decreased transpiration and therefore a reduction in water loss (Antolin *et al.*, 2003). The synthesis of ABA increases as the soil becomes drier, causing an increased effect on transpiration (Davies *et al.*, 1994). When ABA initiates the closure of stomata, it occurs before there are changes in leaf water status, thereby optimising water-use efficiency.

Jasmonic acid (JA) is a plant hormone that is biosynthesised from linolenic acid by the octadecanoid pathway (Weber, 2002). The major functions of JA in regulating plant growth include growth inhibition, senescence and leaf abscission. It has an important role in response to the wounding of plants and systemic resistance. The hormone plays a role in biotic and abiotic stress (Creelman and Mullet, 1995). It will therefore have a role to play during water stress, which can contribute to the regulation of JA biosynthesis. JA can inhibit root growth, and water stress induces the expression of several genes that respond to JA. Loss of cell turgor pressure, which occurs during water deficit, can stimulate the accumulation of JA. This accumulation can take place in the roots and can cause a reduction in the rate of transpiration by being involved in the increase in the betaine level in the leaves, which serves as osmolytes and permits water retention in the leaf cells, JA can therefore be involved in the drought-induced betaine accumulation, as found in pear leaves (Gao *et al.,* 2004).

In order to produce grapes of the highest possible quality, it is necessary to have optimal grapevine growing conditions to allow berry composition to develop optimally in order to use the grapes in winemaking to produce a certain style of wine. Vigour and water status in the vineyard should therefore be optimal and variation should be avoided as far as possible. Measurements of these parameters are therefore very important and the measurement should therefore be very accurate and easily practicable. If vigour and water status are then managed correctly, optimal conditions of grapevine microclimate and competition between reproductive and vegetative growth should follow. Measurement of CID integrates various environmental conditions which influences stomatal conductance and the CO_2 exchange through the stomata. CID measurements reflect ^{13}C absorption by plant material and gives information about transpiration efficiency and photosynthesis. CID measurements can therefore be used to help optimise water use efficiency and promote mild water deficits and to reduce canopy shading to a level suitable for optimal fruit development.

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Chapter 3

Research results

Grapevine water status variability within a *Vitis vinifera* L. Cv Merlot vineyard and carbon discrimination (Wellington region)

Research Results

3.1 INTRODUCTION

Various studies in the past have tested the effects of grapevine physiology reactions on variability in water availability (Choné *et al*., 2001; De Souza *et al*., 2005; Gaudillere *et al*., 2002). Physiological measurements reflect grapevine water status as it is influenced by variability in plant water potential. Carbon isotope discrimination (CID) is a plant physiological measurement that lately has received experimental attention from authors like Gaudillere and Van Leeuwen in order to test the effect on it of grapevine water stress.

In most of those studies, CID measurements were done on mature berry juice sugars to test the effect of variations in soil water availability, often among different varieties. In this study, the seasonal evolution of CID measurements was tested from when the berries were pea-sized, among different irrigation treatments in vines of the same variety. This was done in order to see how six different irrigation treatments could have different effects on grapevine performance throughout a season.

Differences in irrigation causes differences in grapevine water status, and this can have a direct effect on grapevine physiology, influencing grape composition and production (Silvilotti *et al*., 2005). Stomatal conductance (gs) is dependent on soil water availability and it affects the water-use efficiency of grapevines. This dependence on soil water availability is through plant hormones (abscisic acid) from the roots and it will cause reactions in photosynthesis (Schultz, 2000). Differences in gs will lead to differences in CO_2 movement over the stomata, altering the discrimination between ^{12}C and ^{13}C and therefore CID. Physiological measurements like CID can be used to improve vineyard management to optimise irrigation so that the grapevine water potential is such that vegetation is optimal and fruit development occurs in the best possible way to produce quality grapes for quality wine.

Fruit composition at harvest will be altered by differences in the water status of grapevines at different phenological stages within a vineyard (Matthews and Anderson, 1988). Grapevine physiology during these phenological stages will be very important in the determination of fruit composition, and therefore quality. It consequently is important to test the influence of different irrigation regimes, not only on physiological performance, but also on reproductive performance, as juice pH, acidity, sugar content, colour and other quality parameters are influenced by grapevine physiology and by berry size. Berry size is determined by the available water and will influence the dilution and concentration of juice parameters.

Irrigation in a very important tool that can be used by producers to try to establish an optimum grapevine balance between reproductive and vegetative growth, as it will also influence vegetative growth (Hardie and Considine, 1976; Salon *et al*., 2005). Measurements in this study therefore had to include vegetative measurements, as higher water availability can increase leaf area (Intrigliolo and Castel, 2008) due to increased stomatal conductance and photosynthesis. Shoot growth will also increase due to irrigation; this is influenced strongly by the increase in cytokinin from the roots. This increased shoot growth may then improve leaf

positioning for sunlight interception, increasing the photosynthetic capacity of the canopy (Yordanov *et al*., 2003).

This study of water status variability and CID can hopefully be useful to provide information about the importance of irrigation for producing quality fruit as a result of their interactions on the grapevine physiological level through a growing season.

3.2 MATERIALS AND METHODS

3.2.1 CLIMATE

Data for temperature and relative humidity (obtained from ARC, Infruitec-Nietvoorbij, Stellenbosch) was used throughout the season for the analyses, along with seasonal berry ripening data in order to explain some climate-linked developmental changes that occurred.

3.2.2 VINEYARD CHARACTERISTICS

The study was conducted in collaboration with the ARC Infruitec-Nietvoorbij, Stellenbosch (who started the initial study in 2005) during the 2006/2007 growing season in the Wellington region, Western Cape, South Africa. The vineyard was a *Vitis vinifera* L. cv Merlot vineyard, grafted on R99 (*Vitis Berlandieri x Vitis rupestris)* rootstock and was planted in 1989. The vines were planted 3.0 x 1.0 m in a north-south row direction and were trained onto a vertically shoot-positioned four-wire hedge trellis system.

The region has a Mediterranean climate with hot, dry summers, mild winters and winter rainfall. The soil at the site consists mostly of gravely loam with a clay content of approximately 25%.

3.2.3 EXPERIMENTAL LAYOUT

Irrigation was applied with drip emitters (UniRAM 2.3 L/h at 0.75 m spacing). Six different irrigation treatments were applied in a randomised block design, with each treatment having four repetitions. Each repetition consisted of six vines in two adjacent rows, with buffer vines in between treatments (Figure 3.2).

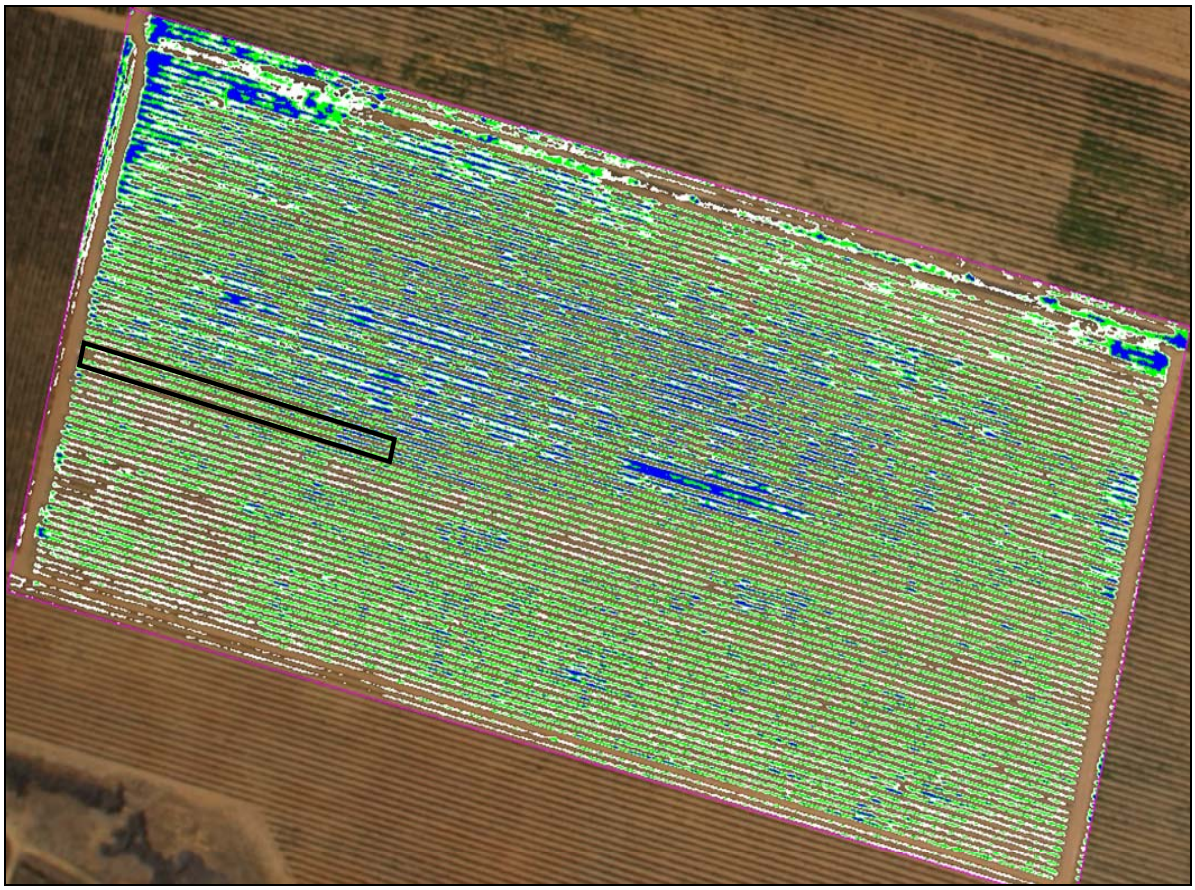

Figure 3.1 Multispectral image of the vineyard indicating vigour differences in the Wellington Merlot block. White areas indicate low vigour, green areas indicate medium vigour and blue areas indicate high vigour.

Experimental plot layout:
O - Experimental grapevines
X – Border grapevines

Plot dimensions:
12 m x 12 m = 144 m^2

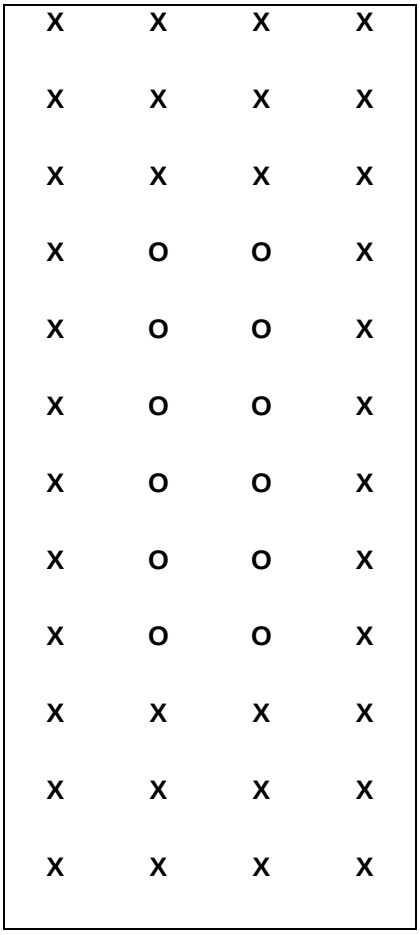

Figure 3.2 Experimental plot layout in the Wellington Merlot vineyard, indicating one repetition unit (obtained from ARC, Infruitec-Nietvoorbij, Stellenbosch).

Table 3.1 Irrigation regimes for the different treatments.

Treatment 1 (T1)	Dryland
Treatment 2 (T2)	Two irrigations on the grapevine row (pea size and véraison)
Treatment 3 (T3)	Irrigation at 20% plant available water depletion on the grapevine row*
Treatment 4 (T4)	Two irrigations in work row (pea size and véraison)
Treatment 5 (T5)	Irrigation at 20% plant available water depletion in work row*
Treatment 6 (T6)	Four irrigations in alternate work rows (partial rootzone drying)

* 2 times per week pre-harvest irrigated, soil water content measurements were used for scheduling of irrigation.

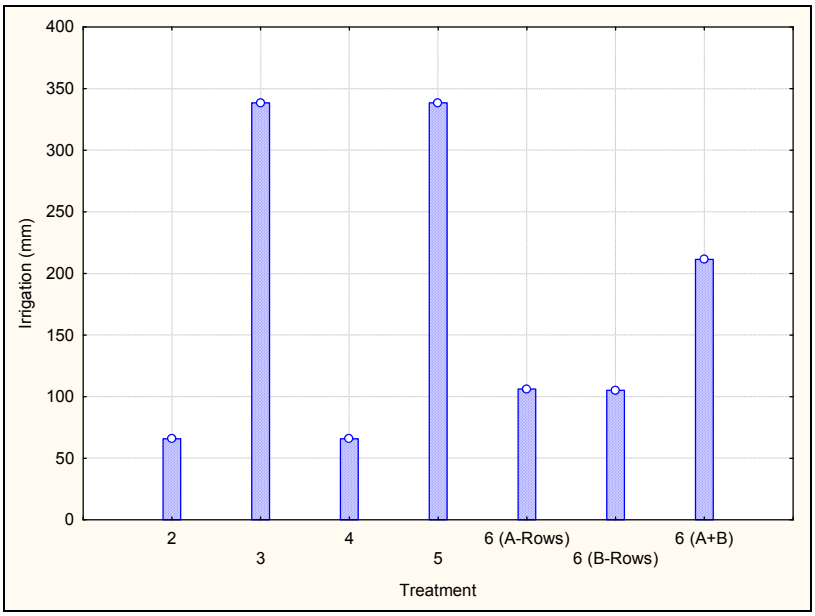

Figure 3.3 Total irrigation (mm) received by the different treatments at the experimental site for the season.

3.2.4 VEGETATIVE MEASUREMENTS

3.2.4.1 WINTER PRUNING MASS

Pruning mass results were obtained for each plot from the ARC Infruitec-Nietvoorbij, Stellenbosch, who was primarily responsible for the vegetative measurements in this project. The vines were pruned to two-bud spurs. Shoot number per vine and pruning mass were recorded for every individual grapevine, and shoot masses were calculated.

3.2.4.2 SHOOT AND LEAF MEASUREMENTS

Destructive measurements of leaf area and shoot length were conducted two weeks prior to harvest. Two shoots from each side of the row were randomly harvested from a representative grapevine for each treatment of each repetition. The shoots were then stored at 4ºC until the measurements were done. Shoot length (both main and lateral) was tape-measured and the number of lateral shoots and bunch numbers were counted. Main and lateral leaf numbers were counted after the leaves were removed from the shoots, and the main and lateral leaf areas of each grapevine were determined separately using a planemometer (Delta-T Devices, Cambridge, UK).

3.2.5 PLANT WATER STATUS MEASUREMENTS

3.2.5.1 STEM WATER POTENTIAL (SWP)

Two healthy, fully expanded leaves on representative vines from both sides of the row were selected for all the treatments and repetitions through the season, and the methods used were adapted from Choné *et al*. (2001). The leaves were chosen on main shoots between the 8th and

10th node. They were enclosed with zip-lock plastic bags covered with aluminium foil while still attached. The leaves were left covered for at least 30 minutes to allow stomatal closure as a result of equilibration with the atmosphere inside the bags. The leaves were then removed by a single cut with a sharp blade after the bags had been removed. They were immediately placed in the pressure chamber (ARIMAD-3000, Israel). Nitrogen gas was allowed to move into the chamber to build up pressure until the first sight of moisture from the petiole was visible, at which time the pressure reading in the chamber was read from the digital screen on the pressure bomb. This reading (-kPa) was noted as the SWP.

3.2.5.2 LEAF WATER POTENTIAL (LWP)

The leaves that were used to measure gs (Section 3.2.6.2) were also used for midday LWP measurement just after the gs measurement was taken. The method used was adapted from Choné *et al*. (2001) and the leaves were removed by a single cut with a sharp blade, and immediately placed in a pressure chamber. Nitrogen gas was allowed to move into the chamber to build up pressure until the first sight of moisture coming out of the petiole was visible, at which time the pressure reading in the chamber was taken from the digital screen on the pressure bomb. This reading (-kPa) was noted as the LWP (Scholander *et al*., 1965).

3.2.6 PHYSIOLOGICAL MEASUREMENTS

3.2.6.1 CARBON ISOTOPE DISCRIMINATION

Twenty μl of fresh juice was extracted for all the samples taken (Section 3.2.7.1) throughout the season up to harvest and was places in Eppindorf tubes and frozen in a -40°C freezer. The samples were sent to the University of Cape Town's stable light isotope laboratory, where the juice was pipetted into a cuvette. The juice was analysed by combustion in a Thermo Finnigan Delta Plus XP stable light isotope mass spectrometer coupled via a Conflo III device to a Thermo 1112 Flash elemental analyser to determine ^{12}C and ^{13}C ratios. The samples were run against in-house reference materials that had been calibrated according to international standards (VPDB for carbon and air for nitrogen). The results are expressed relative to those standards. The conventional expression was used with reference to the Pee Dee Belemnite standard. Carbon isotope composition is expressed as:

$$\delta^{13}C\,‰ = \left(\frac{R_{sample}}{R_{standard}} - 1\right) \times 1000$$

where R_{sample} is the $^{12}C/^{13}C$ for the sample and $R_{srandard}$ is that of the reference material (Farquhar *et al*., 1989).

3.2.6.2 STOMATAL CONDUCTANCE (gs)

Stomatal conductance of two fully expanded, healthy leaves of representative vines of both the sun- and shade-exposed sides of the row were measured during midday periods throughout the season. The leaves were left intact on the grapevine and were chosen to be on main shoots

between the 6th and 8th node. A steady state diffusion leaf porometer, model SC-1 (Decagen devices, inc., Washington, USA), was used for this measurement.

3.2.6.3 LEAF AND CANOPY TEMPERATURES

Canopy and leaf temperatures were collected using a laser-sighted infrared thermometer (Raytek ST20 pro infrared thermometer, Raytek Corporation, USA). The temperature reading was collected five times evenly over a treatment at a distance of one meter and the temperatures were documented (Jones *et al*., 2002).

3.2.7 REPRODUCTIVE MEASUREMENTS

3.2.7.1 WEEKLY BERRY SAMPLING

Berry sampling was performed weekly from pea size to harvest. Thirty berries were randomly sampled for each row of each plot. Sampling was done from the inside and outside of the canopy and from the top, middle and bottom of bunches to obtain a representative sample. The berries were kept in a cooler (4ºC) after being harvested.

A laboratory scale (JW-1000 counting scale, UWE electronic scales, Taiwan) was used to determine the average mass of a berry after calculating the average of the mass of the 30 berries.

The sampled berries (used for weight determination) were crushed by hand in a plastic bag to extract enough fresh juice to measure the pH by using a laboratory pH meter (Crison Basic 20 pH meter, Crison Instruments, Spain).

Enough fresh juice was extracted from the crushed berries to measure the °Brix (total soluble solids) by using a pocket refractometer (ATAGO PAL-1 refractometer, ATAGO Co LTD, Tokyo), zeroed with distilled water. Total soluble solid content was also determined by taking berry size out of the equation (berry mass×°Brix/100).

3.2.7.2 BERRY MEASUREMENTS AT HARVEST

An average of 200 berries was harvested per row of each treatment and each repetition on 31/01/2007. The sampling was done from the inside and outside of the canopy and from the top, middle and bottom part of bunches. The berries were kept in a cooler (4°C) until the analyses were done.

The berry mass was measured for the 200 berries for all the samples collected using a laboratory scale (JW-1000 counting scale, UWE electronic scales, Taiwan). The berries were then crushed in a plastic bag to extract fresh juice. The pH and total soluble solids of the juice were measured.

The titratable acidity (TA) was measured using 150 ml of fresh juice and was analysed with a 785 DMP Metrohm Titrino automatic titration instrument (Metrohm Ltd, UK). Fresh buffers of pH 4 and pH 7 were used for this determination.

Grape juice parameters were determined using a Fourier transform infrared (FT-IR) spectrometer (WineScan®). The juice was first filtered with a filter (type 79500, FOSS Electric, Denmark) that uses filter paper graded at 20 to 25 µm. It was connected to a vacuum pump and

then analysed with a multi-parameter analyser (WineScan® FT 120, FOSS, Denmark) with ready-made calibrations and GrapeScan® software. The WineScan® analyser employs a Michelson interferometer to generate the FTIR spectra. Instrument settings included cell path length of 37 µm, sample temperature set to 40°C, and sample volume of 7 to 8 ml. The samples were pumped through the heat exchanger and the CaF_2-lined cuvette and scanned from 926 to 5012 cm^{-1} at 4 cm^{-1} intervals. The determined parameters included pH, sugars, organic acids and colour. Cleaning was automatically programmed to occur every 5 min. The instrument was zeroed with the zeroing solution (S-6060, Foss Electric) before any set of analyses.

3.2.8 STATISTICAL ANALYSES

The data were analysed using Statistica 8.0 (Statsoft, Inc., Tulsa, OK, USA) with the repeated measures ANOVA mixed model approach (McCulloch *et al.*, 2008).

3.3 RESULTS AND DISCUSSION

3.3.1 CLIMATE

Climate data for the season (figures 3.4 and 3.5) shows that there were periods of steep temperature and/or relative humidity changes during the season. These increases/decreases could have influenced some grapevine development parameters.

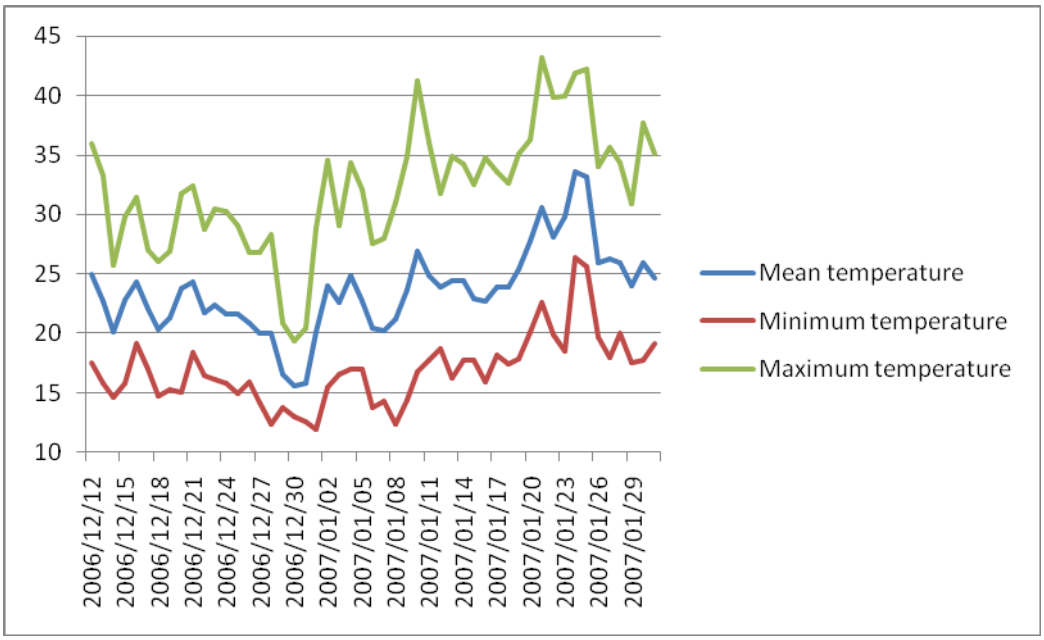

Figure 3.4 Mean, minimum and maximum temperatures (ºC) for the measuring period at the Wellington plot (2007).

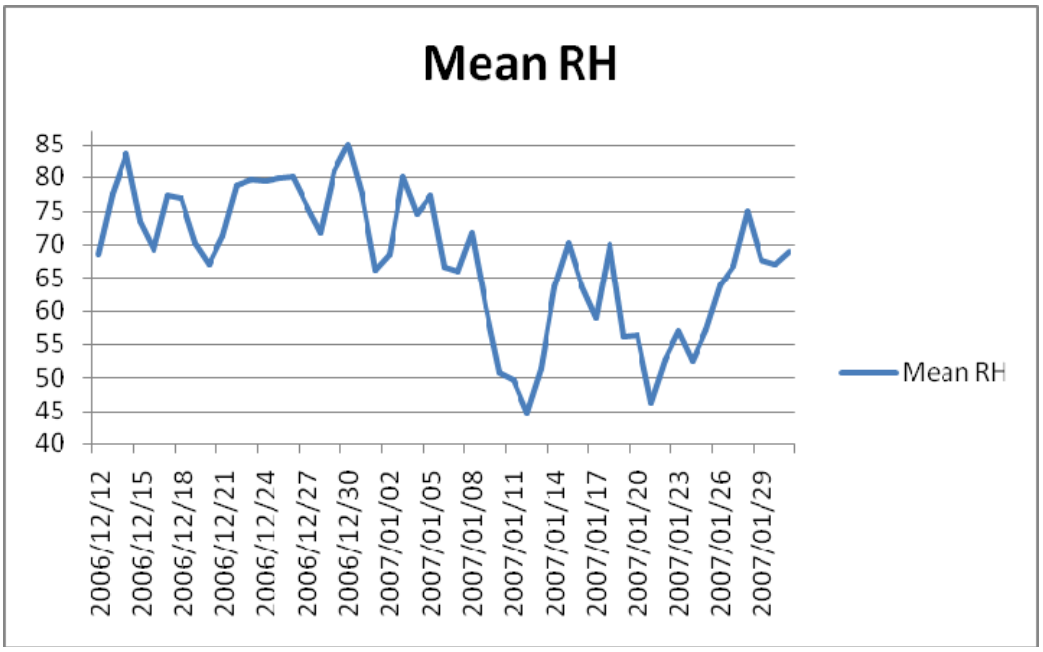

Figure 3.5 Mean relative humidity (%) for the measuring period at the Wellington plot (2007).

There was no rainfall in this area during the measurement season.

3.3.2 VEGETATIVE MEASUREMENTS

3.3.2.1 WINTER PRUNING MASS

Data used for the analyses was obtained from the ARC Infruitec-Nietvoorbij, Stellenbosch. There were significant differences in pruning mass between the irrigation treatments ($P < 0.01$) (figure 3.6). The vines from the dryland treatment (T1) had the smallest pruning mass, followed by the vines that received two irrigations on the grapevine row (T2) and then those that received two irrigations in the work row (T4). Vines that received irrigation at 20% plant available water depletion on grapevine row (T3) had the highest pruning masses, followed by those that received four irrigations in alternate work rows (T6), and then those that received irrigation at 20% plant available water depletion in work row (T5).

There was therefore a definite effect of irrigation regime on vegetative growth and, because experimental work was also conducted on this site in the previous season, the irrigation regime received then could have contributed to grapevine vigour, so it should be an effect of both seasons' irrigation.

It seems that the more frequently irrigated treatments with the larger cane masses had more cytokinin translocation from the roots, leading to more shoot development and causing the larger pruning mass of the vines of those treatments.

The increased irrigation also led to increased stomatal conductance, which should promote photosynthesis, and this could lead to increased vegetative growth.

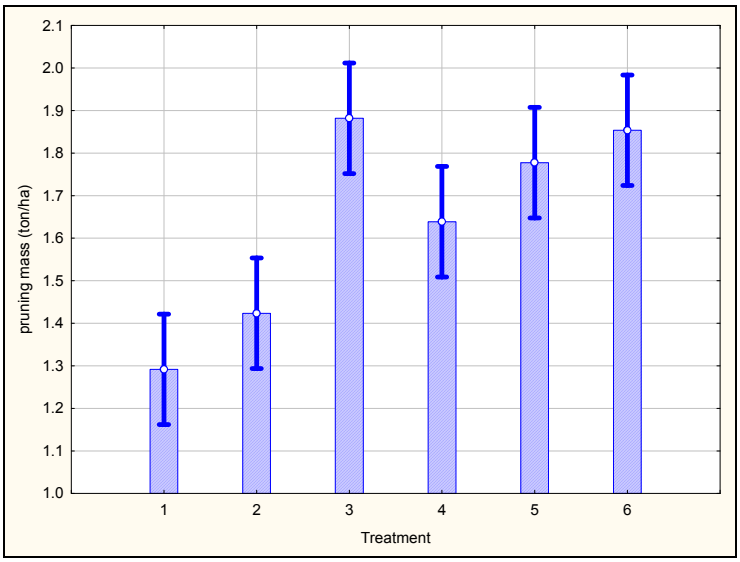

Figure 3.6 ANOVA computed for pruning mass (ton/ha) of vines of all repetitions in the different treatments ($P < 0.01$) (vertical bars denote 0.95 confidence intervals).

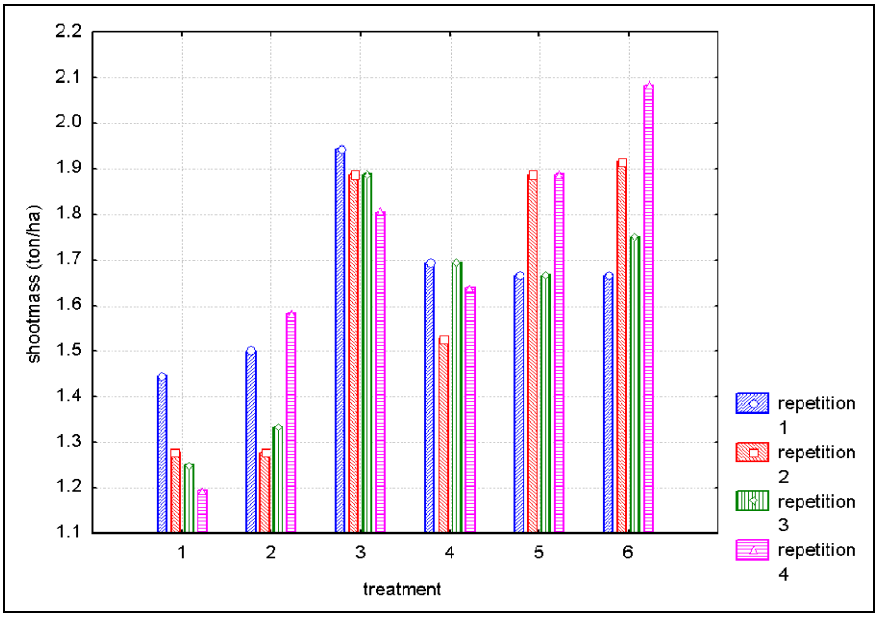

Figure 3.7 Graph indicating grapevine pruning masses (ton/ha) of the repetitions of the different irrigation treatments (vertical bars denote 0.95 confidence intervals).

The grapevine pruning mass in the different repetitions (figure 3.7) indicates that there was large differences between the treatments, but it corresponds with the multi-spectral image (Figure 3.1, Section 3.2.3), which indicates that vines from repetition 4 have a slightly higher vigour, especially for treatment 6. This could have had an effect on the experiment, as the vines in these treatments/repetitions were situated in a part of the vineyard with higher vigour. This is a problem that may arise in irrigation studies, and is also why a study was undertaken, as discussed in the next chapter, to investigate the effect of vigour along with changes in plant water status.

3.3.2.2 SHOOT AND LEAF MEASUREMENTS

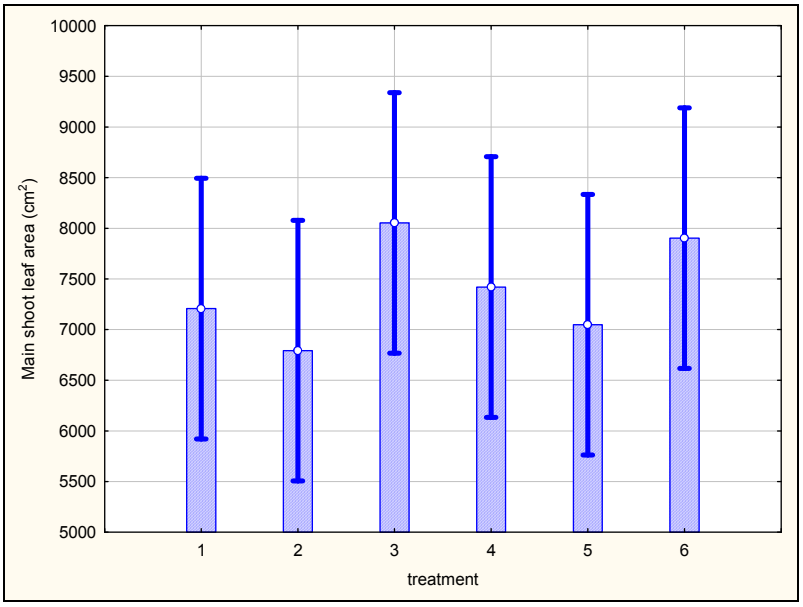

Figure 3.8 ANOVA computed for main shoot leaf areas (cm^2) of vines in the different treatments ($P > 0.1$) (vertical bars denote 0.95 confidence intervals).

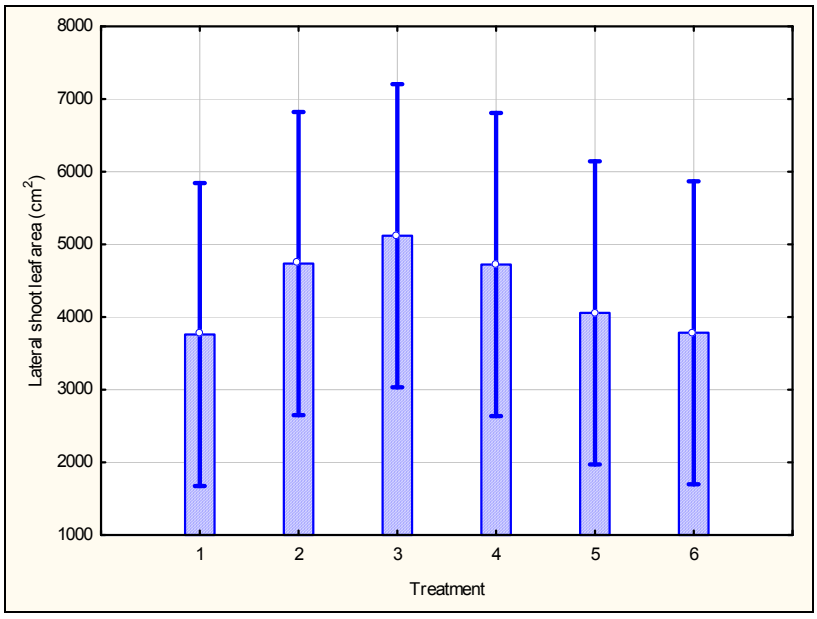

Figure 3.9 ANOVA computed for lateral shoot leaf areas (cm^2) of vines in the different treatments ($P > 0.1$) (vertical bars denote 0.95 confidence intervals).

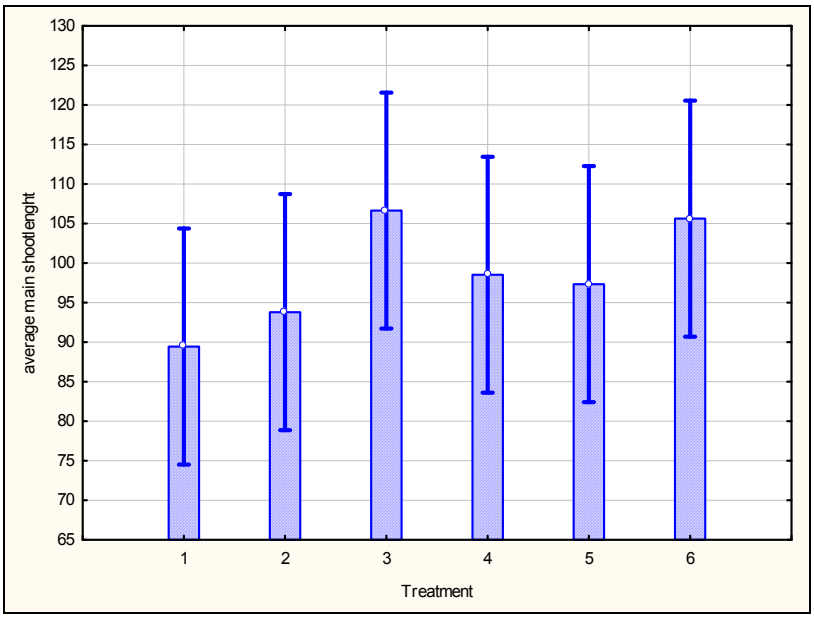

Figure 3.10 ANOVA computed for main shoot length (cm) of vines in the different treatments (2007) (vertical bars denote 0.95 confidence intervals).

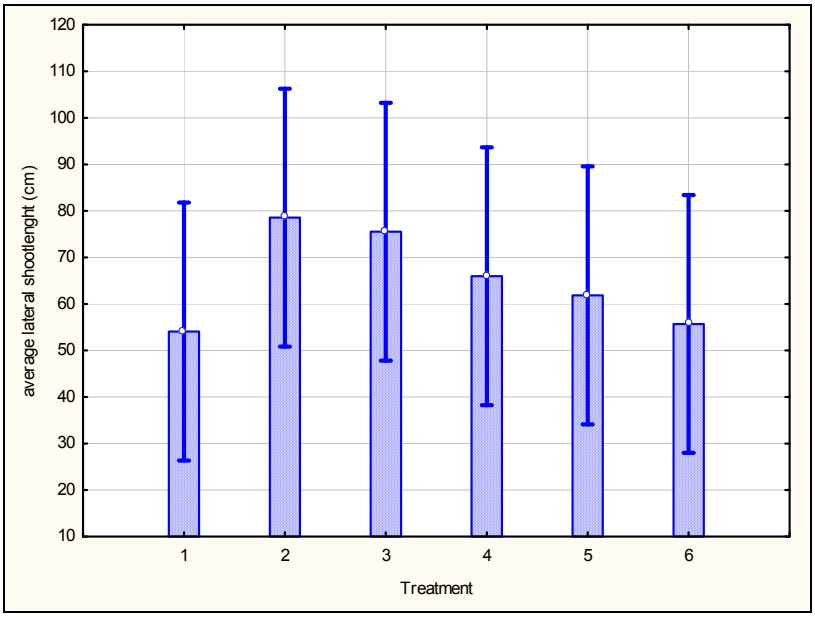

Figure 3.11 ANOVA computed for lateral shoot lengths (cm) of vines in the different treatments (2007) (vertical bars denote 0.95 confidence intervals).

There were no significant differences in total, main (figure 3.10) and lateral (figure 3.11) shoot lengths between the treatments ($P > 0.1$). The differences in total, main (figure 3.8) and lateral (figure 3.9) shoot leaf area were also not significant, and the same was found for areas per leaf (data not shown). Topping of the shoots during the season could have caused there to be no significant differences, while the destructiveness of the measurement could also have contributed to this. It is furthermore noted that the vines from the dryland treatment (T1) had slightly shorter shoot lengths than those from the other treatments, and slightly lower leaf areas than most of the other treatments. Vines of treatment 3 (vines with the highest pruning masses) seem to have had slightly longer shoots and slightly higher leaf areas than vines of the other

treatments. Irrigation regime therefore tended to affect shoot lengths and leaf areas, but this measurement was not a good way to indicate vegetative growth, for the reasons mentioned. It should also be borne in mind that there was large variability within the treatments, which could have influenced these measurements.

3.3.3 PLANT WATER STATUS

Note that water deficit threshold levels (water stress levels) for all the plant water status measurements are described as given in Chapter 2, Table 2.1.

3.3.3.1 STEM WATER POTENTIAL (SWP)

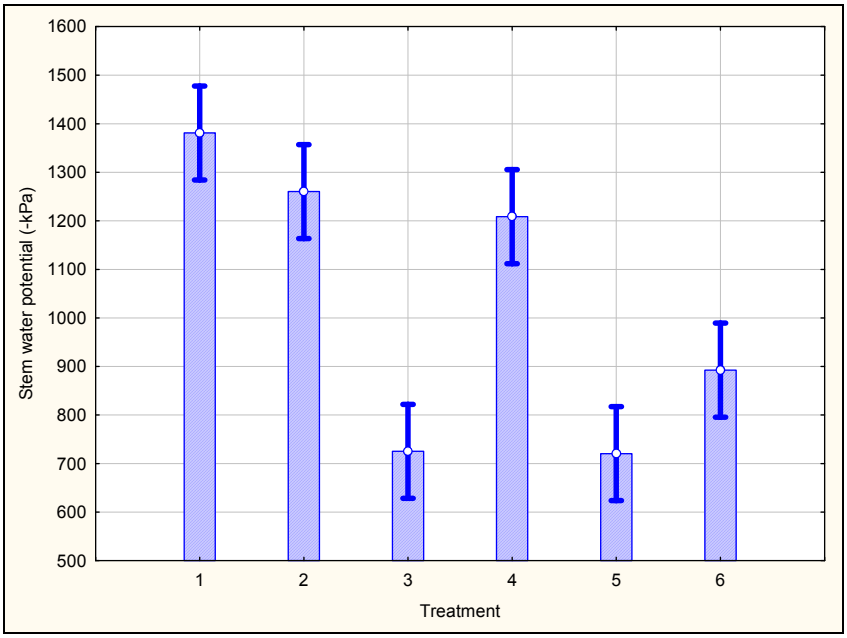

Figure 3.12 ANOVA computed for stem water potentials (-kPa) through the measurement season for the different irrigation treatments ($P < 0.01$) (vertical bars denote 0.95 confidence intervals).

There were some significant differences between SWP measurements for the treatments through the season (figure 3.12). The measurements indicate that dryland vines (T1) were the most stressed throughout the season, showing moderate-severe to severe water deficits. The SWP of vines of treatments 2 and 4, which received two irrigations during the season, did not differ significantly from that of T1, but these vines were slightly less stressed according to the SWP measurements. These vines showed moderate to severe water deficits through the season.

Vines that received four PRD irrigations (T6) had significantly less negative SWP measurements than treatments 1, 2 and 4, and vines that received the PRD treatment had weak to moderate to weak water deficits according to the SWP measurements. It was also found that vines that received irrigation at 20% plant available water depletion had the less negative SWP measurements throughout the season, showing weak water deficit levels.

SWP was therefore highly affected by irrigation regime through the season, with higher irrigation volumes resulting in SWP values indicating less stress.

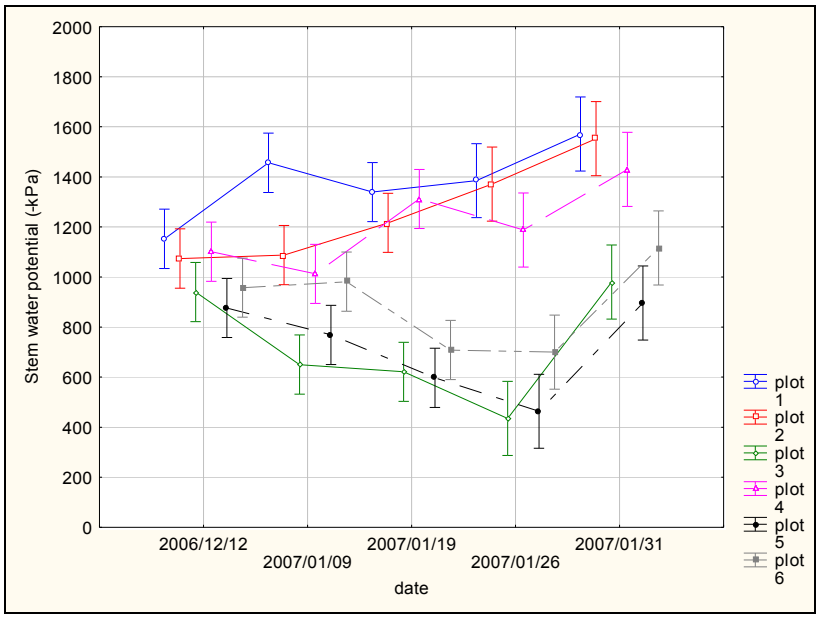

Figure 3.13 ANOVA computed for the seasonal evolution of SWP measurements for the different treatments (vertical bars denote 0.95 confidence intervals).

From the seasonal pattern of SWP measurements for the different treatments (figure 3.13) it can be observed that there was not a big differentiation between the treatments at the start of the measurement season, when the berries were at around pea size. There were still small differences in the stress classifications for the vines of the different treatments at this stage, with T1 showing moderate to severe water deficits, while T2 and T4 showed moderate water deficits, and T3, T5 and T6 showed moderate to weak water deficits.

As the season progressed and water availability between the different treatments started to differ, the differentiation between SWP measurements increased between the treatments.

It can be seen that the vines of T1 immediately showed increased stress, to show moderate-severe to severe water deficits at véraison (around 2007/01/09), while those of T2 and T4 remained stable from pea size to just after véraison due to the two irrigations they received in this period, keeping grapevine stress levels moderate until véraison. The stress levels of the vines of T6 also stayed stable until véraison, while those of T3 and 5 showed decreasing stress levels towards véraison due to the two irrigations they received per week, resulting in weak water deficits at around véraison.

After véraison, large significant differences started to develop between the treatments. The stress levels of the T1 vines did not change very much until five days before harvest, and showed a slight increase in stress after that until harvest time (2007/01/31), with severe water stress during harvest time. The vines of T2 showed a steep increase in stress towards harvest time, as irrigation water was no longer available to the vines and the soil continued to dry out, with SWP levels indicating severe water stress at harvest. For the T4 vines, SWP measurements changed significantly in the next two weeks after véraison, to show moderate to severe water deficits, and stayed that way until a week before harvest, showing moderate-severe to severe water deficits at harvest.

The SWP measurements of vines that received two irrigations a week (T3, 5 and 6) showed decreased stress levels after véraison until about a week before harvest. The stress levels decreased more for the T3 and T5 vines in comparison with the T6 vines during this period. The

SWP measurements of the T3 and T5 vines showed no water deficits at this stage, while those of T6 showed weak water deficits.

In the last week of the season towards harvest there were large increases in water stress for all the treatments, as the vines used a lot of water to ripen the berries and to survive the high temperatures that were recorded during this period. The T6 vines showed moderate water deficits during harvest time, the T3 vines showed moderate to weak water deficits, while the T5 vines showed weak to moderate-to-weak water deficits.

There were no significant differences in the SWP measurements of vines with canopies receiving midday sun and midday shade ($P > 0.1$).

3.3.3.2 LEAF WATER POTENTIAL (LWP)

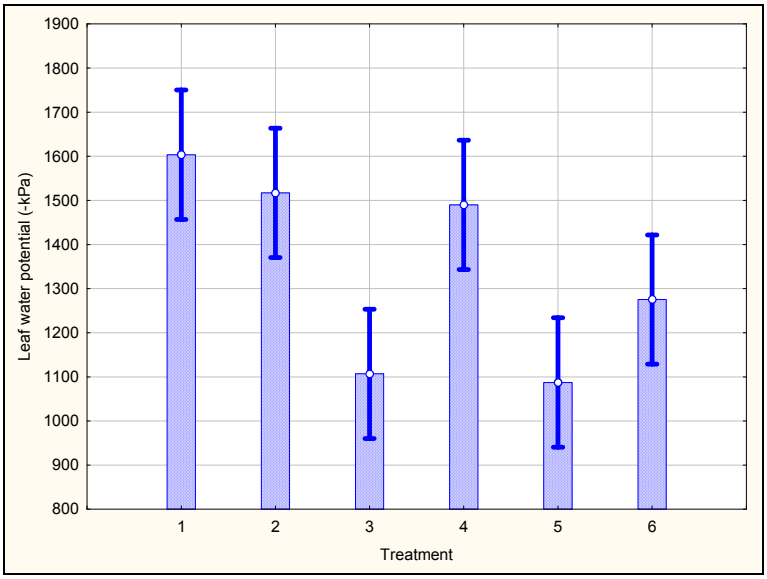

Figure 3.14 ANOVA computed for leaf water potential (-kPa) through the measurement season for the different irrigation treatments (2007) ($P < 0.01$) (vertical bars denote 0.95 confidence intervals).

Leaf water potential measurements between the treatments for the season (figure 3.14) showed similar results to the SWP measurements, although it seems that the SWP measurements had larger significant differences between the treatments. This corresponds to information in the literature, which states that SWP is more discriminating between water status differences (Choné *et al*., 2001).

In the case of vines of T1, T2 and T4, the vines that received the least irrigation in the season showed the highest stress levels according to LWP measurements. During the season, the T1 vines had LWP readings indicating slightly higher stress than T2 and T4 (indicating severe water deficits through the season), while the T2 and T4 vines had moderate-severe to severe water deficits.

The T6 vines had LWP measurements indicating moderate to severe water deficits through the season, while the T3 and T5 vines showed moderate water deficits.

The LWP measurements therefore showed higher stress levels as the availability of irrigation water decreased between the treatments.

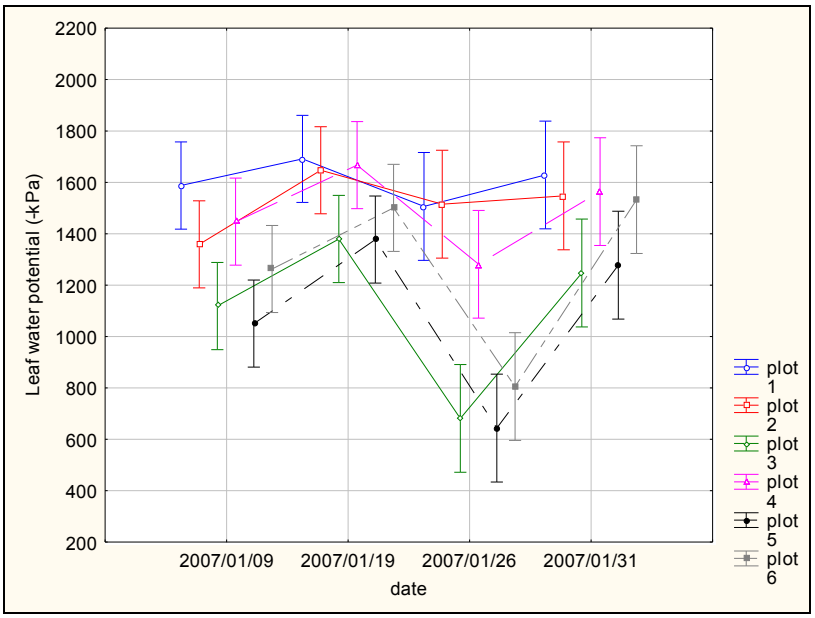

Figure 3.15 ANOVA computed for the seasonal evolution of LWP measurements for the different treatments (vertical bars denote 0.95 confidence intervals).

Seasonal evolution measurements (figure 3.15) show that at the start of LWP measurements, just after véraison, there already was differentiation between the treatments, as the irrigation regimes were well under way. T1 showed the highest stress at this stage, with LWP measurements indicating severe water deficits. The vines of T2 and T4 had already received the two irrigations for the season, and therefore showed a trend of having slightly less stress that T1. The stress levels of the T1 vines stayed this way towards harvest time, while those of T2 increased slightly towards harvest and showed severe water deficits at harvest. The stress levels of T4 varied more as the season progressed, but also showed severe water deficit at harvest.

The LWP stress levels for treatments 3, 5 and 6 increased until 12 days before harvest, as there were increases in the daily temperature and steep decreases in relative humidity. The levels then decreased until five days before harvest, and again showed increases until harvest. At harvest time, the LWP measurements for the T6 vines indicated moderate-severe to severe water deficits, while those of T3 and T5 showed moderate to severe water deficits.

There were no significant differences in LWP between leaves that received midday sun and midday shade ($P > 0.1$).

3.3.4 PHYSIOLOGICAL MEASUREMENTS

3.3.4.1 CARBON DISCRIMINATION

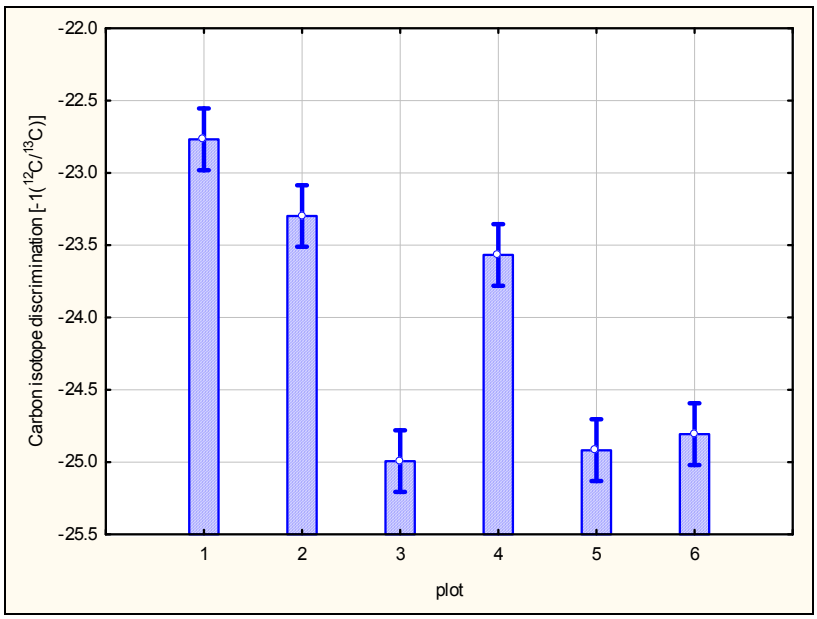

Figure 3.16 ANOVA computed for carbon isotope discrimination ($^{12}C/^{13}C$) through the measurement season for the different irrigation treatments ($P < 0.01$) (vertical bars denote 0.95 confidence intervals).

The sensitivity of the carbon isotope discrimination (CID) measurements can be seen in Figure 3.18, where large significant differences were found between the treatments ($P < 0.01$). The vines of T1 showed the highest stress levels according to the CID measurements, and showed moderate to severe water deficits though the season. Vines of T2 and T4 had significantly lower stress levels according to the CID measurements, and indicated moderate to weak water deficits. The vines of T6 showed CID measurements indicating weak water deficits and did not significantly vary from the vines of T3 and T5.

The vines that received less irrigation had decreased photosynthesis due to lower stomatal conductance and gas exchange, leading to less discrimination by ribulose-1,5-bisphosphate carboxylase oxygenase (Rubisco) on ^{13}C (lower CO_2 supply because of decreased gas exchange), which is why ^{13}C was absorbed more by the more stressed vines, leading to less negative $\delta^{13}C$ values.

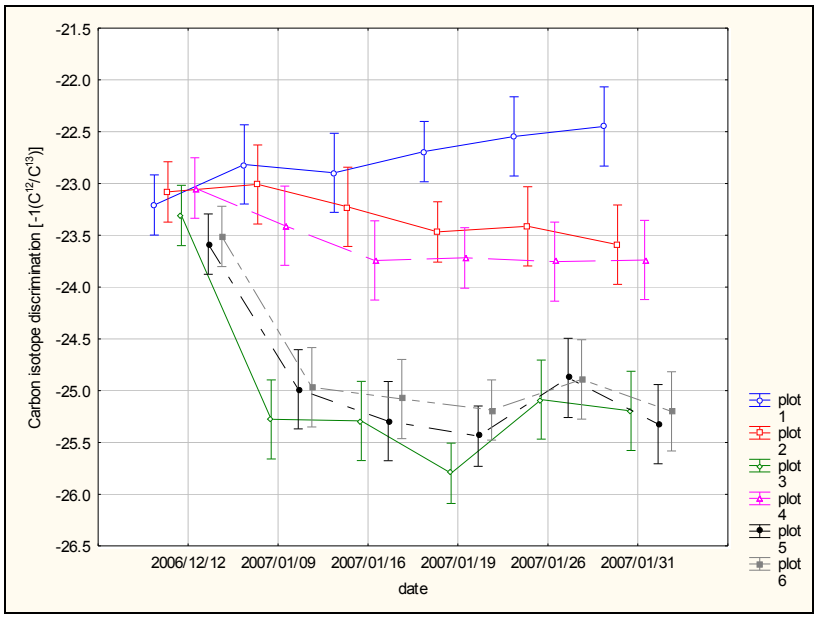

Figure 3.17 ANOVA computed for the seasonal evolution of carbon isotope discrimination ($^{12}C/^{13}C$) measurements for the different treatments (vertical bars denote 0.95 confidence intervals).

The seasonal evolution of the CID measurements (figure 3.17) reflected the measurements of both SWP and gs. This should follow, as stomatal conductance influences the ratio of CO_2 across the internal cells of the leaf and in the atmosphere.

At the start of the CID measurements, when the berries were at around pea size, there was no big difference between the treatments, as the irrigation regimes were yet to be implemented. All the treatments showed moderate water deficits according to CID at this stage. The reason for this might be the berry composition at this stage of the season, with organic acids as the predominant carbon molecules, leading to different CID values than those from sugars due to differences in biosynthetic pathways, possibly leading to the CID values showing “stress” at this stage of the season. This places a question mark behind measuring CID early in the season.

As the season progressed, there was differentiation between the treatments as water availability varied between them, showing that CID measurements can be accumulative throughout the season.

At around véraison (2007/01/09) there were significant differences between the treatments, with treatments that received more irrigation (T3, T5 and T6) showing a steep decrease in water stress, while those that received less water (T2 and T4) did not show strong changes in water stress, although it decreased a little towards harvest. The dryland vines (T1) continued to show increased stress towards harvest.

At véraison, the T1 vines showed moderate to moderate to severe water deficits, while the T2 vines showed moderate water deficits and the T3 vines showed moderate to weak water deficits. The T3, T5 and T6 vines showed weak water deficits during this period. The increased irrigation of T3, T5 and T6 therefore led to steep decreases in the stress level according to the CID. From véraison towards harvest, sugars were the predominant C-molecules in the berries and therefore the values differed a lot from that measured pre-véraison, where it was measured mostly from organic acids.

After véraison towards harvest, the stress level for T1 continued to increase and showed moderate to severe water deficits at harvest. The stress levels of T2 and T4 decreased slightly until harvest and showed moderate to weak water deficits at harvest. The two irrigations received by T2 and T4 therefore caused significant differences between these two treatments and T1, which did not receive irrigation as the season progressed – an effect that was not visible from the measurements of plant water potential and stomatal conductance. This again indicates the sensitivity of the CID measurements to differentiate between irrigation treatments. After véraison there were no changes in the stress levels of the frequently irrigated treatments 3, 5 and 6, which indicated weak water deficits at harvest time. The PRD treatment showed similar CID measurement to T3 and T5, although it received less water.

3.3.4.2 STOMATAL CONDUCTANCE (gs)

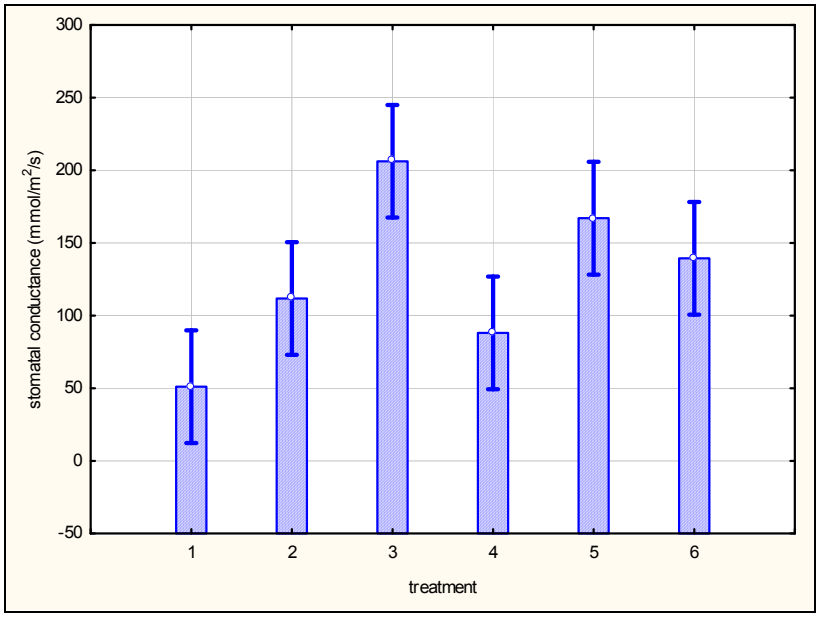

Figure 3.18 ANOVA computed for stomatal conductance measurements through the measurement season for the different irrigation treatments ($P < 0.01$) (vertical bars denote 0.95 confidence intervals).

There were some significant differences in gs measurements between the treatments (figure 3.18), with the T1 vines having the lowest stomatal conductance, confirming that these vines experienced large amounts of stress during the growing season. The T2 and T4 vines had higher gs values than T1, and lower gs values than the more frequently irrigated vines. The T3 vines had the highest gs levels, followed by the T5 and T6 vines. Vines of these three treatments could therefore have had higher transpiration rates and lower water-use efficiencies than the more stressed vines because of the higher gs measurements.

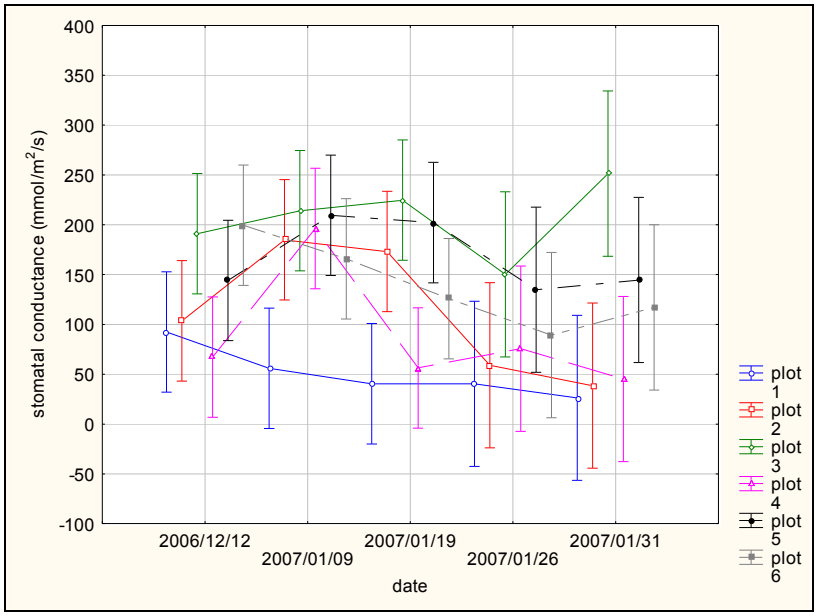

Figure 3.19 ANOVA computed for the seasonal evolution of stomatal conductance measurements for the different treatments (vertical bars denote 0.95 confidence intervals).

The seasonal pattern for gs measurements (figure 3.19) shows that the T1 vines consistently had low gs values, as they were stressed throughout the season. The T2 and T4 vines showed increases in gs after the two irrigations, and then showed decreases towards harvest to have low values at harvest time as the vines became more stressed. The T3 and T5 vines had the highest gs values through the season, while the T6 vines had gs levels a little lower, as the increased ABA transportation from the roots caused partial stomatal closure and reduced transpiration, thus increasing water-use efficiency.

Vines with less water availability, especially T1, and also T2 and T4 at the end of the season, had higher ABA synthesis and transport from the roots, thus increasing stomatal closure, decreasing transpiration and increasing water-use efficiency.

There were significant differences between leaves that received midday sun and midday shade only at a 10% confidence level ($P < 0.1$). Leaves from the shaded side had higher stomatal conductance than leaves on the sun side. This was due to the higher temperatures of leaves on the sun side, which stimulated stomatal closure more than was the case with the leaves on the shaded side.

It seems that the gs measurements were more variable than the SWP and CID measurements.

3.3.4.3 LEAF AND CANOPY TEMPERATURES

Leaf temperature differences between the treatments were not significant ($P > 0.1$) (data not shown). There was a trend showing the highest leaf temperatures for T1, while T2 and T4 vines also had high leaf temperatures. T3, T5 and T6 had slightly lower leaf temperatures. Vines that were exposed to more stressed conditions therefore had higher leaf temperatures because of lower stomatal conductance and therefore less transpiration, which leads to decreased evaporative cooling of the leaves, when compared to vines that were less stressed.

There were no significant differences in leaf temperatures throughout the season on the dates when the measurements were taken (data not shown).

The leaf temperatures of leaves receiving midday sun were significantly higher than those that received midday shade ($P < 0.05$)

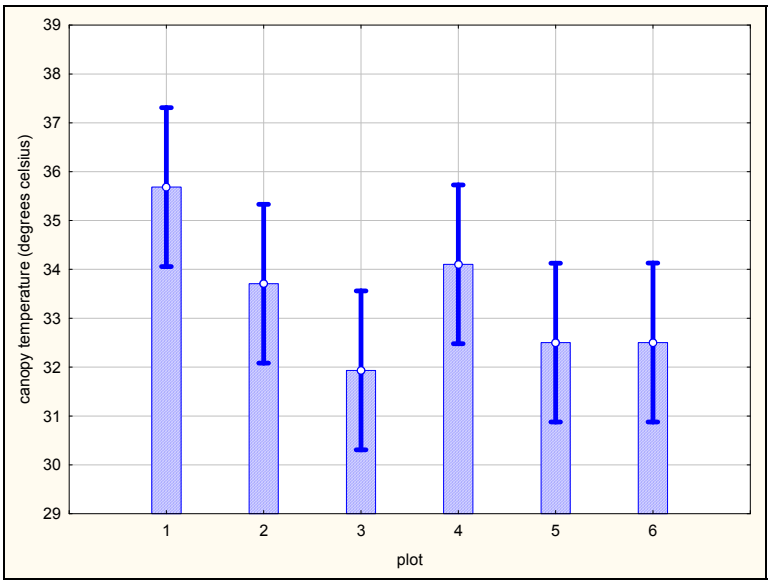

Figure 3.20 ANOVA computed for canopy temperatures (°C) for the different irrigation treatments ($P < 0.05$) (vertical bars denote 0.95 confidence intervals).

Canopy temperatures (figure 3.20) showed better discrimination between the treatments than was shown by leaf temperatures, as there were significant differences between some of the treatments ($P < 0.05$).

It was again found that the T1 vines had the highest canopy temperatures, followed by those of T4 and T2, which only showed a trend towards having lower temperatures than T1, while T3, T5 and T6 had significantly lower temperatures than T1. The more stressed vines had lower stomatal conductance, leading to less cooling of leaves, and the canopy temperature therefore was higher.

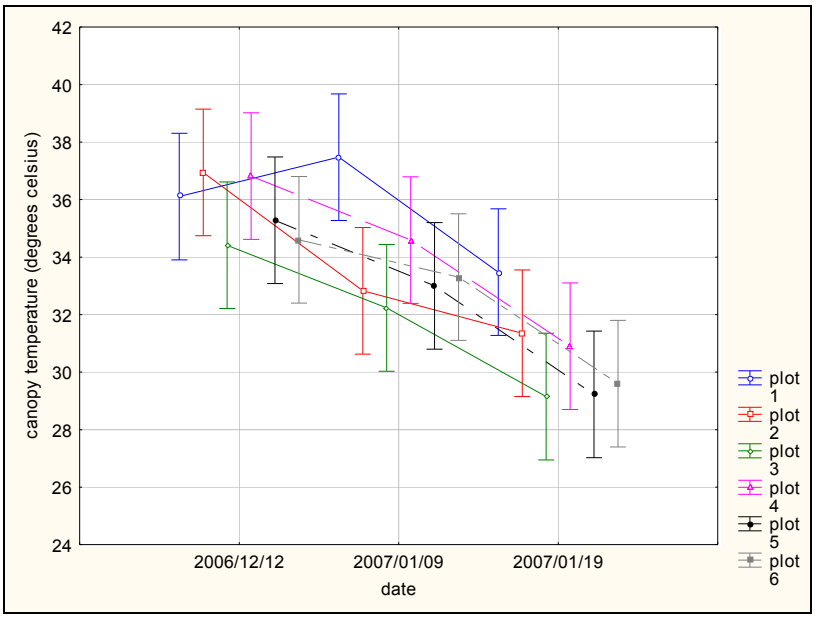

Figure 3.21 ANOVA computed for canopy temperatures (°C) throughout the measurement season for the different irrigation treatments ($P < 0.05$) (vertical bars denote 0.95 confidence intervals).

There were no significant differences throughout the season when canopy temperatures were measured (figure 3.21), although the T1 grapevine canopies showed a trend to having the highest temperatures and the T3 canopy temperatures showed a trend to being the lowest throughout the season. Vines with the highest gs therefore had the coolest canopies, while vines with the lowest gs had the warmest canopies, although the differences were not significant. A reason why there were no significant differences between treatments through the season could be the large variation between vines within treatments.

There were significant differences between canopy temperatures of grapevine rows that received midday shade and midday sun, but only at the 10% confidence level ($P < 0.1$), with those that received midday sun having higher canopy temperatures.

3.3.5 REPRODUCTIVE MEASUREMENTS

3.3.5.1 BERRY DEVELOPMENT

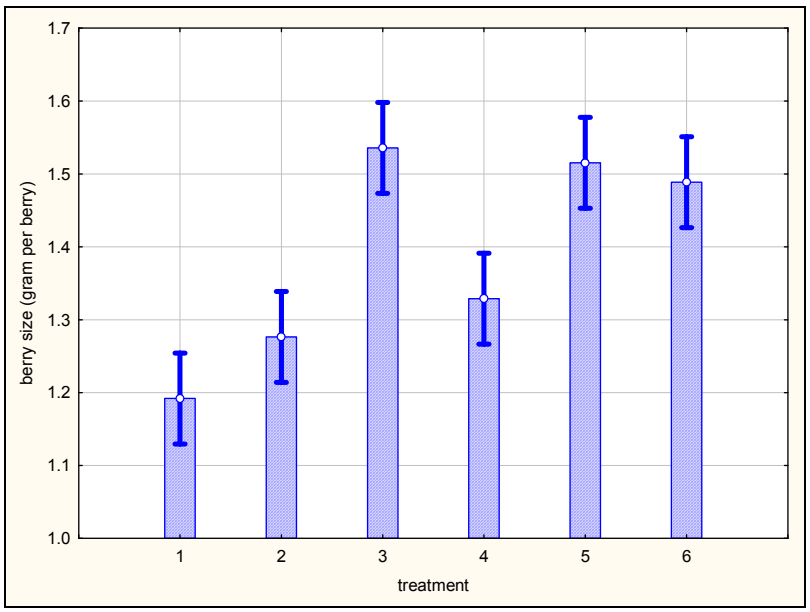

Figure 3.22 ANOVA computed for berry mass for the different irrigation treatments ($P < 0.01$) (vertical bars denote 0.95 confidence intervals).

Plant water availability affected berry growth significantly (figure 3.22), as the wetter treatments (T3, T5 and T6) had the largest berries. The berries of T1 were significantly smaller than those of T4, T3, T5 and T6, and had the smallest berries. T1, T2 and T4 had significantly smaller berries than T3, T5 and T6. Increased water availability therefore led to increased berry mass. Unfortunately, berry volume data was not collected for this study.

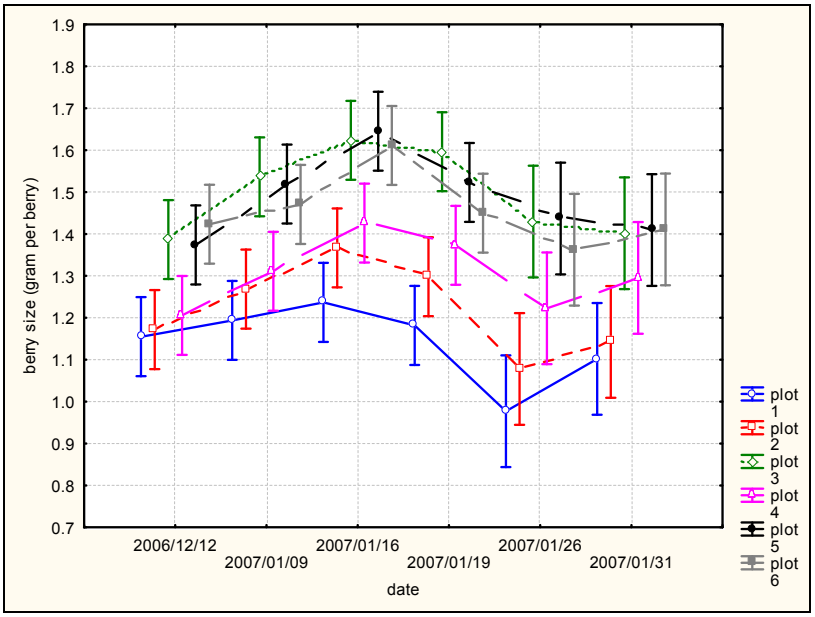

Figure 3.23 ANOVA computed for berry size evolution through the measurement season for the different irrigation treatments (vertical bars denote 0.95 confidence intervals).

Seasonal evolution data (figure 3.23) show that the berry mass of T1, T2 and T4 was similar at that at the start of measurements, while that of T3, T5 and T6 was larger. The higher vigour for treatments 3, 5 and 6 due to the influence of the previous season's irrigation could have caused these differences. As the season progressed, berry mass for all the treatments started to increase, with that of T2 and T4 increasing more rapidly than that of T1 after the two irrigation regimes, which increased berry cell expansion more than that of the dryland vines. The vines that received two irrigations a week also showed steep increases in berry mass and had significantly larger berries than those of treatments 1, 2 and 4 at about two weeks before harvest. It therefore can be seen how increased water availability leads to berry mass expansion.

Berry sizes for all the treatments showed slight decreases until five days before harvest. The high temperatures and low relative humidity recorded during this period could have contributed to this by increasing berry evapotranspiration. The berry mass then increased slightly towards harvest.

3.3.5.2 BERRY CHEMICAL COMPOSITION

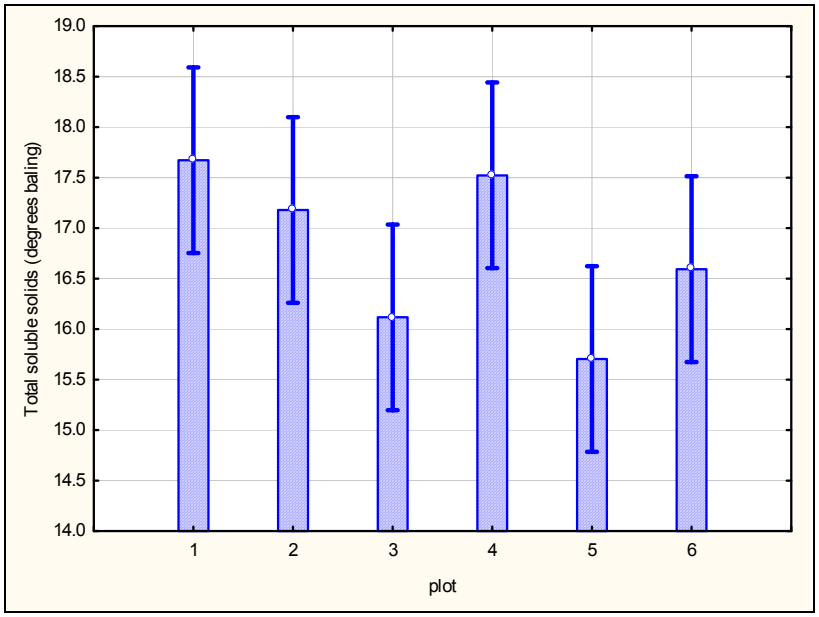

Figure 3.24 ANOVA computed for berry juice total soluble solids (°B) for the different irrigation treatments ($P < 0.05$) (vertical bars denote 0.95 confidence intervals).

There were significant differences in juice total soluble solid content between the treatments ($P < 0.05$) (figure 3.24). Vines from the dryland treatment had significantly higher contents than those from T5 and showed trends for having higher contents than the other treatments as well. The treatments that received two irrigations (T2 and T4) showed trends to having higher contents than those that received more irrigation; T4 had significantly higher contents than T5. Treatments that received more irrigation had the lowest content and it therefore can be seen that vines with larger berries had lower total soluble solids. This might be due to a dilution effect, with higher berry volumes leading to increased dilution of sugars in the berry juice.

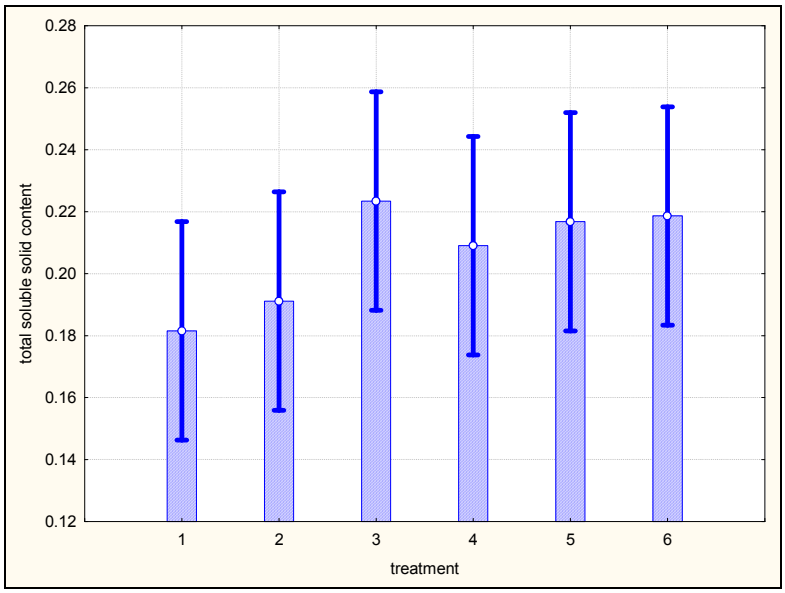

Figure 3.25 ANOVA computed for berry juice total soluble solid content for the different irrigation treatments (P > 0.1) (vertical bars denote 0.95 confidence intervals).

When berry size was taken out of the equation (figure 3.25), there were no significant differences between juice total soluble solids between the treatments. There was only a trend for T1 and T2, which were the driest treatments, to have lower soluble solids than the wetter treatments. The reason might be lower photosynthesis for these vines due to reduced stomatal conductance, leading to lower sugar production.

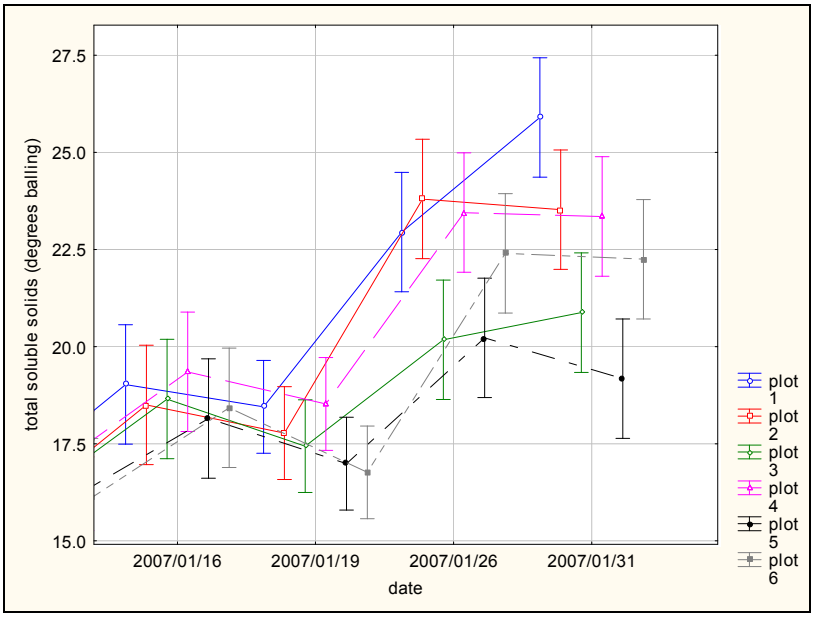

Figure 3.26 ANOVA computed for the seasonal evolution of berry juice total soluble solids throughout the measurement season for the different irrigation treatments (vertical bars denote 0.95 confidence intervals).

Seasonal evolution (figure 3.26) shows that there were no differences in juice total soluble solids until about two weeks before harvest. All the treatments showed steep increases until véraison as the berries ripened. Sugar production then slowed down and increased again significantly in the last couple of weeks towards harvest.

At the end of the season, as berry mass started to differ substantially between the treatments, there was a differentiation in juice soluble solids, as the larger berries from the wetter treatments showed increased dilution in the measurements.

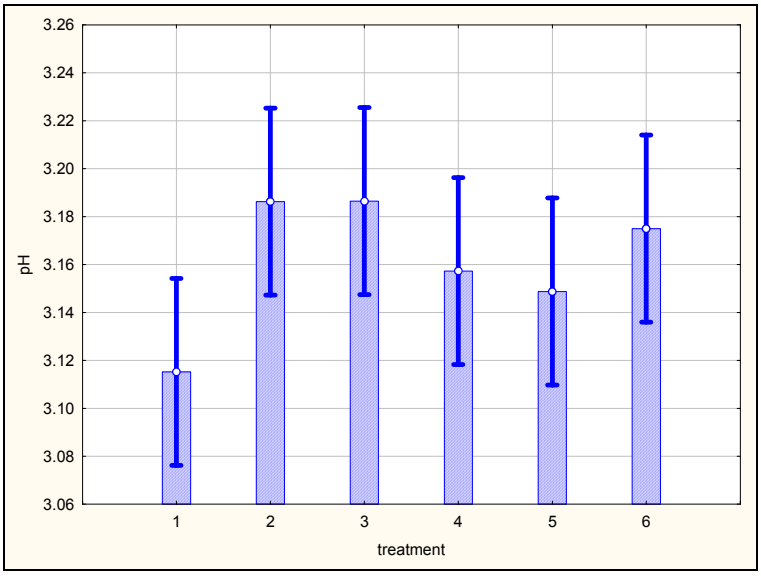

Figure 3.27 ANOVA computed for berry juice pH for the different irrigation treatments (P > 0.1) (vertical bars denote 0.95 confidence intervals).

Differences in juice pH were not significant between the treatments (figure 3.27), although the vines of treatment 1 had a trend towards the lowest pH. The small berries of the vines of treatment 1 might have led to increased concentrated acids, and thus a lower pH.

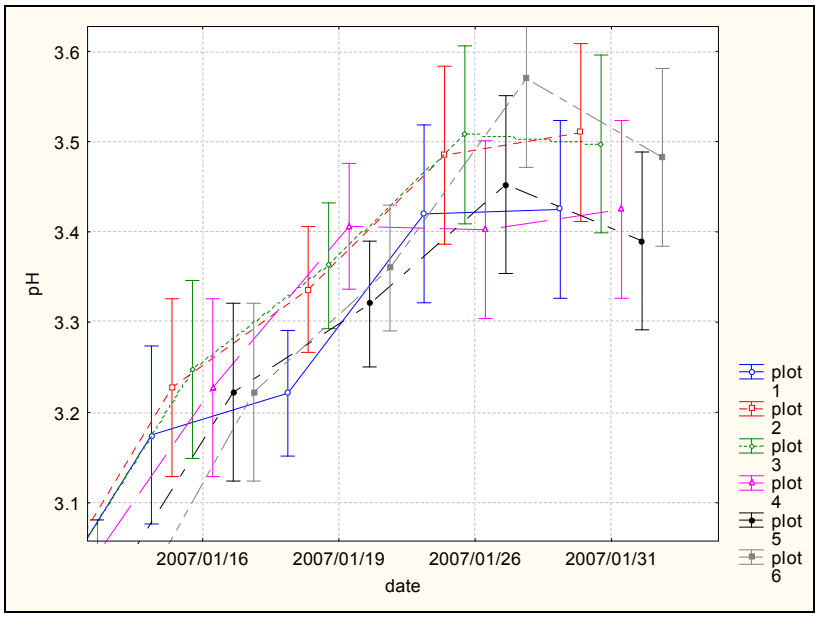

Figure 3.28 ANOVA computed for the seasonal evolution of berry juice pH throughout the measurement season for the different irrigation treatments (vertical bars denote 0.95 confidence intervals).

Seasonal evolution of juice pH (figure 3.28) shows that the increases in juice pH was fast in the beginning of the season until just after véraison, when the increase started to slow down as the berries ripened and the ratio of acid salts and free acids increased as acid synthesis reduced.

The increase in pH in vines from treatments 1 and 4 started to slow after about two weeks before harvest. This might be due to the reduced berry size, causing the acids to become more concentrated in the juice, and thus leading to the slower increase in pH. There were no significant differences between the treatments in the seasonal evolution of juice pH.

3.3.5.3 BERRY MEASUREMENTS AT HARVEST

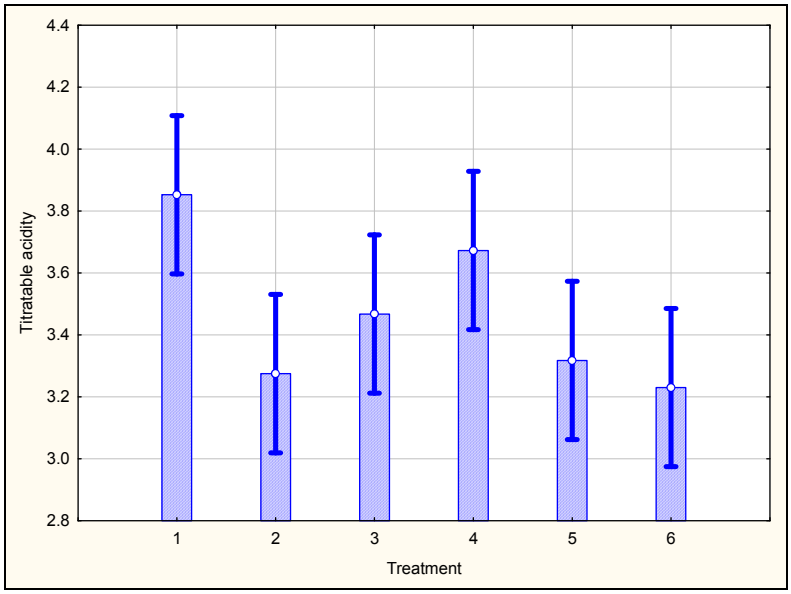

Figure 3.29 ANOVA computed for berry juice titratable acidity for the different irrigation treatments (P < 0.1) (vertical bars denote 0.95 confidence intervals).

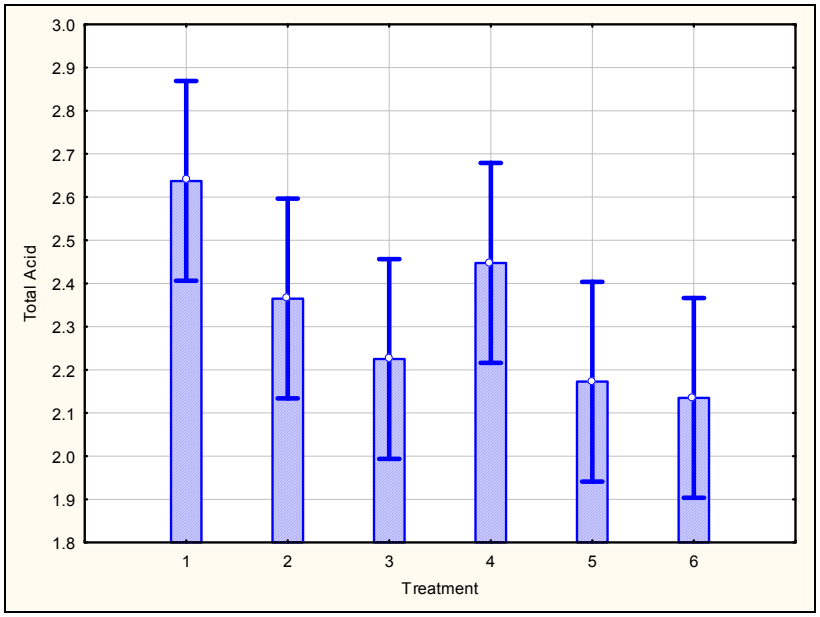

Figure 3.30 ANOVA computed for berry juice total acid concentration for the different irrigation treatments (P < 0.1) (vertical bars denote 0.95 confidence intervals).

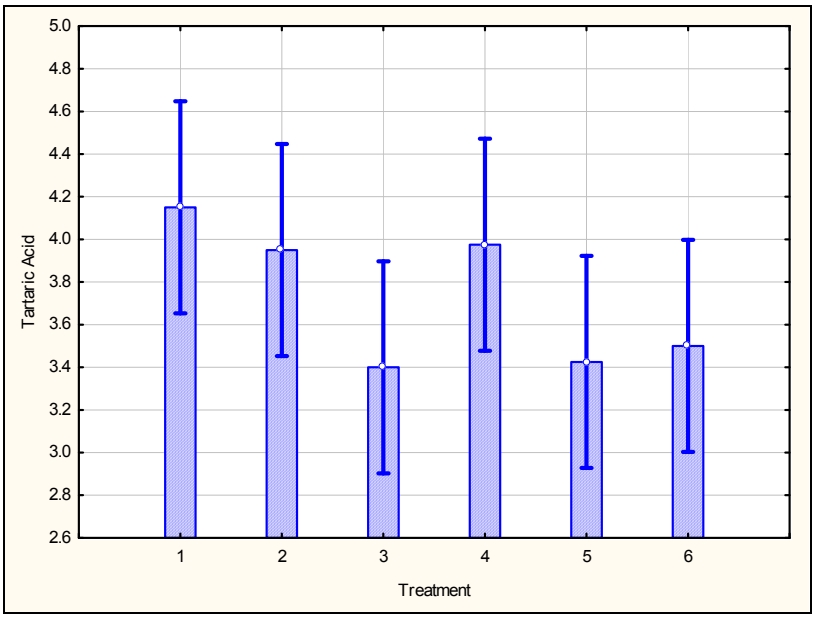

Figure 3.11 ANOVA computed for berry juice tartaric acid concentration for the different irrigation treatments ($P < 0.1$) (vertical bars denote 0.95 confidence intervals).

Vines from treatment 1 tended to have the highest titratable acid (figure 3.29), total acid (figure 3.30) and tartaric acid (figure 3.31) (not significant) concentrations, and this might be due to delayed ripening for these stressed vines, causing delays in the reduction of acid synthesis and therefore more acid synthesis than the other treatments. Similar results were found for the T4 vines, although not as much as for T1, as the T4 vines were a little less stressed. These acid concentrations were not highly affected by irrigation regime, as there were no significant differences between the treatments when the influence of berry mass was not included in the results. The larger berries of the T3, T5 and T6 vines might also have led to less concentrated acids in the juice and therefore a trend towards lower total and tartaric acids for those treatments.

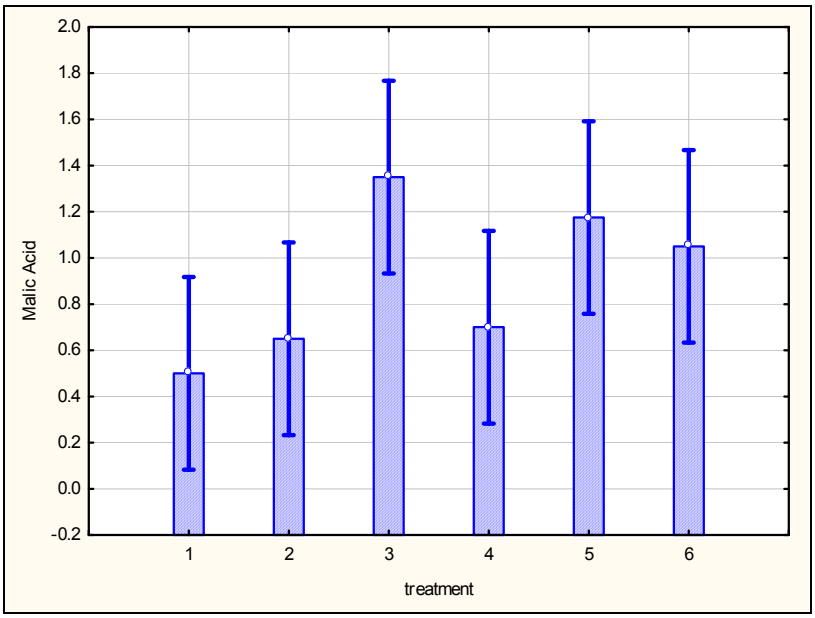

Figure 3.32 ANOVA computed for berry juice malic acid concentration through the measurement season for the different irrigation treatments ($P < 0.05$) (vertical bars denote 0.95 confidence intervals).

The effect of irrigation on malic acid concentration (figure 3.32) was significant between T1 and T3 and showed trends of differences between all the treatments ($P < 0.05$). This supports the information in the literature, which states that irrigation regime influences malic acid concentration throughout the season due to microclimate, vegetative growth and yield differences between the treatments (Esteban *et al*., 1999). The wetter treatments had higher malic acid concentrations and this should follow, as malic acid degradation slows down under wetter conditions. This might be caused by delayed ripening because of higher yields for wetter vines, and/or because of microclimate influences due to higher vigour. The lowest concentration was found for dryland vines, where malic acid concentration did not slow down as rapidly.

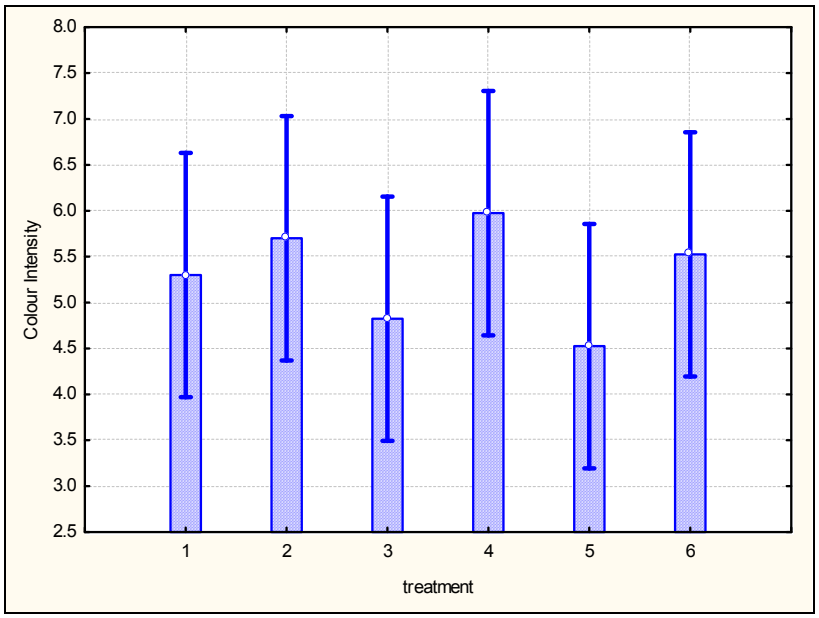

Figure 3.33 ANOVA computed for berry juice colour intensity through the measurement season for the different irrigation treatments ($P < 0.05$) (vertical bars denote 0.95 confidence intervals).

Treatment 4 had tended to have the highest colour intensity, followed by treatment 2, although this trend was not significant (figure 3.33). It does indicate that these mildly stressed vines had a trend to have better colour development than the more frequently irrigated vines. Treatment 6 also had higher colour intensity than treatments 4 and 5, and a little more than treatment 1. The PRD effect might thus have had a positive effect on colour intensity. Treatments 3 and 5 have lower colour intensities, probably due to a dilution effect because of their bigger berries.

3.4 WELLINGTON TRIAL CONCLUSION

The different irrigation regimes in this trial influenced soil water availability, which affects root water uptake and subsequently plant water status. Root development therefore could have differed between the treatments and this would have affected grapevine vegetative growth due to the link between root and shoot growth.

The differences in plant water status due to the differences in irrigation regime could be quantified through plant water status measurements of SWP and LWP. SWP measurements indicated the nature of plant water status better than LWP, as there was better differentiation between the treatments throughout the season. This corresponds with the information in the literature, which states that SWP is more differentiating and could be a better indicator of grapevine water transport than LWP. The different grapevine water potentials could have led to differences in chemical signalling within the grapevine, and there was thus a direct effect of water status on grapevine physiology.

ABA synthesis and transport could have been higher for the dryer treatments (T1, T2 and T4), while cytokinin synthesis and transport could have been higher for the wetter treatments (T3, T5 and T6). This then influenced stomatal conductance, with increased gs for the wetter treatments and lower gs for the dryer treatments due to the influence of these chemical signals on guard cell turgidity. Apart from this effect of water status on gs, there could have been effects due to the vigour differences implemented by these water status differences on gs, because of microclimate and sunlight interception differences between the different vigour levels.

These variations in gs between the treatments could have led to differences in leaf gas exchange, photosynthesis and transpiration. The dryer treatments had reduced transpiration and therefore increased water-use efficiency, while the wetter treatments had increased transpiration and therefore reduced water-use efficiency.

Carbon discrimination, a measurement that can indicate water-use efficiency, was influenced by these changes in gs because of the variation in CO_2 gas exchange caused by it. The wetter treatments with higher gs had more leaf internal CO_2 because of increased gas exchange, which promotes discrimination against ^{13}C and leads to a more negative $\delta^{13}C$ than found in the dryer treatments. It must borne in mind that the measurements of CID in this study reflected environmental conditions that influenced the ratio of CO_2 over the stomata (which is affected mostly by water status), but it also reflects the seasonal development of the berries, as its composition changes throughout the season. The organic compounds in berry juice differ in different periods of the season, consisting mostly of organic acids before véraison and mostly of sugars after véraison. These molecules have different CID values because of differences in their biosynthesis, which influences discrimination. The CID measurements showed very good

discrimination between the treatments and therefore showed how sensitive this measurement can be to illustrate integrative long-term effects of differing irrigation treatments on grapevine physiology.

The differences in physiology induced by the different irrigation regimes led to some differences in fruit composition. Vines from the wetter treatments might have had increased competition for nutrients between vegetative growth and berry development when compared to the dryer treatments. Berry size differed between the treatments, as the treatments with more water availability had larger berries as more water was available for cell expansion. This caused the dilution of some berry compounds like total soluble solids, while it also caused berry acids to be less concentrated (total acids). Irrigation regimes in this trial therefore led to modifications in aspects of grapevine performance with effects on vegetative development, plant physiology and reproductive development.

Destructive measurements of leaf area and shoot length did not show significant differences between the treatments and the reason might well have been the destructiveness of the measurement and because of topping that was implemented. Such measurements are therefore difficult to be used accurately in irrigation trials in order to evaluate vigour and would be more accurate in vineyards where topping is not implemented. Measurements of shoot growth rate could also be a good indicator of the effect of different irrigation regimes on vigour and could have contributed to this study.

To exclude the effect of seasonal berry development on CID measurements and therefore to have CID measurements that largely indicate the effect of water deficits and other aspects affecting CO_2, one should do measurements only on berry sugars throughout the season. Organic acids should therefore be removed from the juice samples, leading to sugars being the main organic compounds present in the juice during the season. The CID in the leaves should then be reflected, as sucrose production during photosynthesis is then reflected from CID measurements in berry sugars. Sucrose transportation to the berries and the conversion to glucose and fructose will also contain the fixed carbon isotopes. This can eliminate the effect of the biosynthetic pathways of different molecules on CID measurements.

This study can contribute to the determination of quality expressed through irrigation by its effects on grapevine physiology, which will determine possible wine quality.

Future studies such as this should therefore include CID measurements on berry sugars after the removal of acids. Wine parameters could also be studied to evaluate the effect of different CID measurements on wine quality as a result of different irrigation regimes (it is interesting to note that, during the Nietvoorbij trial, tasting panels consistently preferred the wine of T1). Through CID measurements, irrigation can be controlled by grape buyers without having to do labour-intensive measurements in the vineyard, while they may also be useful as a determinant of wine quality.

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Chapter 4

RESEARCH RESULTS

Grapevine water status variability and vigour interactions within a *Vitis vinifera* L. Cv Merlot vineyard and carbon discrimination (Stellenbosch region)

Research Results

4.1 INTRODUCTION

Studies of the influence of grapevine water status on carbon isotope discrimination (Chapter 3) have been done by various authors (De Souza *et al*., 2005; Gaudillere *et al*., 2002). However, not many studies have looked at the effect of vigour on carbon discrimination and this is why the focus in this study was on the effect of water status and vigour variability on carbon discrimination.

Variability of vigour within a vineyard can be caused by various parameters, including soil water availability, soil type variation, diseases, pruning and plant material variation (Long, 1997).

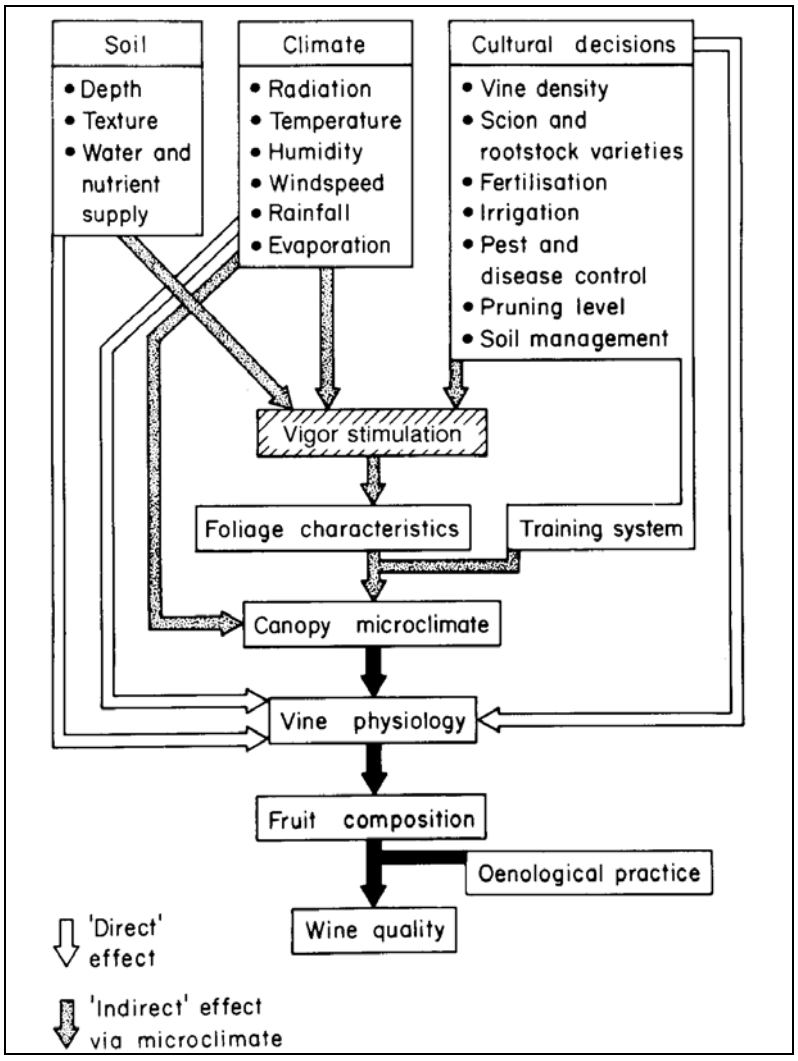

Figure 4.1 Conceptual model to show how soil, climate and cultural practices can affect wine quality via effects on canopy microclimate (from Smart *et al.,* 1985).

From Figure 4.1 it can be seen that various parameters influence vigour stimulation, ranging from soil conditions and climate to management.

In South Africa, soil type variability is probably the biggest cause of vigour variability in vineyards, as it influences root development, which will affect above-ground grapevine growth. The availability of soil water also influences root growth and subsequent above-ground growth.

Grapevine vigour is a determinant of canopy microclimate; this is due to canopy shading, as vigour determines foliage and its arrangement in space (Smart *et al*., 1985). Variability in the canopy microclimate will have an indirect effect on grapevine physiology, and will then also affect fruit composition. Vigour variability will therefore have an important effect on physiology and fruit quality.

Stomatal conductance (gs) can be influenced by vigour, as sunlight interception is one of the parameters affecting gs (Farquhar *et al*., 1982). Higher-vigour vines with more shoots and leaf layers increase canopy shading, which reduces sunlight interception and therefore influences stomatal control and photosynthesis. This will affect grapevine physiology and then fruit composition. Vines with a vigour that is too low can lead to high leaf temperatures and closure of the stomata, which will also negatively affect physiology and fruit composition. Variability in grapevine vigour can therefore influence carbon isotope discrimination (CID), as it influences CO_2 gradients across the leaf stomata, which influence the discrimination against ^{13}C and therefore the ratio of $^{12}C/^{13}C$.

Vines with too high vigour (shaded canopies) can result in increased pH, malic acid, colour intensity and anthocyanin, and in reduced tartaric acid and sugar in the berries. Vines with too low vigour can have high leaf temperatures and experience closure of the stomata, influencing physiology and berry composition.

The effect of available water on CID has been well described (Chapter 3), as the soil water content affects plant water status and stomatal conductance, and thus also CID. Water status (more directly, through hormonal control) and vigour (more indirectly) will therefore influence stomatal conductance and this will affect photosynthesis. Fruit composition can be influenced by both water status and vigour, as it is influenced by grapevine physiology (Smart *et al*., 1985).

Variability in vigour and water status within a vineyard would therefore have an influence on CID. Vigour can be negatively correlated with CID (Stamatiadis *et al*., 2007), while increased water deficits lead to less negative CID values. This study can hopefully help to understand how these parameters influence grapevine physiology and especially CID in order to try to use CID as a management tool for the control of vigour and water status in the vineyard.

4.2 MATERIALS AND METHODS

4.2.1. CLIMATE

Data for temperature and relative humidity was used throughout both seasons for the analyses, along with seasonal berry ripening data in order to explain some developmental changes that occurred.

4.2.2 VINEYARD CHARACTERISTICS

The study was conducted in the 2006-2007 and 2007-2008 growing seasons in the Stellenbosch region, Western Cape, South Africa. The vineyard was a Merlot (*Vitis vinifera* L. cv Merlot) clone MO 9 vineyard, grafted on R110 (*Vitis berlandieri x Vitis rupestris*) rootstock on an Oakleaf soil form from the 2110 soil family (MacVicar *et al*., 1977). The area has a Mediterranean climate with hot, dry summers and cold, rainy winters. The vines were planted 2.7 x 1.5 m in an ENE-WSW direction on a seven-wire movable hedge trellis system. Canopy management included shoot positioning and mechanical shoot topping.

4.2.3 EXPERIMENTAL LAYOUT

The vineyard was divided into three vigour classes, namely low, medium and high. Irrigation treatments were implemented in the vineyard, so that treatments of dryland, partially irrigated and irrigated vines were imported into each of the vigour classes. Each plot consisted of four rows with 12 vines each. The two side rows were used as buffer rows, while the two middle rows were used as measurement rows. Two representative vines from each plot were chosen to use for grapevine-level measurements. In 2006-2007, 16 plots were used, while 18 plots were used in the 2007-2008 growing season. Irrigation was applied through drippers (2.6 l/h), at a spacing of 75 cm per dripper. Irrigation scheduling was done according to pre-dawn (PDWP) and stem water potential (SWP) measurements, where wet treatment vines were irrigated when SWP and PDWP measurement were between -1000 and -1200 and -200 and -300 kPa respectively. Irrigation for the dry treatment vines were conducted when SWP and PDWP measurements were between -1200 and -1400 and – 300 and -400 respectively.

To study the effect of grapevine water status, the effect of vigour was separated from the data by using it as a covariate when statistical analyses were done to assess the effect of grapevine water status. The plots were also categorised into wet and dry treatments in order to see the effect of the variability in grapevine water status. The plots were originally categorised into irrigation treatments throughout the vineyard, but lateral water movement due to slopes and the effect of rainfall resulted in plots not reacting to the treatments as expected.

The measurements at each plot for plant- or soil-based water status were used to classify plots into “new” treatments (relative “wet” and “dry”) based on the predominant global reaction of certain parameters for each season. Plots were therefore grouped accordingly for statistical analyses (table 4.1).

Table 4.1 Indication of plot classification of water status.

Season	Plots	Soil water content (count ratio)	Carbon discrimination -1(C12/C13)	Stem water potential (-kPa)	Predawn leaf water potential (-kPa)	Stomatal conductance (mmol/m^2/s)	Leaf water potential (-kPa)	Berry size (gram/berry)
2007	1, 4, 5, 8, A2, A10, B5	Wet (high values)	Wet (more negative values)	Wet (less negative values)	Wet (less negative values)	Wet (high values)	Wet (less negative values)	Wet (higher berry mass)
2007	2, 3, 6, 7, A3, A6, A9, A12	Dry (low values)	Dry (less negative values)	Dry (more negative values)	Dry (more negative values)	Dry (low values)	Dry (more negative values)	Dry (lower berry mass)
2008	1, 4, 5, 6, 8, A2, A3, A6, A10, A12	Wet (high values)	Wet (more negative values)	Wet (less negative values)	Wet (less negative values)	Wet (high values)	Wet (less negative values)	Wet (higher berry mass)
2008	2, 3, 7, B5, B8, B10, B12	Dry (low values)	Dry (less negative values)	Dry (more negative values)	Dry (more negative values)	Dry (low values)	Dry (more negative values)	Dry (lower berry mass)

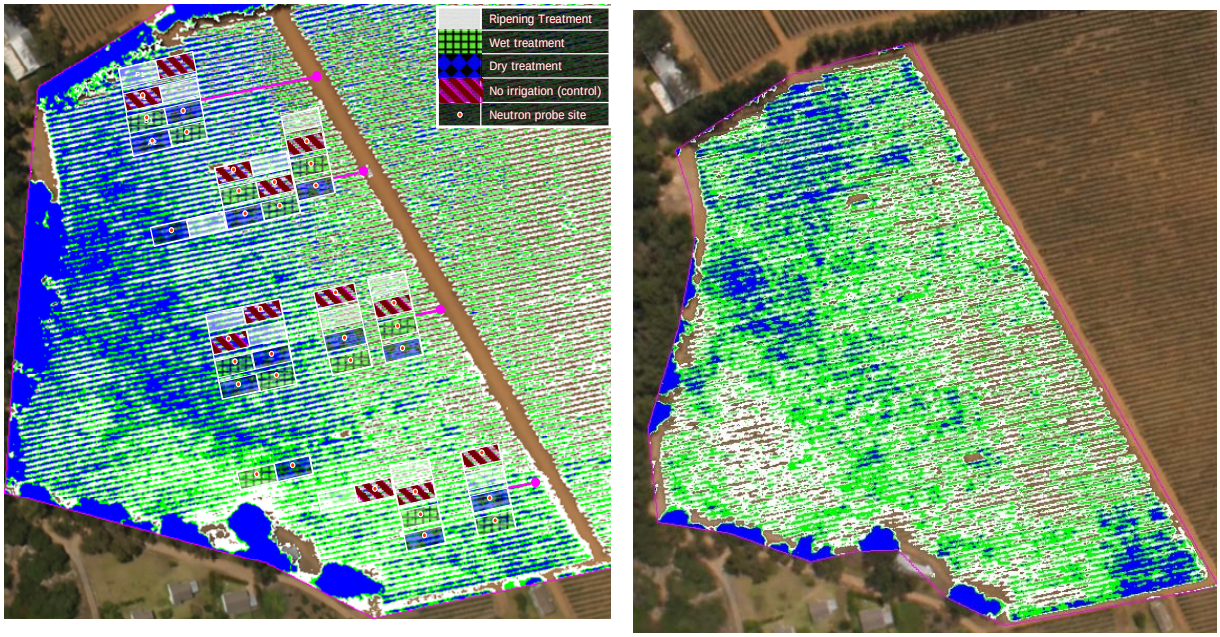

Figure 4.2 Multispectral images of the vineyard (left 2007; right 2008). The layout of the treatments is indicated on the 2007 image. Blue areas indicate high vigour, while green areas indicate medium vigour and white areas indicate low vigour.

4.2.4 VEGETATIVE MEASUREMENTS

4.2.4.1 WINTER PRUNING MASS

Winter pruning was conducted in the winter of both seasons – in July 2007 and July 2008. During pruning, the vines were pruned to two-node spurs. Number of canes per grapevine and pruning mass were recorded for every individual grapevine, and cane mass were calculated.

4.2.4.2 SHOOT GROWTH RATE

Two representative shoots from each of the chosen vines in every plot were marked in both the 2007 and 2008 seasons. Shoot lengths were measured weekly from two weeks before flowering, and ceased when topping was carried out by the producer.

4.2.4.3 SHOOT AND LEAF MEASUREMENTS

Destructive measurements of leaf area and shoot length were conducted shortly after harvest in both seasons on the chosen vines in all the plots. One representative shoot from each cordon of the vines was harvested and immediately stored at 4ºC until measurements were done. Main and lateral shoot lengths were tape-measured and the number of lateral shoots was counted. Main and lateral leaf numbers were counted after the leaves were removed from the shoots, and the main and lateral leaf areas of each grapevine were determined separately using a planemometer (Delta-T Devices, Cambridge, UK).

4.2.5 SOIL WATER CONTENT

The measurement of soil water content was conducted by means of the neutron scattering technique. A neutron probe (503DR Hydroprobe Neutron depth moisture gauge, Campbell Pacific Nuclear International Inc., USA) was used, along with polyvinyl chloride (PVC) tubes, which were installed in the grapevine rows in a central position in the plot, 50 cm from a representative grapevine. Neutron count values were measured at 30, 60 and 90 cm soil depths by collecting a 30 second reading at each depth. Count ratios were determined as follows: Cr = Neutron count/water drum standard. The water drum standard was a 200 litre water drum containing a PVC pipe to obtain a reference standard in the form of a water-saturated environment.

4.2.6 PLANT WATER STATUS

4.2.6.1 PRE-DAWN LEAF WATER POTENTIAL (PDWP)

Pre-dawn leaf water potentials were measured weekly during both seasons in selected plots according to a method adapted from Choné *et al*., (2001). Five healthy, fully-expanded leaves on main shoots were removed by a single cut with a sharp blade. After a leaf had been removed, it was immediately placed in a leaf pressure chamber (ARIMAD-3000) and nitrogen gas was allowed to enter the chamber. The pressure measurement was taken when the first sign of moisture was visible from the petiole (Scholander *et al*., 1965).

4.2.6.2 STEM WATER POTENTIAL (SWP)

SWP was determined in both seasons for two leaves from all the two chosen vines in every plot: Two healthy, fully-expanded leaves from each grapevine were chosen and the measurement of SWP was conducted between 11:00 and 14:00 on clear days. The method of measurement was adapted from Choné *et al*. (2001). Leaves were chosen on main shoots between the 8^{th} and 10^{th} node. While still attached to the plant, the leaves were enclosed with a combination zip-lock plastic and aluminium foil bag. The leaves were left covered for at least 30 minutes to allow

stomatal closure as a result of equilibration with the atmosphere inside the bags. The leaves were then removed by a single cut with a sharp blade after the bags had been removed. They were immediately placed in the pressure chamber (ARIMAD-3000, Israel). Nitrogen gas was allowed to move into the chamber to build up pressure until the first sight of moisture coming out of the petiole was visible, at which time the pressure reading in the chamber was taken from the digital screen on the pressure bomb. This reading (-kPa) was then noted as the SWP.

4.2.6.3 LEAF WATER POTENTIAL (LWP)

LWP was determined for leaves on the selected vines in both seasons. The leaves that were used to measure gs (Section 4.2.7.2) were also used for midday LWP measurement, just after the gs measurement was conducted. Methods used by Choné *et al*. (2001) were adapted and the leaves were removed by a single cut with a sharp blade and immediately placed in a pressure chamber (Section 4.2.7.3). This reading (-kPa) was then noted as the LWP (Scholander *et al*., 1965).

4.2.7 PHYSIOLOGICAL MEASUREMENTS

4.2.7.1 CARBON ISOTOPE DISCRIMINATION

Twenty μl of fresh juice was extracted from the crushed berries (Section 4.2.8.1) with a 3 ml plastic pipette from all the collected samples throughout both seasons up to harvest, and was placed in Eppindorf tubes and frozen in a -40°C freezer. The samples were sent to a stable light isotope laboratory (UCT, Cape Town), where the juice was pipetted into a cuvette. The juice was analysed by combustion in a Thermo Finnigan Delta Plus XP stable light isotope mass spectrometer, coupled via a Conflo III device to a Thermo 1112 Flash elemental analyser to determine ^{12}C and ^{13}C ratios. The samples were run against in-house reference materials that had been calibrated according to international standards (VPDB for carbon and air for nitrogen). The results are expressed relative to those standards. The conventional expression was used with reference to the Pee Dee Belemnite standard. Carbon isotope composition is expressed as:

$$\delta^{13}C\,‰ = \left(\frac{R_{sample}}{R_{standard}} - 1 \right) \times 1000$$

where R_{sample} is the $^{12}C/^{13}C$ for the sample and $R_{srandard}$ is that of the reference material (Farquhar *et* al.,1989)

4.2.7.2 STOMATAL CONDUCTANCE (gs)

Stomatal conductance (mmol/m^2/s) was measured during the 2006-2007 growing season using a porometer, model SC-1 (Decagon Devices, Inc., Washington, USA), for the selected vines in all the plots.

During the 2007-2008 growing season, gs was measured using a CIRAS-1 infra red gas analyser (PP Systems, North America). The instrument was set up to measure gs and the CO_2

assimilation rate under nearly ambient conditions of light, temperature and humidity (Bunce, 1998). The measurements were conducted close to solar noon on two representative, fully-expanded, healthy leaves on the 8th to 10th node of main shoots while the leaves were still intact.

4.2.7.3 LEAF PHOTOSYNTHETIC RATE AND LEAF GAS EXCHANGE

A CIRAS-1® photosynthesis instrument (PP Systems, North America) with an infrared gas analysis instrument (IRGA) was used during the 2007-2008 season.

Photosynthetic rate (Pn) in mmol/m^2/s, intercellular CO_2 (*C*i), leaf temperature, evapotranspiration and gs were measured for the leaves described in Section 4.2.7.2. The instrument makes measurements possible through differential or absolute changes caused by leaf gas exchange, and calculates photosynthesis from the gain or loss in CO_2. The instrument has an open system, allowing constant air flow though the measuring chamber, which minimises its effect on leaf gas exchange.

During the measurements, the photosynthetically active radiation (PAR), CO_2 concentration and relative humidity (RH) of the ambient air were maintained at similar levels in order to minimise the effect of the measuring chamber on leaf photosynthesis. A ceptometer (AccuPAR LP-80 PAR/LAI ceptometer, Decagon Devices, Inc., Washington, USA) was used to determine ambient light intensity and, after the average of the ceptometer readings was calculated, the internal light source in the CIRAS® was set to provide the light intensity. The chamber CO_2 was controlled by the CIRAS® instrument and kept equivalent to atmospheric CO_2 concentrations. Atmospheric RH was measured manually with a humidity sensor at atmospheric levels, and the chamber RH was then controlled by CIRAS® to be equivalent to the measured RH.

After the chosen leaf was clamped in the leaf chamber, the instrument was allowed to stabilise, as determined by real-time monitoring of the Pn levels within the system.

4.2.8 REPRODUCTIVE MEASUREMENTS

4.2.8.1 WEEKLY BERRY SAMPLING

Berry sampling was performed weekly during both seasons. Thirty berries were randomly sampled each time and were picked on both sides of the canopies (midday sun and shade-exposed sides) of every plot. Sampling was done from the inside and outside of the canopy and from the top, middle and bottom of bunches in order to get a representative sample of each treatment. The berries were immediately placed in a cooler at about 4°C after being removed from the vines.

The mass of the berries was measured using a laboratory scale (JW-1000 counting scale, UWE electronic scales, Taiwan) and the average mass of a berry was determined. The sampled berries were crushed in a plastic bag by hand to extract enough juice to measure the pH for all sets of samples with a laboratory pH meter (Crison Basic 20, Crison Instruments, Spain). Sufficient amounts of fresh juice were extracted from the crushed berries to measure the °Brix (total soluble solids) using a pocket refractometer (ATAGO Pal-1 refractometer, ATAGO Co LTD., Tokyo), zeroed with distilled water. Total soluble solid content was also determined by taking berry size out of the equation (berry mass×°Brix/100).

4.2.8.2 YIELD AND BERRY MEASUREMENTS AT HARVEST

An average of 200 berries was sampled at harvest time for both seasons from the chosen vines of both rows of every plot. The berries were sampled from the inside and outside of the canopy and from the top, middle and bottom of the bunches. They were placed in the 4°C cooler immediately after removal from the grapevine. The number of bunches per grapevine were counted and weighed using a digital scale to determine the yield per grapevine.

Berry mass was determined by counting out 200 berries per sample and measuring them with a laboratory scale (JW-1000 counting scale, UWE electronic scales, Taiwan). Average berry mass could be determined using this procedure. The berries were then crushed by hand in a plastic bag to extract enough fresh juice. Some of the juice was used to determine pH and total soluble solids (see 4.2.8.1).

Titratable acidity (TA) was measured using 150 ml fresh juice that was analysed with a 785 DMP Metrohm Titrino automatic titration instrument. Fresh buffers of pH 4 and pH 7 were used for this determination.

Grape juice quality parameters were determined using Fourier transform infrared (FT-IR) spectrometry (WineScan®). The juice was first filtered with a filter (type 79500, FOSS Electric, Denmark) that uses filter paper graded at 20 to 25 µm. It was connected to a vacuum pump and then analysed with a multi-parameter analyser (WineScan® FT 120, FOSS, Denmark) with ready-made calibrations and GrapeScan® software. The WineScan® analyser employs a Michelson interferometer to generate the FTIR spectra. Instrument settings included cell path length of 37 µm, sample temperature set to 40°C, and sample volume of 7 to 8 ml. The samples were pumped through the heat exchanger and the CaF_2-lined cuvette and scanned from 926 to 5012 cm^{-1} at 4 cm^{-1} intervals. The determined parameters included pH, sugars, organic acids and colour. Cleaning was automatically programmed to occur every 5 min. The instrument was zeroed with the zeroing solution (S-6060, Foss Electric) before any set of analyses.

4.2.9 DIURNAL PHYSIOLOGICAL MEASUREMENTS

During the 2007/2008 season, a diurnal cycle was conducted separately over three days to include all measured plots. This was done over an average of two-hour time intervals from 6:00 until 17:00 in November and December 2007.

For carbon discrimination measurements during these cycles, petiole sap was extracted by placing leaves in the pressure chamber (Section 4.2.6). Nitrogen gas was then allowed to enter the chamber until enough petiole sap (20 µl) could be collected from one leaf by means of a pipette. The carbon isotope ratio was subsequently determined for the carbohydrates present in the petiole sap. It must be remembered however that xylem sap, coming from the roots contains few sucrose and can cause questionable results when used for measuring CID. This method was used however, as the berries was still too small at this stage of the season in order to get juice extracted for CID measurements while not using too much berries and because very few sugars are present in berry juice during this stage.

During the diurnal cycles, gs was also measured for all the plots for every one of the three days at the time intervals mentioned. This was done on both sides of the row.

LWP, SWP, photosynthetic rates, evapotranspiration and leaf temperatures were also measured during the diurnal cycles at all plots on both sides of the row for the time intervals mentioned.

4.2.10 EXTREME STRESS EXPERIMENT

In the 2008 season, it was decided to do measurements on an extra plot (plot Z), which was situated in a seemingly highly stressed part of the vineyard. The plot had very low vigour and showed signs of limiting conditions throughout its development. The reason for the high stress in this part of the vineyard might be due to its location right next to big trees, which could have acted as competition for nutrients and water. The soil in this part of the vineyard was also very sandy and rocky, with possible low water holding capacity, and not as suitable for grapevine growth as in other parts of the vineyard. Measurements were compared with those of another plot that was already moderately to severely stressed (plot B12). This was done to test the effect of severe stress on grapevine water status measurements, physiology and reproductive development. This was done for the parts of the season from when measurements for Plot Z were implemented from about a week after véraison.

4.2.11 STATISTICAL ANALYSES

The data were analysed using Statistica 8.0 (Statsoft, Inc., Tulsa, OK, USA) with the repeated measures ANOVA mixed model approach (McCulloch *et al*., 2008), with pruning mass included as the covariate.

4.3 RESULTS AND DISCUSSION

4.3.1 CLIMATE

Climatic data for both seasons (figures 4.3 to 4.8) show that there were periods of large fluctuations in temperature and/or relative humidity. During the 2008 season, average mean temperatures and rainfall were slightly higher than in 2007, while average relative humidity was slightly lower in 2008.

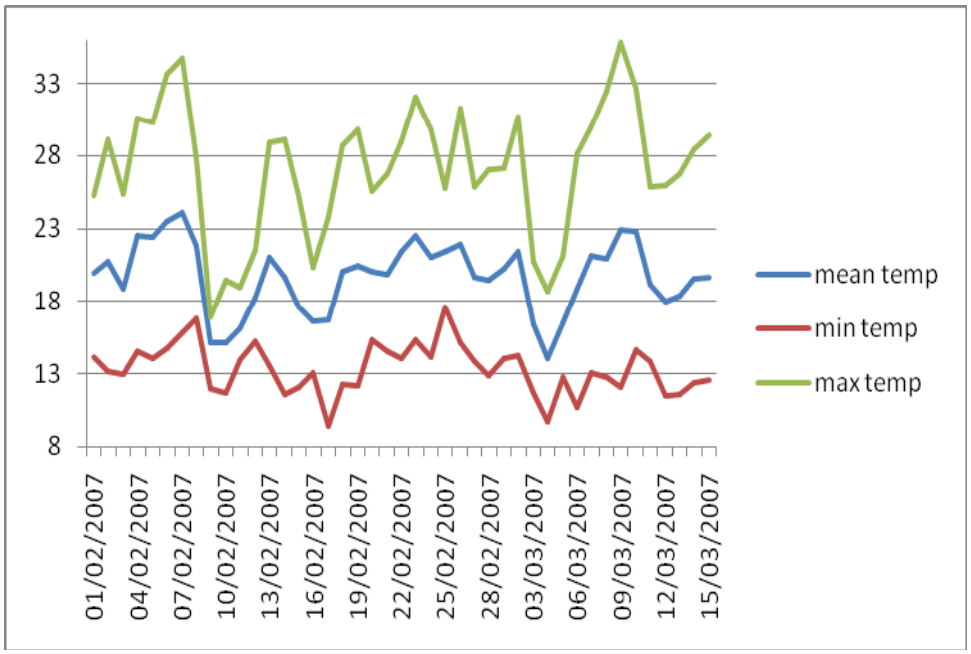

Figure 4.3 Mean, minimum and maximum daily temperatures (°C) through the measurement season (2007).

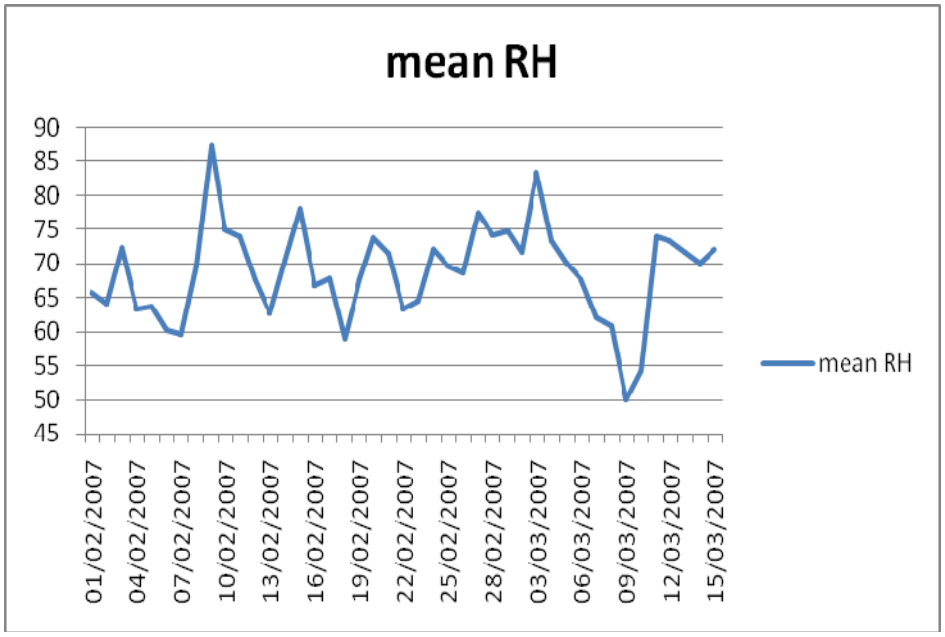

Figure 4.4 Mean daily relative humidity (%) measurements through the measurement season (2007).

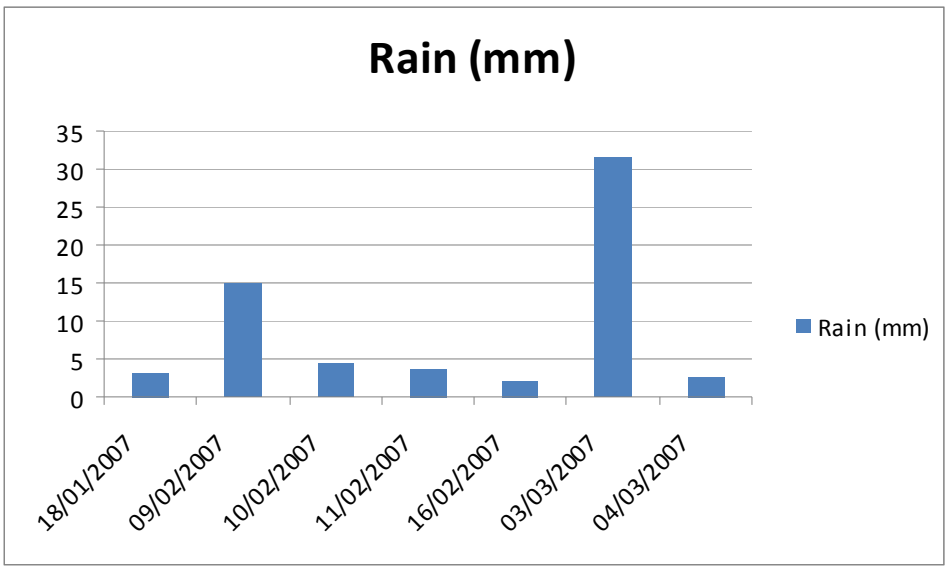

Figure 4.5 Rainfall (mm) during the 2007 season.

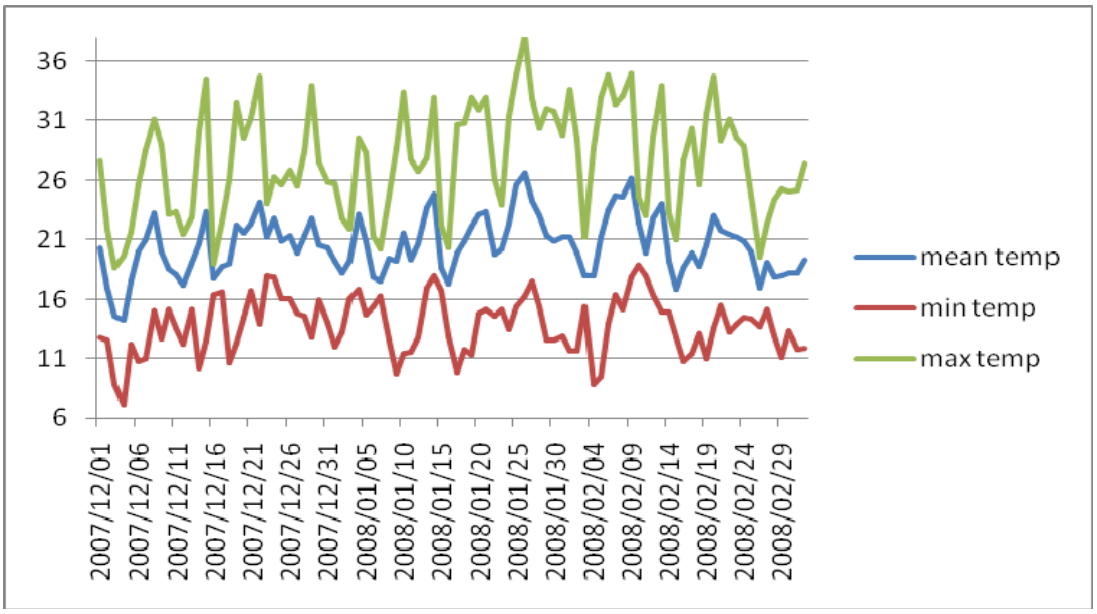

Figure 4.6 Mean, minimum and maximum daily temperatures (°C) through the measurement season (2008).

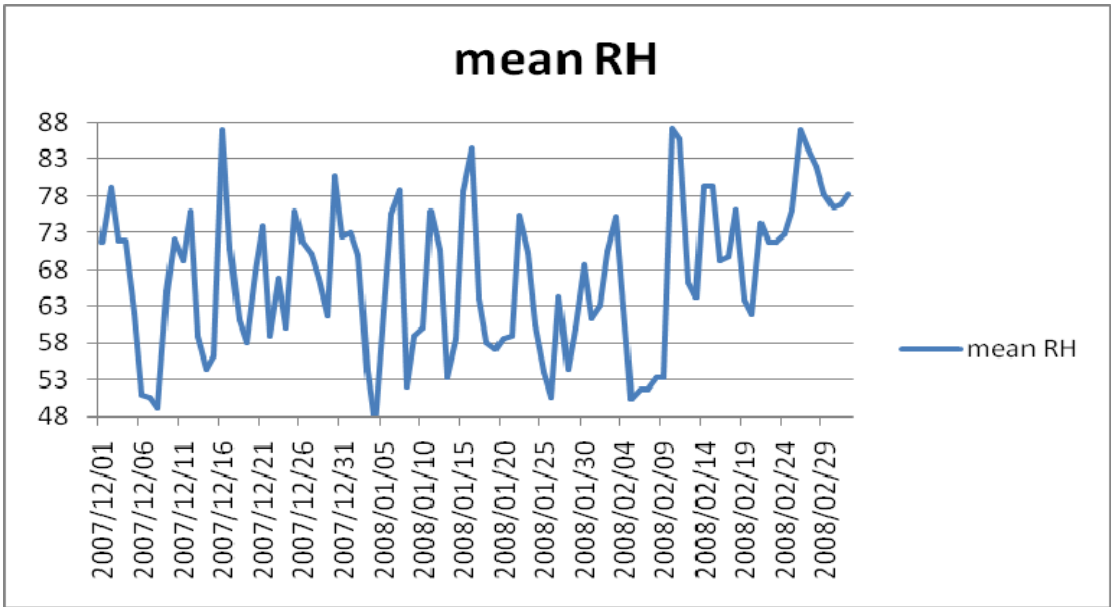

Figure 4.7 Mean daily relative humidity (%) measurements through the measurement season (2008).

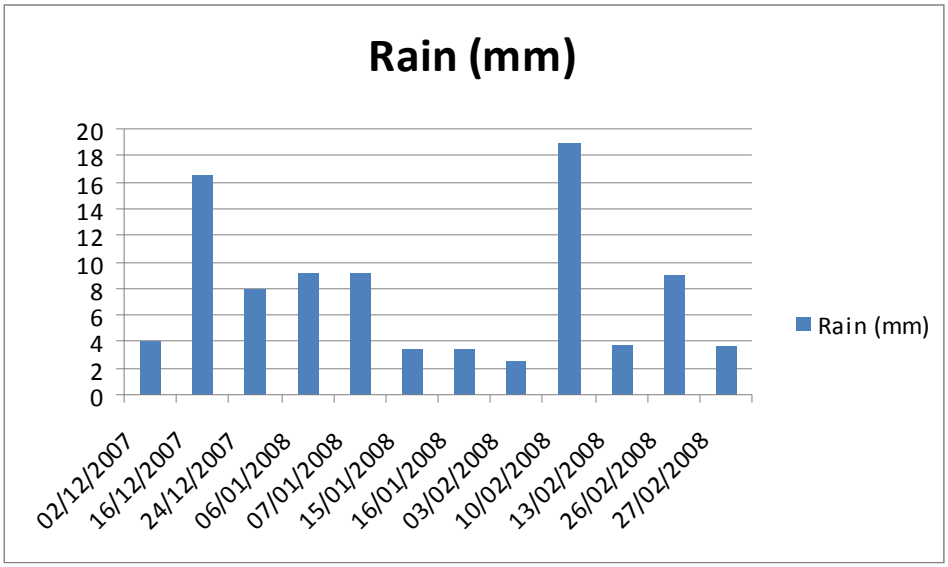

Figure 4.8 Rainfall (mm) during the 2008 season.

4.3.2 VEGETATIVE MEASUREMENTS

4.3.2.1 WINTER PRUNING MASS

Pruning mass (kg/vine) for the various plots in the vineyard are shown for both seasons (Figures 4.9 and 4.11). The pruning masses (kg/vine) for the two vines used in each plot for grapevine level measurements are also shown for both seasons (Figures 4.10 and 4.12). It is interesting to note that the mean pruning mass of all plots for the 2007 season was 0,88 kg per vine, while it was 1,08 kg per vine for the 2008 season. The higher rainfall during the 2008 season therefore contributed to higher growth vigour in the vineyard. It can further be observed that the variation in pruning mass between plots seems to have been bigger for the 2007 season than the 2008 season. The reason for this might be the higher general vigour, and therefore less vines with low vigour.

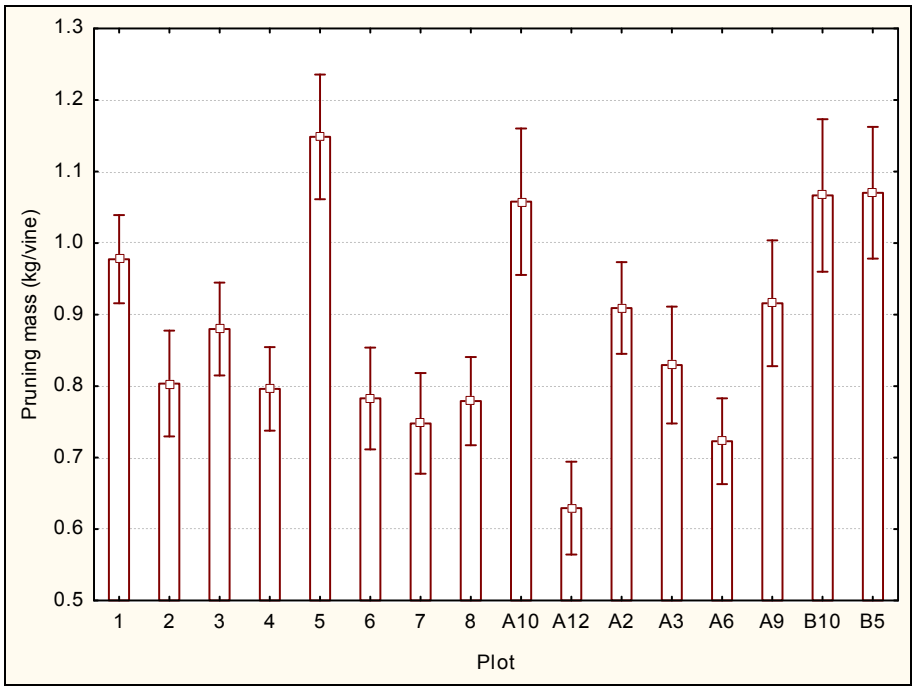

Figure 4.9 Means with error plot of the pruning mass **(kg/vine)** for the different plots (2007) (vertical bars denote 0.95 confidence intervals).

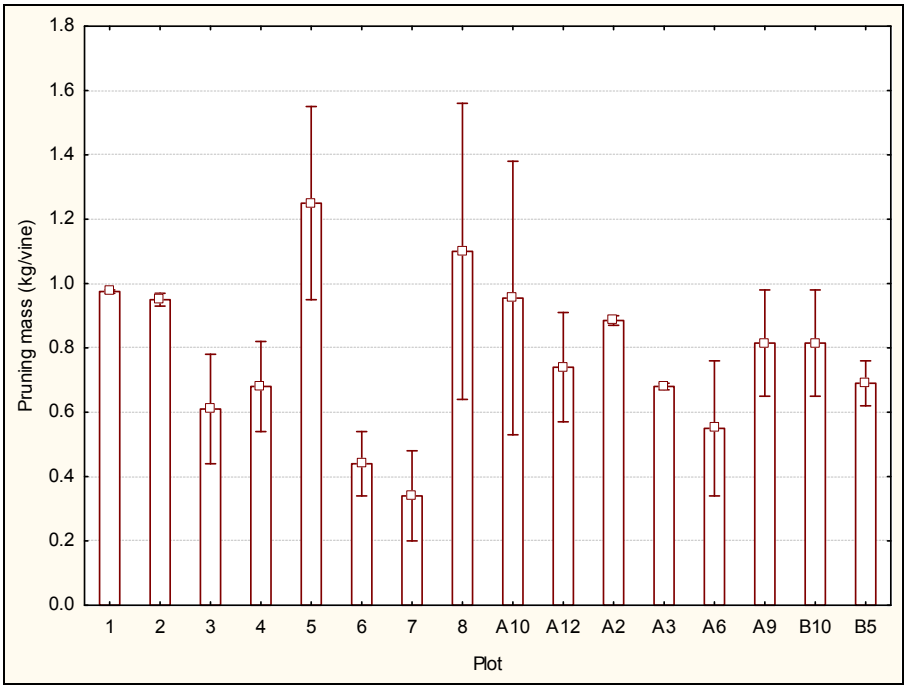

Figure 4.10 Means with error plot of the pruning mass **(kg/vine)** of the two measurement vines per plot (2007) (vertical bars denote means, minimum and maximum values).

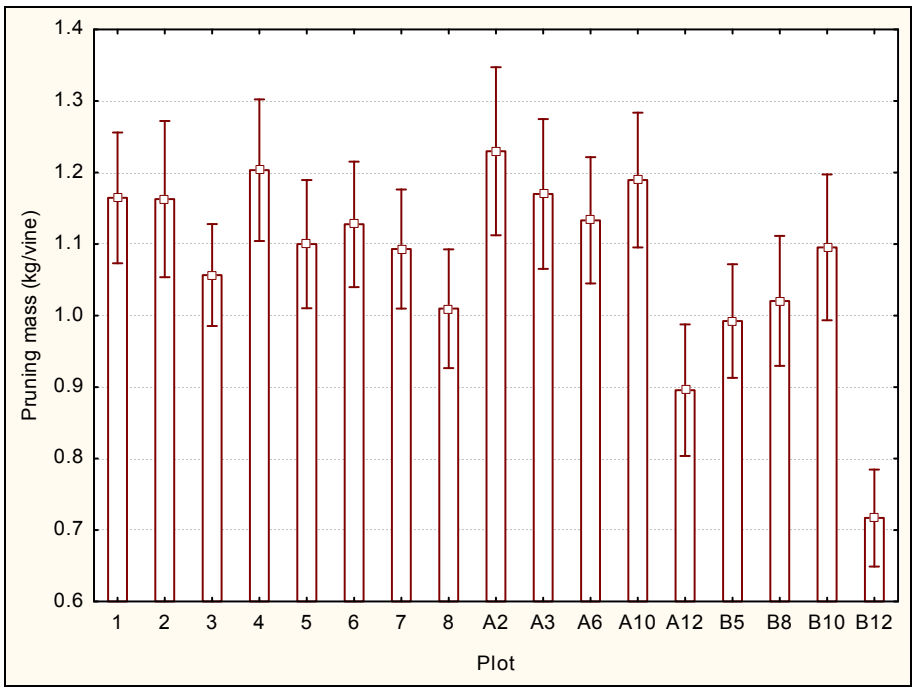

Figure 4.11 Means with error plot of the pruning mass **(kg/vine)** for the different plots (2008) (vertical bars denote 0.95 confidence intervals).

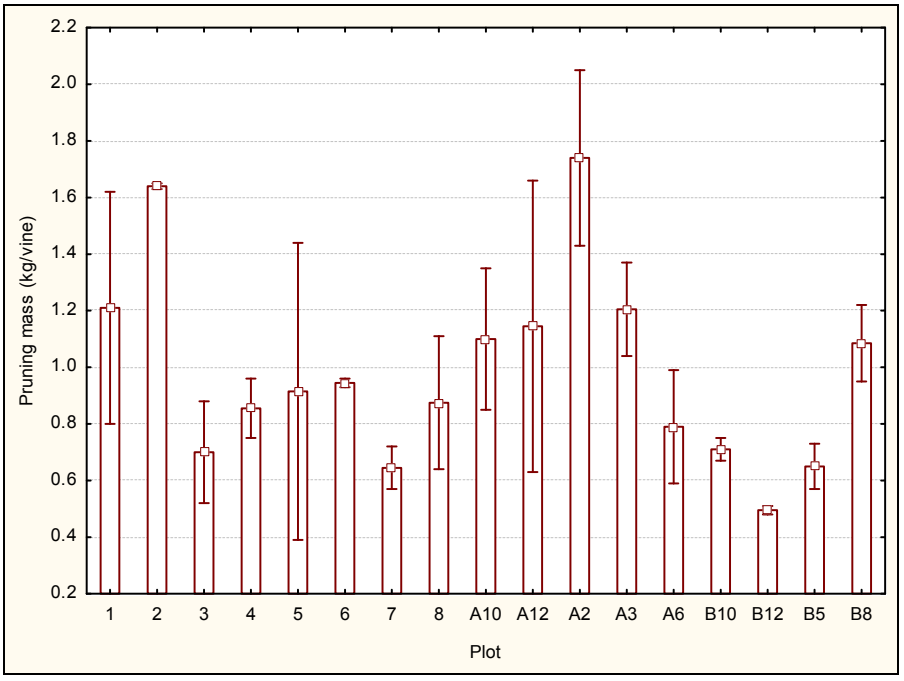

Figure 4.12 Means with error plot of the pruning mass **(kg/vine)** of the two measurement vines per plot (2008) (vertical bars denote means, minimum and maximum values).

4.3.2.2 SHOOT GROWTH RATE

There were no significant differences between the wet and dry treatments in shoot length development during the 2007/2008 season for the duration of these measurements until topping was implemented (after 6 December 2007)($P > 0.1$).

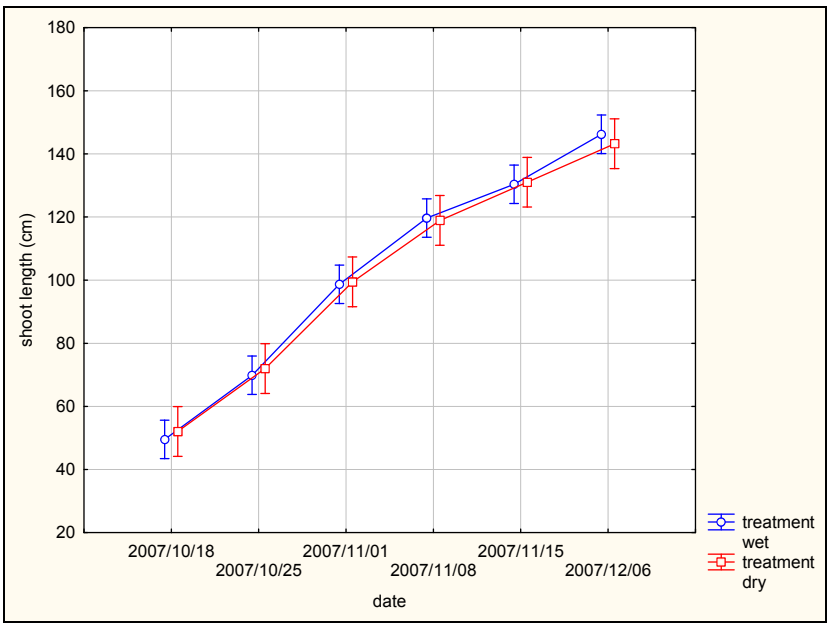

Figure 4.13 ANOVA computed for covariates at their means of the seasonal evolution of shoot length development for the wet and dry treatments (2007) (vertical bars denote 0.95 confidence intervals).

The seasonal growth pattern of the shoots (figure 4.13) indicates that the shoots became significantly longer as the season progressed, especially during the early parts of the season. There were no large differences between the shoot lengths of the wet and dry treatments through the season, as no significant differences were found at any stage of the measuring period. Water status therefore did not influence shoot length during this part of the season, although it might have influenced shoot growth later in the season as the effect of irrigation regimes became more significant.

The covariate (vigour) had a strong, significant effect on shoot length development ($P < 0.01$); higher-vigour vines had longer shoots, as was expected, and the growth of the shoots with higher vigour was faster than that with lower vigour (data not shown). A moderate strength correlation was found between pruning mass and mean shoot length development per plot, plots with higher pruning masses had stronger shoot development ($r^2=0.4789$; $P < 0.01$).

4.3.2.3 SHOOT AND LEAF MEASUREMENTS

The implementation of topping in the vineyard during both seasons made it difficult to find measurement differences between the treatments, and there therefore were no significant differences for leaf areas or shoot lengths between the treatments. The topping that was implemented resulted in shoots that did not significantly differ in length between the treatments, and therefore also led to no significant differences in leaf area. When topping is implemented, one would expect increased lateral shoot growth, but the topping action was implemented on

these shoots as well. It was done until late in the season, when shoot growth was no longer very active, resulting in no significant differences in lateral shoot lengths either.

No significant differences in shoot length and leaves per vine or leaf area were caused by treatment variation in both seasons (Table 4.2). There were only slight trends in 2008, showing longer shoots for wet treatment vines (probably due to increased cytokinin synthesis from the roots). Wet treatment vines therefore had slightly more leaves and a greater leaf area.

Table 4.2 Significance of differences in shoot length, leaves per vine and leaf area between the treatments in both seasons.

	2007			2008		
	Shoot length	Leaves per vine	Leaf area	Shoot length	Leaves per vine	Leaf area
Treatment	P > 0.1	P > 0.1	P > 0.1	P > 0.1	P > 0.1	P > 0.1

Vigour (the covariate) affected the leaf area of the main shoot significantly in 2008 (Table 4.3), with increased leaf area for vines with a higher vigour. In 2007 this was not significant, with trends of higher leaf area for higher-vigour vines only. This effect might have been stronger if topping did not have such a vast effect on shoot length and leaf area.

Areas per leaf for the main shoot leaves showed similar trends, with no significant differences caused by vigour in both seasons.

Vigour effects on lateral leaf area were weakly significant in 2007 and not significant in 2008, higher-vigour vines had slightly higher lateral leaf areas in 2007 and showed a similar trend in 2008. Area per lateral leaf was also significantly affected by vigour in 2007, while the effect was not significant in 2008. Lateral leaf development was therefore influenced by vigour to a greater extent in 2007 than in 2008, probably due to the higher variation in vigour in 2007.

Total leaf area was affected by vigour with weakly significant differences in 2007 and significant differences in 2008, higher-vigour vines had increased total leaf area in both seasons.

Vigour affected average main shoot length with weak significance in 2008 and with no significance in 2007. Vines with a higher vigour only had slightly longer main shoots than lower-vigour vines in 2008 and a similar trend was found in 2007. Main shoot length was influenced by topping.

The effect of vigour on total lateral shoot length was weakly significant in 2007 and not significant in 2008. Vines with a higher vigour had increased lateral shoot lengths in 2007 and a similar trend was found in 2008. The effect was bigger in 2007 than in 2008, probably due to the increased effect of topping on the lateral shoots in 2008 because of the higher vegetative growth in that season.

Vigour affected total shoot length in both seasons with weak significance, and the vines with higher vigour had longer shoots.

There were no significant effects of vigour on the amount of lateral shoots in both seasons, although there were trends in both seasons for the higher-vigour vines to have more lateral shoots.

Table 4.3 Significance in the effect of the covariate on vegetative measurements and correlations between pruning mass and the measurements.

Vegetative measurement	Covariate (P)	Correlation with pruning mass (r^2)*
2007		
Main shoot leaf area	> 0.1	0.2134
Area per leaf (main)	> 0.1	0.2214
Lateral leaf area	< 0.1	0.1814
Area per leaf (lateral)	< 0.05	0.3515
Total leaf area	< 0.1	0.3518
Main shoot length	> 0.1	0.3445
Lateral shoot length	< 0.1	0.2538
Total shoot length	< 0.1	0.4874
Lateral shoots	> 0.1	0.2948
2008		
Main shoot leaf area	< 0.05	0.2258
Area per leaf (main)	> 0.1	0.2112
Lateral leaf area	> 0.1	0.2061
Area per leaf (lateral)	> 0.1	0.0394
Total leaf area	< 0.05	0.3649
Main shoot length	< 0.1	0.1510
Lateral shoot length	> 0.1	0.1589
Total shoot length	< 0.1	0.3014
Lateral shoots	> 0.1	0.1461

*All P values of correlation analyses with pruning mass were significant.

The differences in vigour between 2007 and 2008 were that vigour was higher in 2008, with less variation in vigour between plots. This could have contributed to the larger effect of the covariate on main shoot length and therefore also leaf area in 2008. It seems that lateral shoot length and leaf area were affected more in 2007 than in 2008, and one reason might be a larger effect of topping on lateral shoots due to the higher vigour in 2008.

4.3.3 SOIL WATER CONTENT

Significant differences were found between the count ratios determined for wet and dry treatments in the 2007 season ($P < 0.01$) (figure 4.14). This was for the combined count ratios measured at a soil depth of 30, 60 and 90 cm. The soils of the wet treatments were therefore significantly wetter than those of the dry treatments throughout the soil profile in 2007.

In the 2008 season, the difference in count ratios between the wet and dry treatments was not significant (figure 4.15), but it showed a similar trend to 2007 ($P > 0.1$). The reason for this might be the higher rainfall during the 2008 growing season, causing less variation in soil water between the treatments.

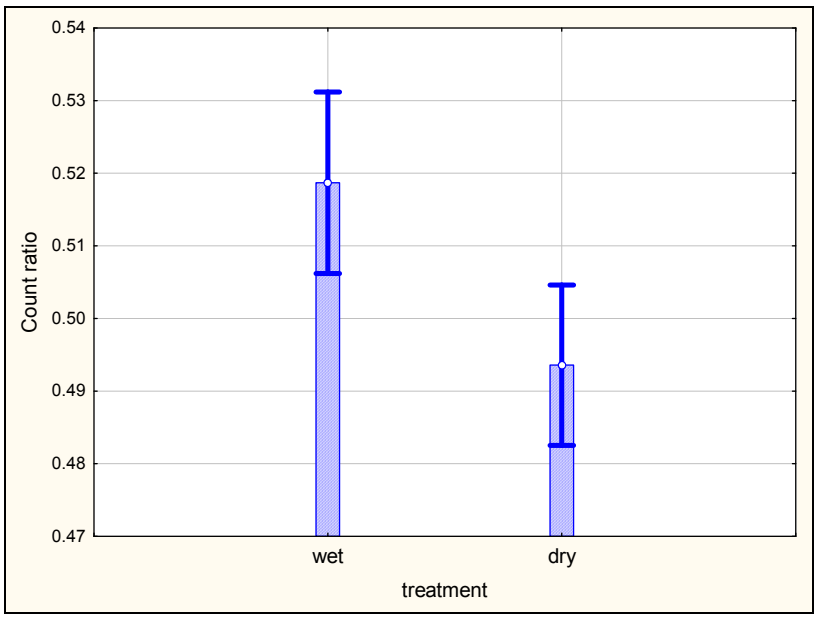

Figure 4.14 ANOVA computed for covariates at their means of the count ratios for the wet and dry treatments at soil depths of 30, 60 and 90 cm (2007) ($P < 0.01$) (vertical bars denote 0.95 confidence intervals).

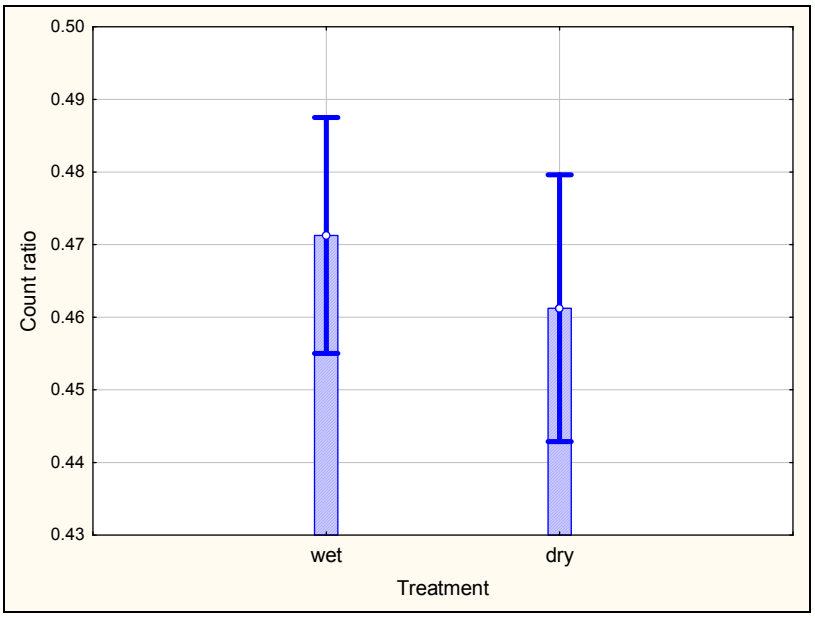

Figure 4.15 ANOVA computed for covariates at their means of the count ratios for the wet and dry treatments for at soil depths of 30, 60 and 90 cm (2008) ($P > 0.1$) (vertical bars denote 0.95 confidence intervals).

The seasonal pattern for soil water content also shows more differentiation between the treatments in 2007 throughout the season (figure 4.16), while this was not the case in 2008 (figure 4.17) because of the higher rainfall.

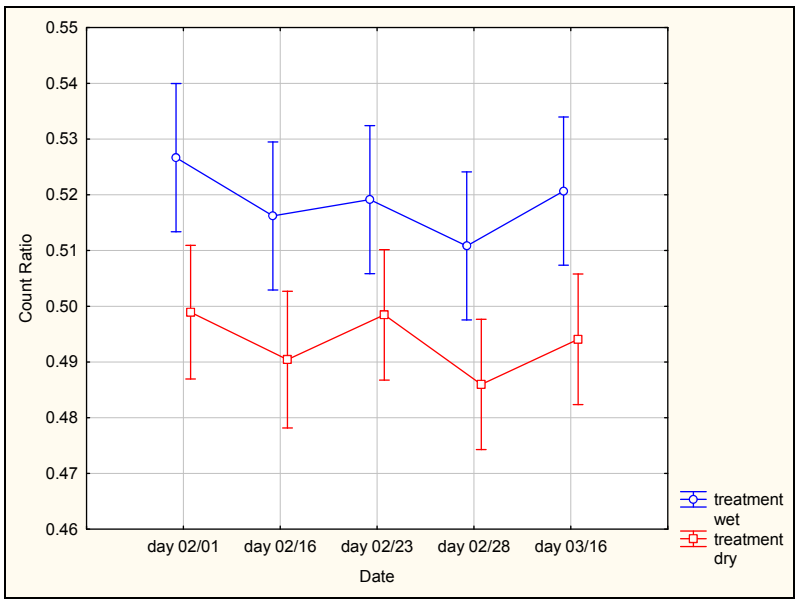

Figure 4.16 Repeated measures ANOVA computed for covariates at their means of the seasonal evolution of count ratios for the wet and dry treatments (2007) (vertical bars denote 0.95 confidence intervals).

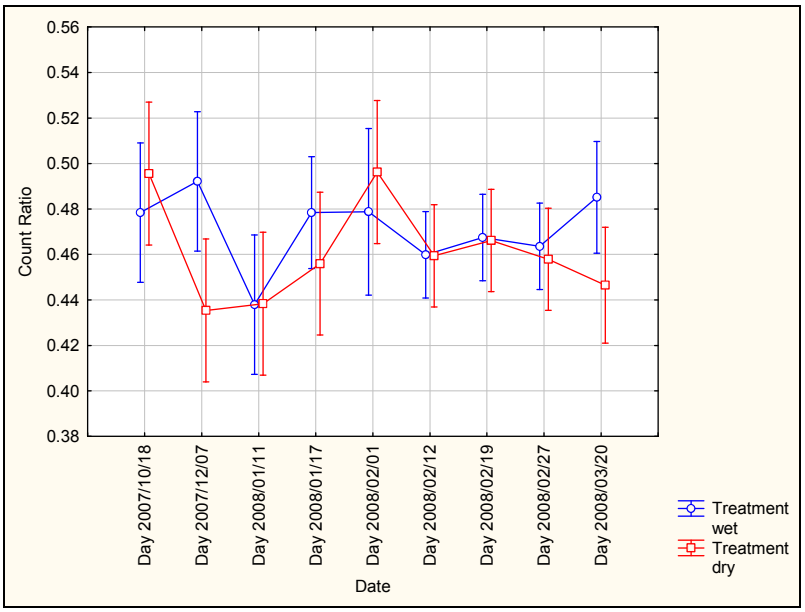

Figure 4.17 Repeated measures ANOVA computed for covariates at their means of the seasonal evolution of count ratios for the wet and dry treatments (2008) (vertical bars denote 0.95 confidence intervals).

4.3.4 PLANT WATER STATUS

4.3.4.1 PRE-DAWN LEAF WATER POTENTIAL (PDWP)

Significant differences could be found for PDWP measurements between the wet and dry treatments for both seasons (figures 4.18 and 4.19), with a stronger significance in 2008 (2007: $P < 0.05$ and 2008: $P < 0.01$). Vines from the dry treatment had more negative PDWP readings than those from the wet treatment, and were therefore more water stressed than the latter throughout the season. This might result from the wetter root zones for the wet-treatment vines, which is correlated with PDWP. This correlation exists as water potentials are equilibrated in the

leaves and the soil during the pre-dawn period as there is no transpiration during this stage. The higher rainfall in 2008 led to less negative PDWP values than in 2007.

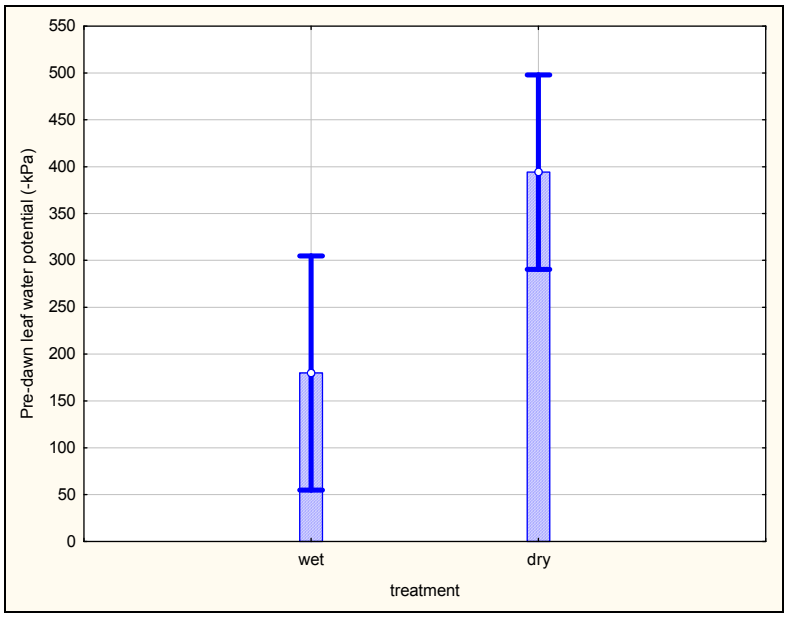

Figure 4.18 ANOVA computed for covariates at their means of pre-dawn leaf water potential for the wet and dry treatments (2007) ($P < 0.05$) (vertical bars denote 0.95 confidence intervals).

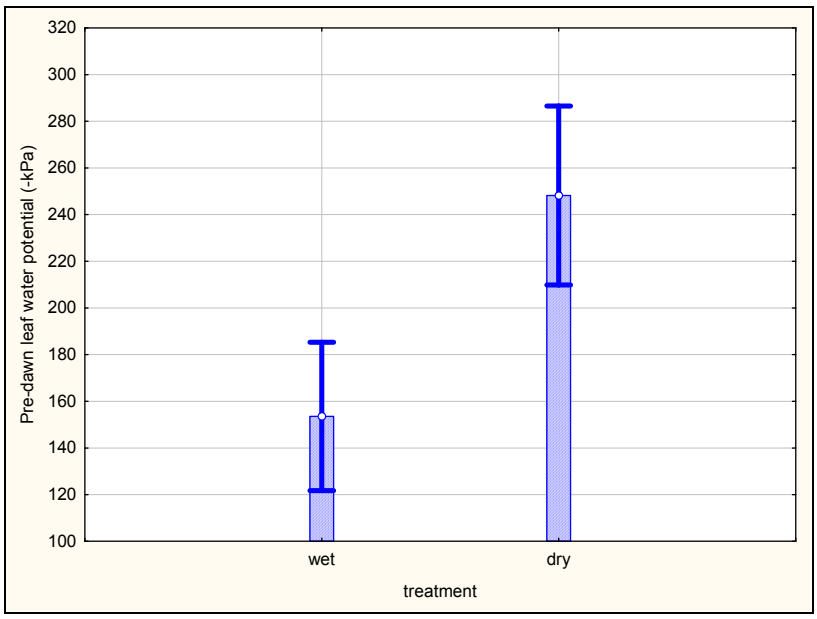

Figure 4.19 ANOVA computed for covariates at their means of pre-dawn leaf water potential for the wet and dry treatments (2008) ($P < 0.01$) (vertical bars denote 0.95 confidence intervals).

The seasonal patterns of the PDWP measurements show good differentiation between the wet and dry treatments. During the 2007 season (figure 4.20), the PDWP measurements indicated absent to weak water deficits for the vines from the wet treatment and moderate water deficits for the vines from the dry treatment (Van Leeuwen *et al*., 2007) when the measurements started (at about véraison). The PDWP measurements then started showing more stress to become weakly stressed, while the vines from the dry treatment remained moderately stressed (about a week after véraison). The stress levels then reduced slightly for both treatments, when the vines from the wet treatment showed PDWP measurements indicating no water deficits,

while those from the dry treatment showed values indicating weak to moderate water deficits. The measurements then slowly reduced and, at two weeks before harvest, the vines from the wet treatment showed PDWP measurements indicating absent to weak water deficits, while those from the dry treatment had PDWP measurements indicating moderate to weak water deficits.

During the 2008 season (figure 4.21), the PDWP for both treatments showed no water deficits at the start of the seasonal measurements (123 days after bud burst, at about véraison). The PDWP stress levels then increased significantly for both treatments (until about 137 days after bud burst) to show weak water deficits for the wet treatments and moderate to weak water deficits for the dry treatments. The high temperatures and low relative humidity recorded during this period could have contributed to this steep increase in plant water stress according to PDWP. The PDWP measurements then decreased significantly to show no water deficits for both treatments. One reason for this might be the high rainfall recorded in this period. The measurements showed more stress towards harvest and, at two weeks before harvest, the vines from the wet treatment showed PDWP values indicating very little to no water deficits, while those from the dry treatment showed weak water deficits.

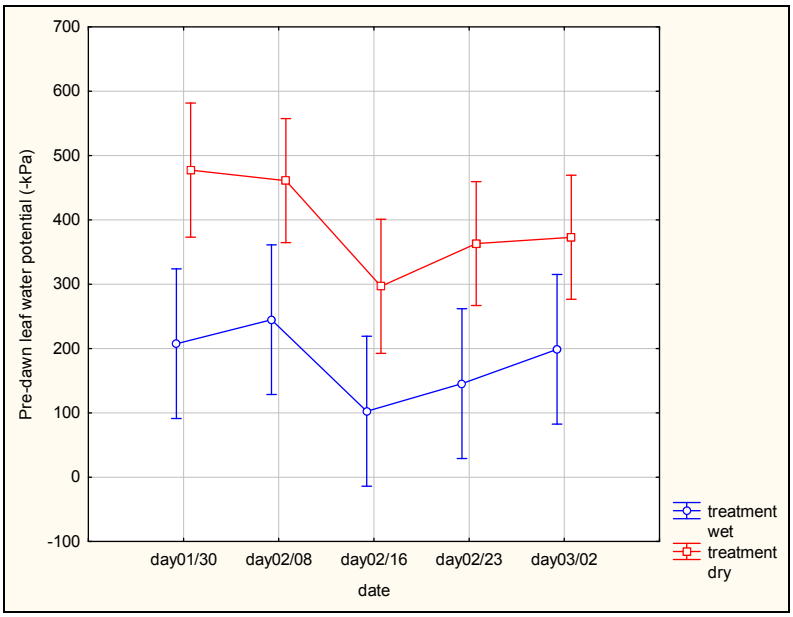

Figure 4.20 ANOVA computed for covariates at their means of the seasonal evolution of pre-dawn leaf water potential for the wet and dry treatments (2007) (vertical bars denote 0.95 confidence intervals).

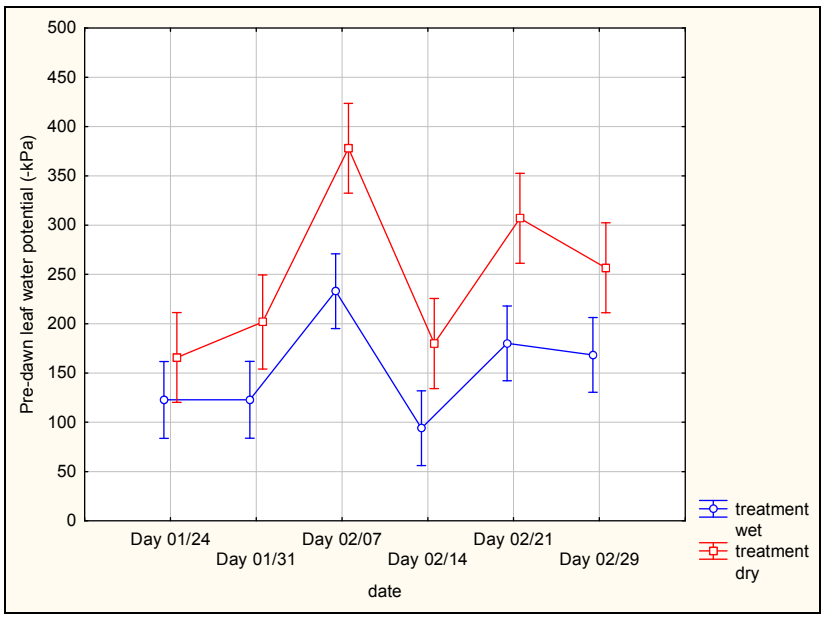

Figure 4.21 ANOVA computed for covariates at their means of the seasonal evolution of pre-dawn leaf water potential for the wet and dry treatments (2008) (vertical bars denote 0.95 confidence intervals).

The effect of the covariate was significant on the PDWP in 2007 ($P < 0.05$). Figure 4.22 indicates that, when pruning mass was used as covariate (A) and the effect of vigour on PDWP is accounted for, it increased the significance between the differences of the wet and dry treatments throughout the season. Without the incorporated effect of vigour, the result was negative PDWP levels for the dry treatments and less negative values for the wet treatments, showing that vigour had an effect on PDWP. Plots with more stressed PDWP measurements corresponded slightly to higher pruning masses, probably due to higher water usage by those vines during the day because of increased transpiration (larger leaf area), while the vines could not completely recover as efficiently as the lower-vigour vines during the night. In 2008 the effect of the covariate on PDWP was weakly significant ($P < 0.1$), with slightly more negative PDWP measurements for higher-vigour vines.

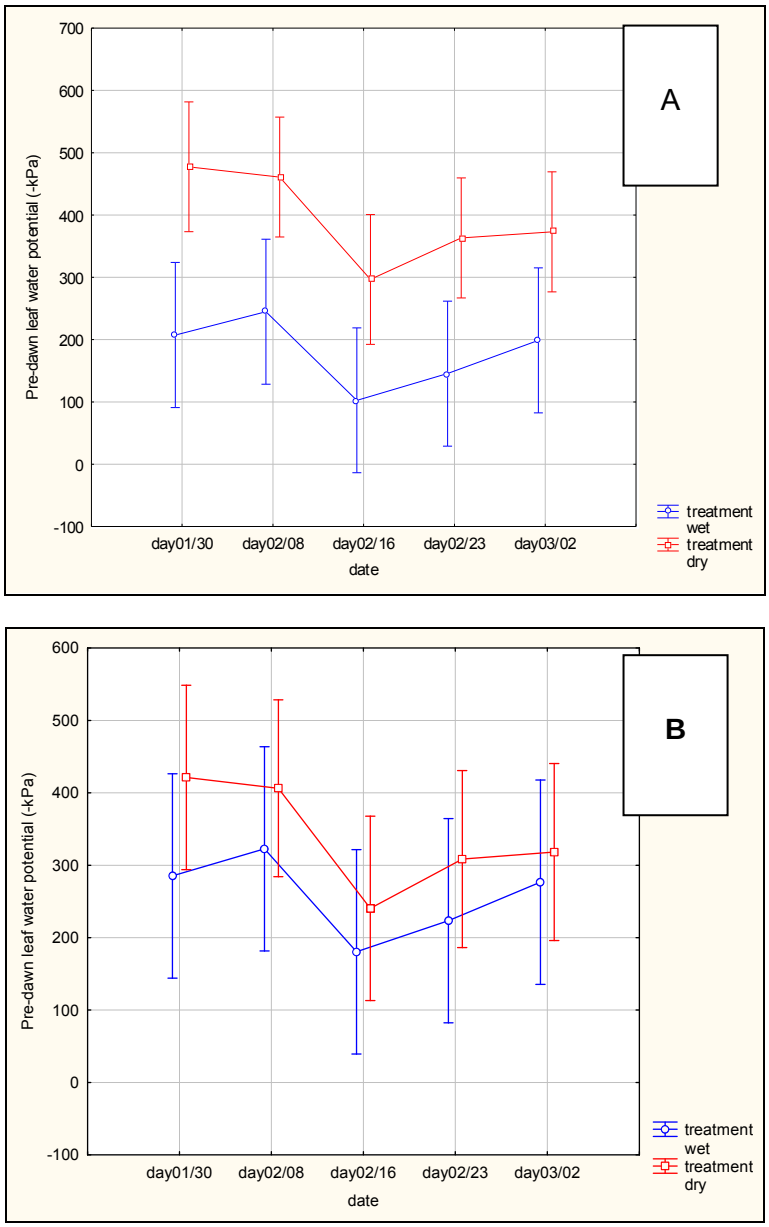

Figure 4.22 ANOVAs of pre-dawn leaf water potentials (A: computed for covariates at their means and B: no covariate computed) (2007) (vertical bars denote 0.95 confidence intervals).

4.3.4.2 STEM WATER POTENTIAL (SWP)

A strong, significant difference could be found between SWP measurements for the vines from the wet and dry treatments in the 2008 season ($P < 0.01$) (figure 4.24), with those from the wet treatment showing less water deficit stress (less negative SWP values) than those from the dry treatment. A weak significant effect was found for this in 2007 ($P < 0.1$) (figure 4.23). This effect was again expected, as SWP should reduce (become more negative) due to increased water deficits. A reduction in xylem water potentials follows as less water is available from the roots (Jones, 2004; Intrigliolo and Castel, 2007).

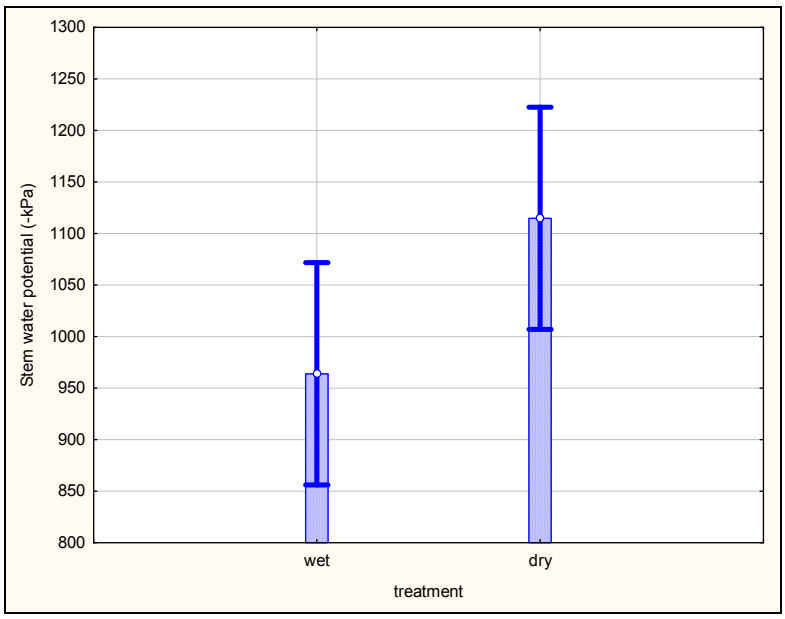

Figure 4.23 ANOVA computed for covariates at their means of stem water potential for the wet and dry treatments (2007) (P < 0.1) (vertical bars denote 0.95 confidence intervals).

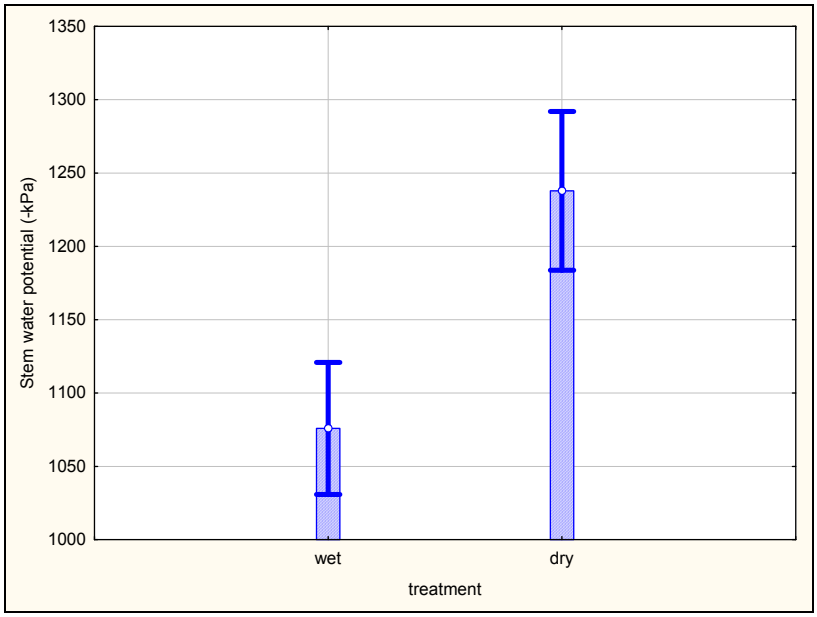

Figure 4.24 ANOVA computed for covariates at their means of stem water potential for the wet and dry treatments (2008) (P < 0.01) (vertical bars denote 0.95 confidence intervals).

In the 2007 season (figure 4.25), SWP showed the most stress around the first couple of weeks when the measurements were started – at 137 days after bud burst (08/02/2008). Wet treatment vines showed moderate water deficits, while dry treatment vines showed moderate to severe deficits during this stage. SWP readings for both treatments then showed significant decreases in water deficit until a week before harvest, when no or weak water deficits were present. The values reduced to indicate significantly increased stressed levels for both treatments until harvest (more so for the vines from the dry treatment). Wet treatment vines had weak to moderate water deficits at harvest, while dry treatment vines had moderate to severe deficits. There were no significant differences between wet and dry treatment vines at any stage of the season, although dry treatment vines showed a trend to have SWP values indicating more stress throughout the season.

During the 2008 season (figure 4.26), SWP measurement showed the lowest stress levels for both treatments in the beginning of the measurements, about a week before véraison, indicating weak water deficits for both treatments because the vines were not stressed at this early part of the season. SWP measurements then significantly reduced until about a month before harvest, showing an increase in the differentiation between wet (moderately water stressed) and dry treatments (moderate to severe water stress). The SWP measurements for both treatments stabilised as the season moved closer to harvest, with a decrease in water deficit stress for the wet treatments from two weeks before harvest. The reduction and stabilisation in stress might be due to the rainfall recorded during this period.

No significant differences could be found between SWP measurements for midday sun-exposed and midday shade leaves during both seasons ($P > 0.1$)

The effect of the covariate on SWP was not significant in both seasons ($P > 0.1$), which means that grapevine vigour did not significantly affect SWP. There were only small trends in both seasons for vines with larger pruning masses to have less negative (less stressed) SWP values, with no significant effects (2007, $r^2=0.0416$; $P > 0.1$ and 2008, $r^2=0.0456$; $P > 0.1$).

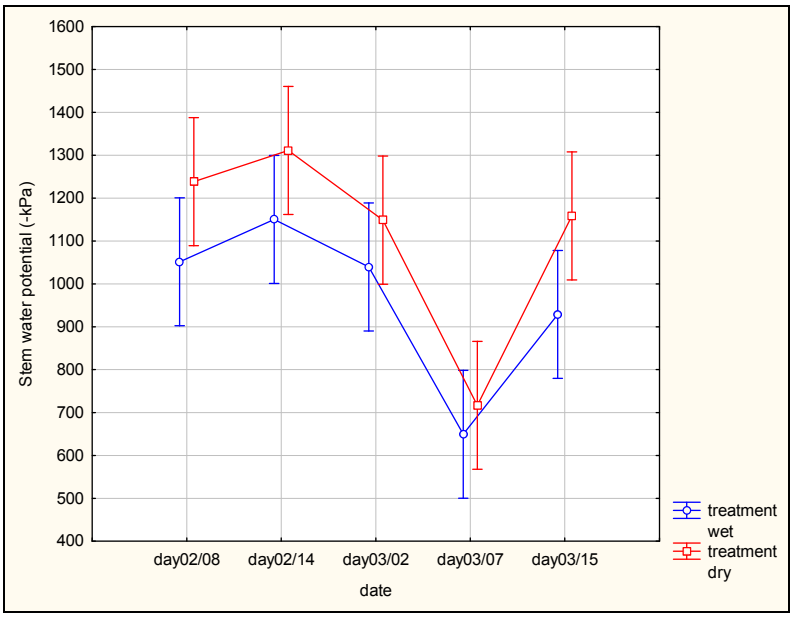

Figure 4.25 ANOVA computed for covariates at their means of the seasonal evolution of stem water potential for the wet and dry treatments (2007) (vertical bars denote 0.95 confidence intervals).

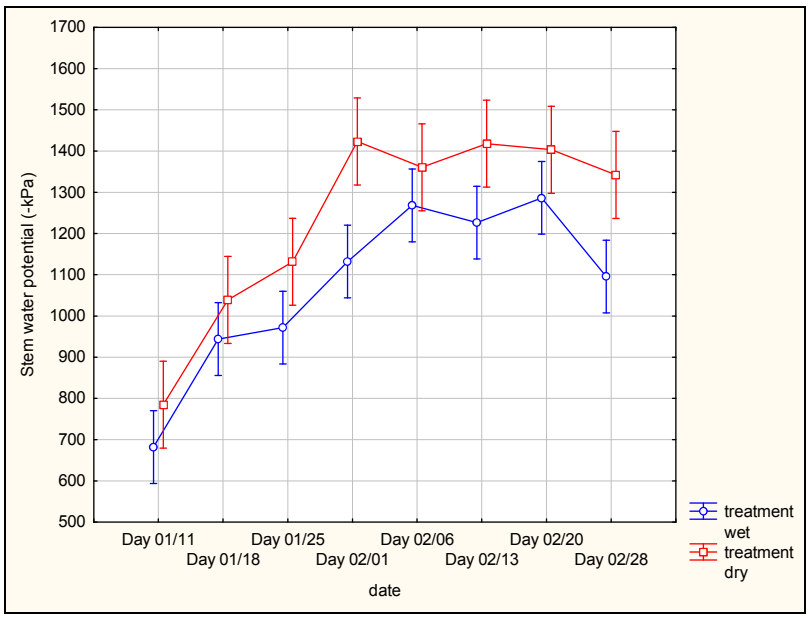

Figure 4.26 ANOVA computed for covariates at their means of the seasonal evolution of stem water potential for the wet and dry treatments (2008) (vertical bars denote 0.95 confidence intervals).

4.3.4.3 LEAF WATER POTENTIAL (LWP)

No significant differences could be found in LWP measurements between the wet and dry treatments over both seasons (figures 4.27 and 4.28). In both seasons there was a tendency for the vines from the dry treatment to have more negative LWP values than those from the wet treatment ($P > 0.1$). This corresponds with findings in the literature that LWP can indicate water deficits (Grant *et al*., 2007), but that the reading is not the best measurement to indicate grapevine water status (Schultz, 2003), as the environment has a combined effect on the measurement. The more negative values for the dry treatment were expected, as this follows due to increased water deficits because less water moves from the roots through the xylem to leaf cells, giving it a reduced (more negative) LWP.

It can also be observed that the SWP showed much more differentiation between the wet and dry treatments than LWP and therefore was a better indicator of grapevine water status.

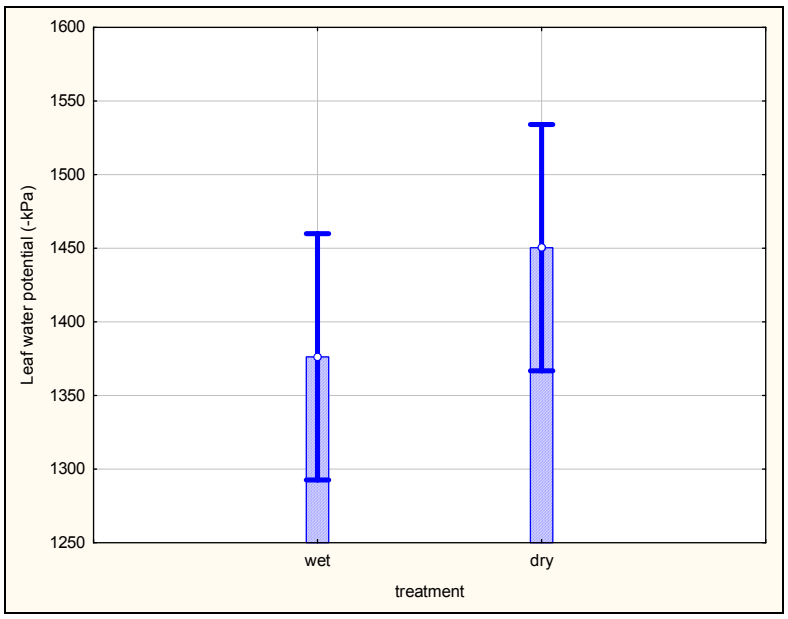

Figure 4.27 ANOVA computed for covariates at their means for leaf water potential for the wet and dry treatments (2007) (P > 0.1) (vertical bars denote 0.95 confidence intervals).

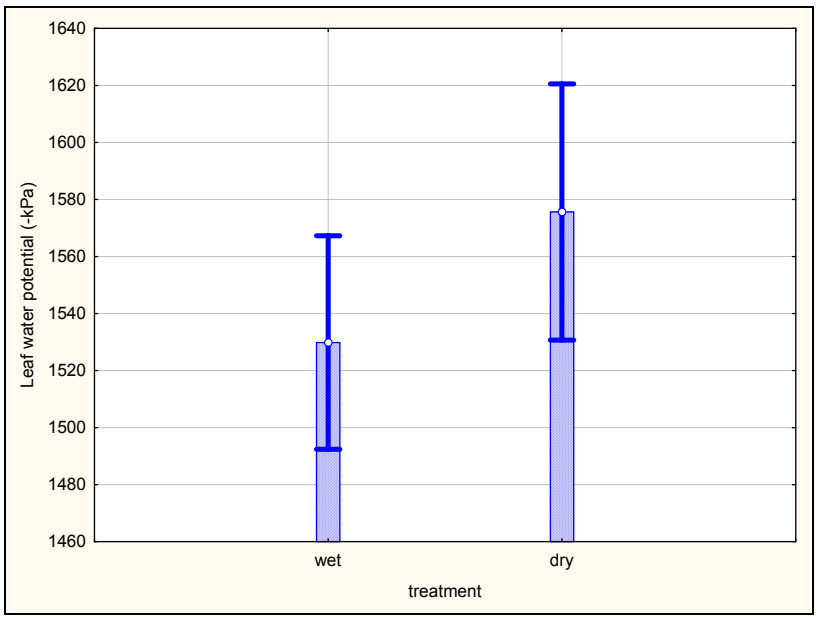

Figure 4.28 ANOVA computed for covariates at their means of leaf water potential for the wet and dry treatments (2008) (P > 0.1) (vertical bars denote 0.95 confidence intervals).

The seasonal progression of the LWP measurements for the 2007 season (figure 4.29) indicates that, when the measurements started at 137 days after bud burst (08/02/2007), LWP measurements indicated high water deficits for both the wet (moderate to severe) and dry (severe) treatment vines. Water deficit stress levels then increased slightly for both treatments until about one month before harvest, when both showed severe water deficits. The levels decreased significantly for both treatments until a week before harvest, indicating weak water deficits for wet treatments and weak to moderate deficits for dry treatments. The water deficit stress levels according to LWP then increased significantly until harvest, when both treatments showed moderate to severe water deficits. The increase in stress after a week before harvest

might be due to steep increases in temperatures and decreases in relative humidity recorded in this period.

In the 2008 season (figure 4.30), LWP measurements showed increased water deficits from the beginning of the measurements at about a week before véraison, when the readings indicated weak to moderately weak water deficits for the wet treatments and moderate to weak water deficits for the dry treatments, when both treatments had the lowest water deficits for the season according to their LWP measurements. The water deficit stress according to LWP then increased until about three weeks before harvest. This increase was more consistent for the vines from the wet treatment than those from the dry treatment, which had a much more inconsistent pattern in the increase in water deficit in this period. This might be due to the increased influence of rainfall on the LWP readings for the vines from the dry treatment than those from the wet treatment, because LWP can be affected by environmental conditions such as rainfall. Decreases in temperature and increases in relative humidity after 13/02/2008 might also have contributed to the reduction in stress according to LWP. The LWP values showed severe stress for both treatments at this stage. From then towards harvest there was a more volatile effect between the LWP readings for the dry and wet treatments, with both showing signs of decreasing water deficits. The vines from the wet treatment showed a steeper decline in water stress than those from the dry treatment, which showed only a very slight decrease during this period.

There were no significant differences between the LWP measurements for the midday sun-exposed and midday shade leaves during both seasons ($P > 0.1$).

The effect of the covariate on LWP was not significant in both seasons ($P > 0.1$), as vigour did not significantly influence plant water status expressed by LWP. There were only trends in both seasons for vines with larger pruning masses to have slightly less negative LWP measurements (less stressed) with no significant effects (2007, $r^2=0.0604$; $P > 0.1$ and 2008, $r^2=0.0676$; $P > 0.1$).

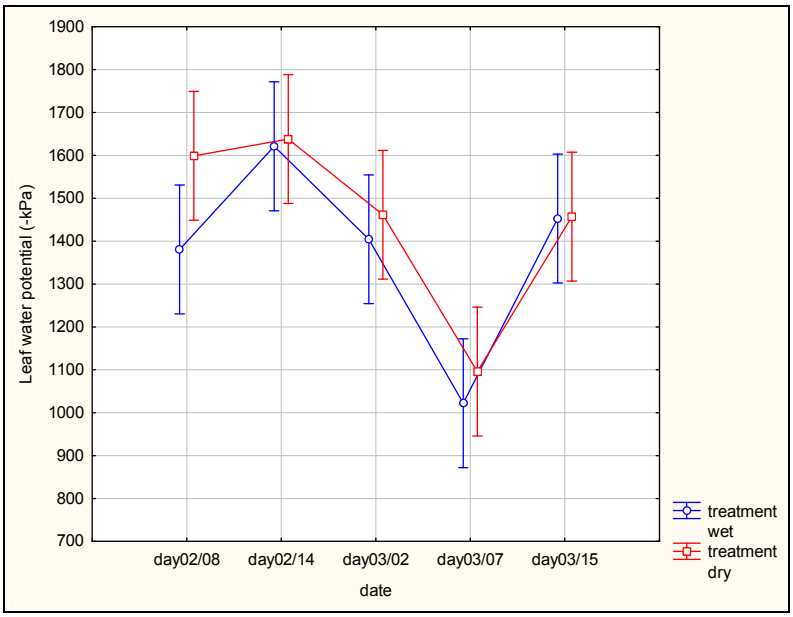

Figure 4.29 ANOVA computed for covariates at their means of the seasonal evolution of leaf water potential for the wet and dry treatments (2007) (vertical bars denote 0.95 confidence intervals).

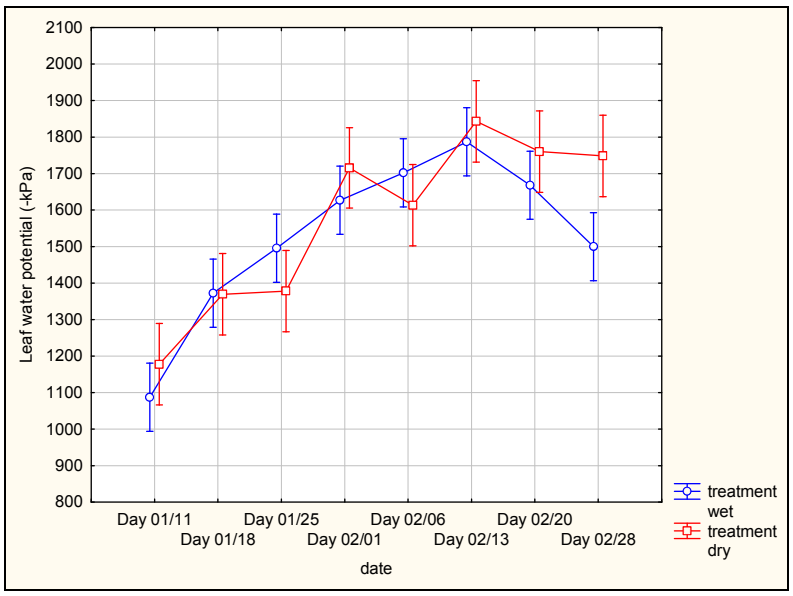

Figure 4.30 ANOVA computed for covariates at their means of the seasonal evolution of leaf water potential for the wet and dry treatments (2008) (vertical bars denote 0.95 confidence intervals).

4.3.5 PHYSIOLOGICAL MEASUREMENTS

4.3.5.1 CARBON ISOTOPE DISCRIMINATION

There were significant differences in carbon isotopic discrimination between the wet and dry treatments in both the 2007 (figure 4.31) and 2008 (figure 4.32) seasons ($P < 0.01$). The CID measured for the vines from the wet treatment had more negative values than those measured for the vines from the dry treatment. This corresponds with the literature, which shows that increased water deficit leads to decreased discrimination against ^{13}C and a less negative CID value (Bodin and Morlat, 2006). The results of the effect of the water deficit status on CID can be attributed to lower stomatal conductance for the dry treatment vines, resulting in decreased gas exchange and photosynthesis and less leaf internal CO_2. This leads to less CO_2 supply to Rubisco (Lal *et al*., 1996) and more ^{13}C absorption for these vines than those from the wet treatment.

The values of the measurements lie in the range of -24 to -26.8 (indicating moderate water deficits) for the 2007 season and -25.2 to -26.4 (indicating weak to absent water deficits) for the 2008 season (Van Leeuwen *et al*., 2007). The measurement for the 2008 season shows this result, due to it being a wetter season than the 2007 season. It is also important to note that the measurement season in 2008 covered a longer period than that of 2007, which could also have influenced measurements due to berry development effects (see discussion later).

Differences between the treatments seem to have been bigger in 2008 than in 2007 and this was also noted for plant water measurements. Plant water status therefore effected grapevine physiology and carbon discrimination.

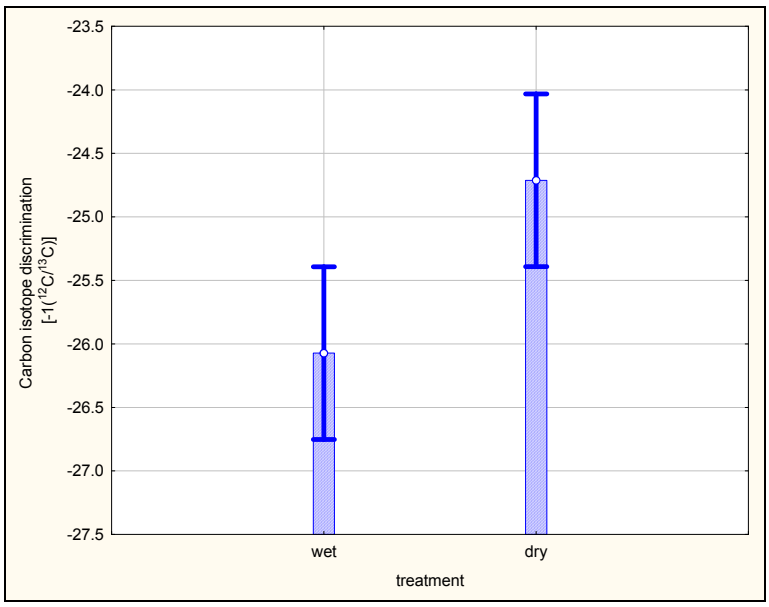

Figure 4.31 ANOVA computed for covariates at their means of the carbon isotope discrimination ratios for the wet and dry treatments (2007) (P < 0.01) (vertical bars denote 0.95 confidence intervals).

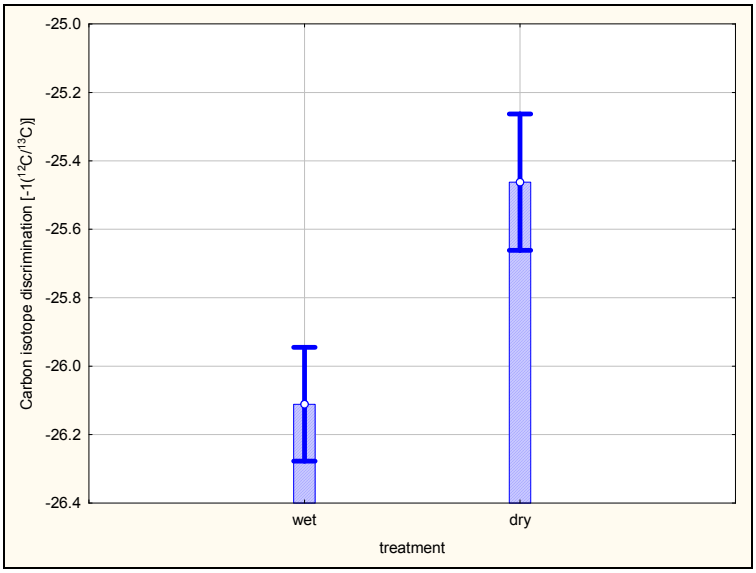

Figure 4.32 ANOVA computed for covariates at their means of the carbon isotope discrimination ratios for the wet and dry treatments (2008) (P < 0.01) (vertical bars denote 0.95 confidence intervals).

It seems that, as the seasons progressed, the significance of the difference between the CID values for the wet- and dry-treated vines increased. This can easily be seen by looking at the results for the 2008 season (figure 4.34), which had a much longer measuring period than the 2007 season (figure 4.33). This result might follow from the accumulative nature of the CID measurement in reaction to stress. The seasonal development of CID for the 2008 season shows that the vines had some of the highest stress levels at the start of the measuring season (both treatments had CID values indicating weak to moderate water deficits) when vines were at the pea-size phenological stage, and the stress level declined after that and reached a minimum when the vines were at véraison (both treatments had CID values indicating no stress). This corresponds with plant water potential measurements, which also showed some of the lowest

water deficits during this stage. The values before véraison indicated higher stress levels, probably because of juice composition consisting of high levels of organic acids at this stage (see discussion later). At véraison there was a turning point in the CID measurements and this should have a lot to do with juice composition, as the predominant carbon molecules change from acids to sugars (see discussion later). CID measurements after véraison indicated increases in water stress, which for the wet treatments showed increases for the subsequent two weeks, after which it remained stable until harvest. The CID measurements for the dry-treatment vines showed increased stress after véraison until about two weeks before harvest, when they indicated weak to moderate water deficit stress until harvest time. Increased water deficit after véraison again corresponds with increased deficits according to plant water potential measurements.

The seasonal development of the CID values could not easily be seen during the 2007 season because of the short length of the measuring period, which was focussed on the latter part of the season, but differences in the measurements between the wet and dry treatments can be seen throughout the season. The CID measurements in this study did not only indicate plant stress because of changes in stomatal aperture and leaf internal CO_2, but because the measurements were done on raw berry juice they were also influenced by the seasonal development of the berries. Organic acids are the predominant carbon molecules in the early season until véraison, while sugars are the predominant carbon molecules after véraison. These molecules have different biosynthetic pathways (Gaudillere, personal communication, 2008), which influence their CID values. This can probably explain the CID values at around pea size, which indicated the high stress in the 2008 season.

CID measurements on raw juice after véraison should be more trustworthy than that before véraison as it seems that the turning point at véraison can be instrumental in the separation of water deficit and berry development effects on CID. The fact that there existed a link between seasonal CID measurements and plant water potential measurements (PDWP, SWP and LWP) from véraison as both showed low water deficits at véraison and increased water deficit stress after véraison, could show that CID reacts to plant water status during this stage. It must also be remembered that CID is an accumulative measurement and it should therefore be the most accurate at harvest, when berry organic acids will also not have a large influence on the measurement.

There were no significant differences between the CID measured in samples taken from the shade and the sun parts of the rows in both seasons, but the CID values for the sun-exposed vines were a little less negative in the 2008 season that the shade-exposed vines, with a weak significant difference ($P < 0.1$). This indicated slightly higher stress for the samples taken from vines receiving more direct midday sun, corresponding with slightly lower photosynthetic rates for these vines, which could have influenced carbon fixation.

In the 2007 season, there was no significant effect of the covariate on CID ($P > 0.1$). The covariate, however, had a significant effect on CID in 2008 ($P < 0.05$). There was a weak correlation between pruning mass and CID ($r^2=0.2006$, $P < 0.1$), with vines with a higher vigour having more negative (less stressed) CID ratios in 2008. A weakly significant difference was found between CID ratios of vines with different vigour. No correlation was found in 2007, with

no significant differences (P > 0.1). This effect in 2008 corresponds to claims in the literature, which state that pruning mass can be negatively correlated with CID (Stamatiadis *et al*., 2007). This negative correlation could be due to microclimate differences between the vigour, influencing leaf stomatal conductance. The stomatal conductance for the vines with higher vigour was higher than that of vines with lower vigour in 2008 (Section 4.3.5.2), leading to increased leaf internal CO_2 and subsequent carbon discrimination, as more CO_2 is available for discrimination by Rubisco.

It can further be said that CID was therefore influenced more by water status than vigour, and this may be expected, as stomatal conductance and leaf gas exchange may be influenced more by water status than exposed leaf area. The reason for this is that root hormonal signals (influenced by root water status) are a large determinant of stomatal aperture (Davies *et al*., 2002), influencing CO_2 supply to Rubisco and the ability of the enzyme to discriminate against ^{13}C.

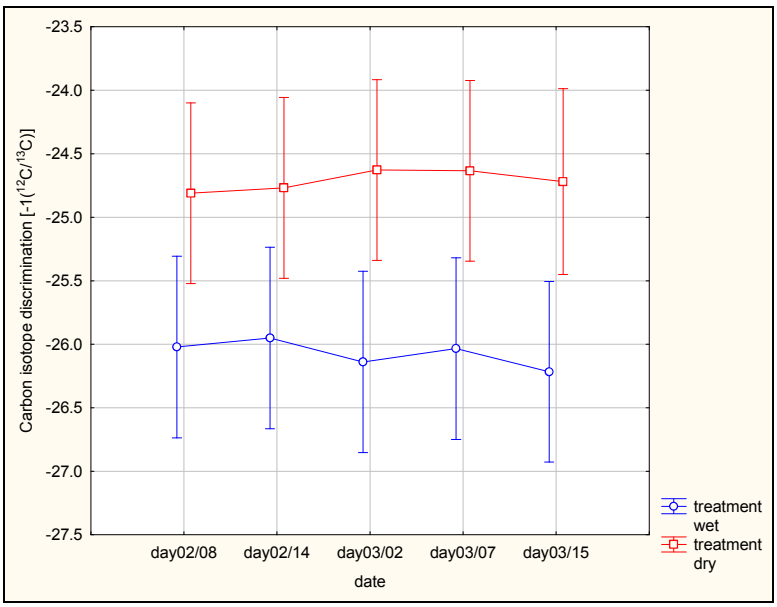

Figure 4.33 ANOVA computed for covariates at their means of the seasonal evolution of carbon isotope discrimination ratios for the wet and dry treatments (2007) (vertical bars denote 0.95 confidence intervals).

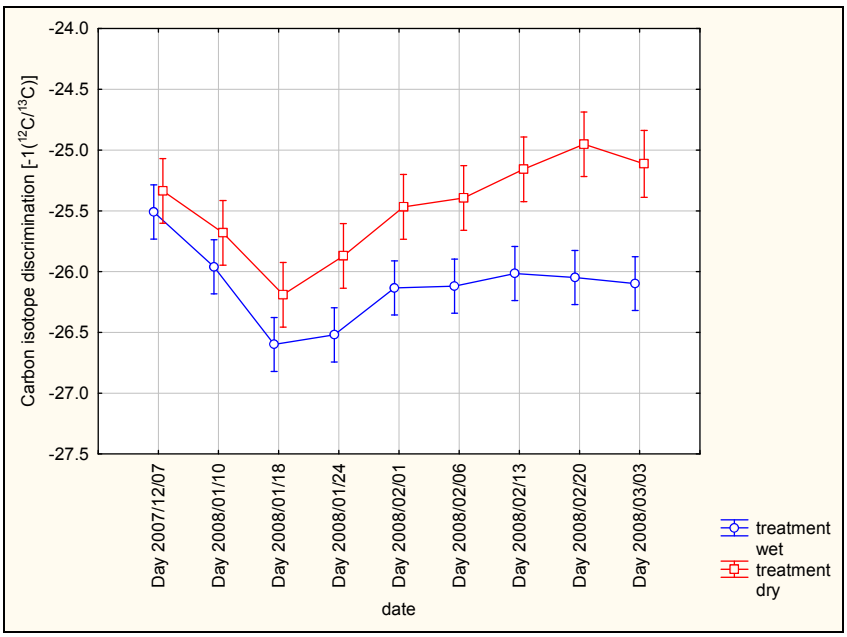

Figure 4.34 ANOVA computed for covariates at their means of the seasonal evolution of carbon isotope discrimination ratios for the wet and dry treatments (2008) (vertical bars denote 0.95 confidence intervals).

4.3.5.2 STOMATAL CONDUCTANCE (gs)

Measurements of stomatal conductance (gs) for the wet and dry treatments in the 2007 season showed no significant differences (figure 4.35), but the values were lower for the dry treatments ($P > 0.1$). For the 2008 season (figure 4.36), significant differences could be found between the wet and dry treatments ($P < 0.01$), with vines from the wet treatment having higher gs values than those from the dry treatment. This was expected, as soil drying causes increased ABA supply from the roots, which stimulates stomatal closure and therefore lower stomatal conductance due to loss of guard cell turgor. The transpiration rate in the vines from the dry treatment should therefore have been lower than that of the wet vines, and the dry treatment should also have had better water-use efficiency. Stomatal conductance was higher in 2008 than in 2007 due to the higher rainfall and more soil water being available to the vines.

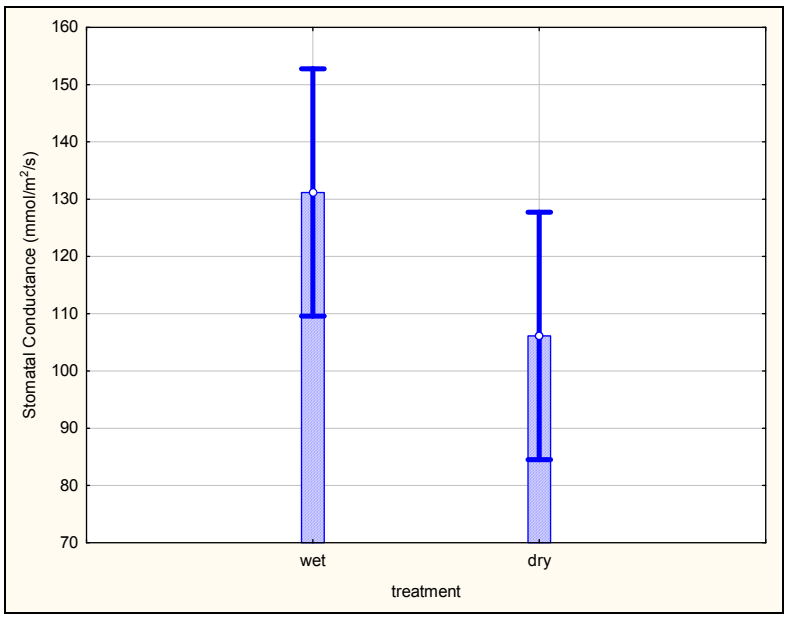

Figure 4.35 ANOVA computed for covariates at their means of leaf stomatal conductance for the wet and dry treatments (2007) (P > 0.1) (vertical bars denote 0.95 confidence intervals).

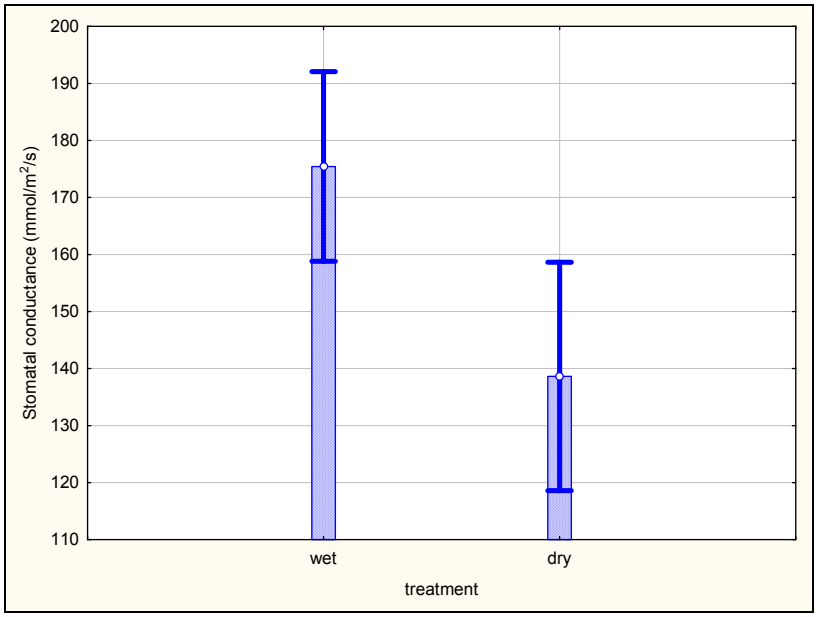

Figure 4.36 ANOVA computed for covariates at their means of leaf stomatal conductance for the wet and dry treatments (2008) (P < 0.01) (vertical bars denote 0.95 confidence intervals).

The seasonal pattern of the gs measurements is clearly visible in the 2008 season (figure 4.38), but less so in the 2007 season (figure 4.37), as the 2008 measurements were spread much more widely over the season. In the 2008 season, gs was the highest at the beginning of the measurement season (a week before véraison), while it declined from then onwards until about a month before harvest, when it stayed more constant for the rest of the ripening period until harvest. This result corresponds to the results for CID, where both showed the lowest stress values during the season around the véraison period, and then showed increasing stress until about a month before harvest. At this stage the stress status became more stable until harvest, probably because of the high levels of rainfall during this period. This trend could be seen for both the wet and the dry treatments. It can therefore be said that there is a relation

between gs and CID and that gs contributes to CID by affecting leaf gas exchange and therefore leaf internal CO_2 (Lajtha and Marshall 1994; Stamatiadis *et al*., 2007).

No differences could be found between the gs measurements of the midday sun-exposed and shade vines in both seasons.

The effect of the covariate was not significant in both seasons in its effect on stomatal conductance ($P > 0.1$). This was no surprise, as gs may be affected to a greater extent by water status than by exposed leaf area, as stomatal aperture is influenced largely by chemical signalling induced by root water status (Davies *et al*., 2002). A weak correlation (r^2=0.3009) were found in the 2008 season, when leaves from vines with higher pruning masses tended to have higher stomatal conductance, although this was not significant.

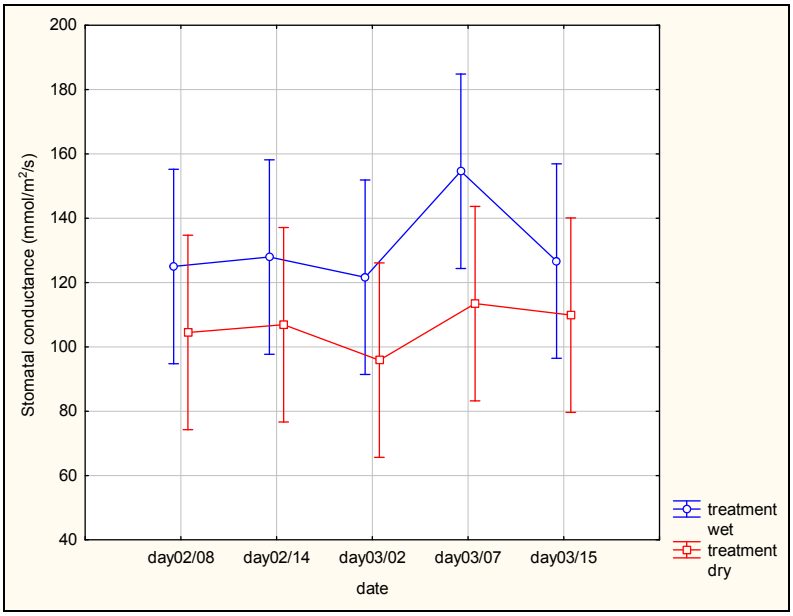

Figure 4.37 ANOVA computed for covariates at their means of the seasonal evolution of leaf stomatal conductance for the wet and dry treatments (2007) (vertical bars denote 0.95 confidence intervals).

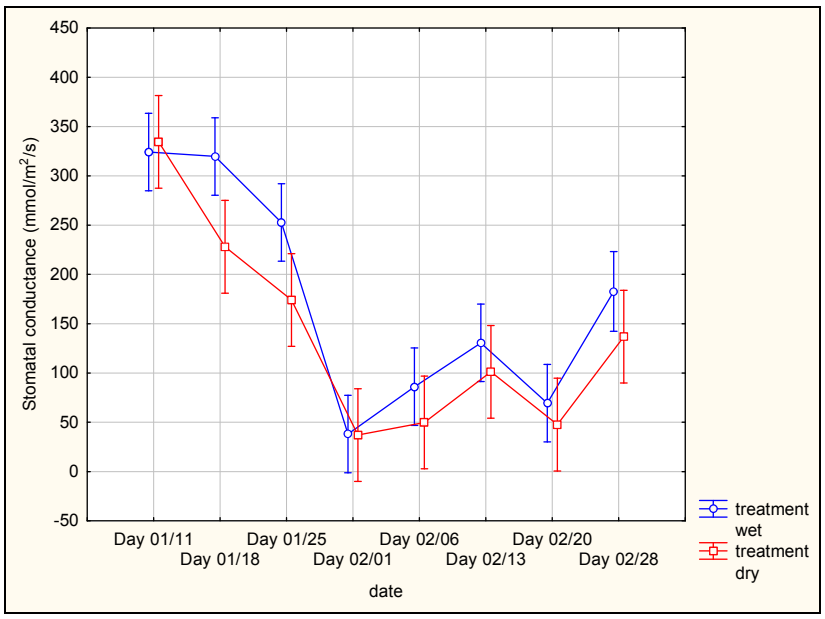

Figure 4.38 ANOVA computed for covariates at their means of the seasonal evolution of leaf stomatal conductance for the wet and dry treatments (2008) (vertical bars denote 0.95 confidence intervals).

4.3.5.3 LEAF PHOTOSYNTHETIC RATE AND LEAF GAS EXCHANGE

A strongly significant difference was found between the leaf photosynthetic rates (Pn) of the vines from the wet and dry treatments in the 2008 season ($P < 0.01$) (figure 4.39). The irrigation treatment therefore had an effect on photosynthesis for the season.

Vines from the wet treatment had higher photosynthetic rates than those from the dry treatment, which was to be expected, as reduced stomatal aperture due to increased water deficits leads to reduced photosynthesis. The reason for this is that less CO_2 movement from the atmosphere through the stomata takes place when gs reduces, decreasing the Pn rate (Medrano *et al*., 2003).

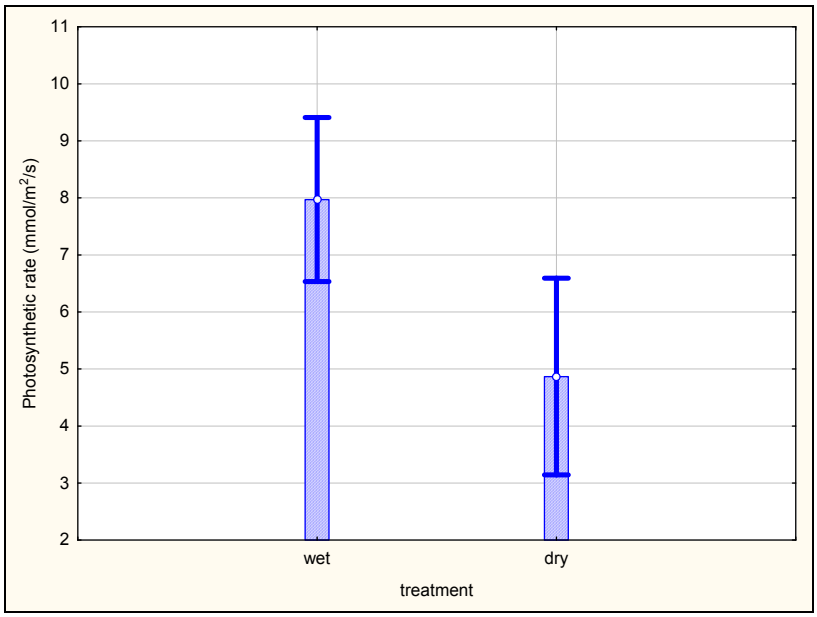

Figure 4.39 ANOVA computed for covariates at their means of leaf photosynthetic rates for the wet and dry treatments (2008) ($P < 0.01$) (vertical bars denote 0.95 confidence intervals).

The seasonal pattern of the Pn measurements (figure 4.40) shows that the photosynthetic rate was the highest in both treatments at the start of the season (about a week before véraison), and that it was similar in both the treatments. As the season progressed, the rate of photosynthesis decreased significantly, which corresponds to large increases in plant stress according to plant water potential measurements. The rates for both treatments stayed rather stable until about two weeks before harvest, and then increased towards harvest time. A reason for this stabilisation might be the high levels of rainfall recorded in this period.

No significant differences ($P > 0.1$) were found between the Pn rates of leaves on vines receiving midday sun and leaves on vines receiving midday shade, although vines that received more midday shade had slightly higher photosynthetic rates than vines that received midday sun.

No significant effect of vigour variation was found on leaf photosynthetic rate in both seasons, as the effect of the covariate was non-significant ($P > 0.1$). No correlation was found between pruning mass and Pn, as stomatal conductance was not significantly affected by vigour.

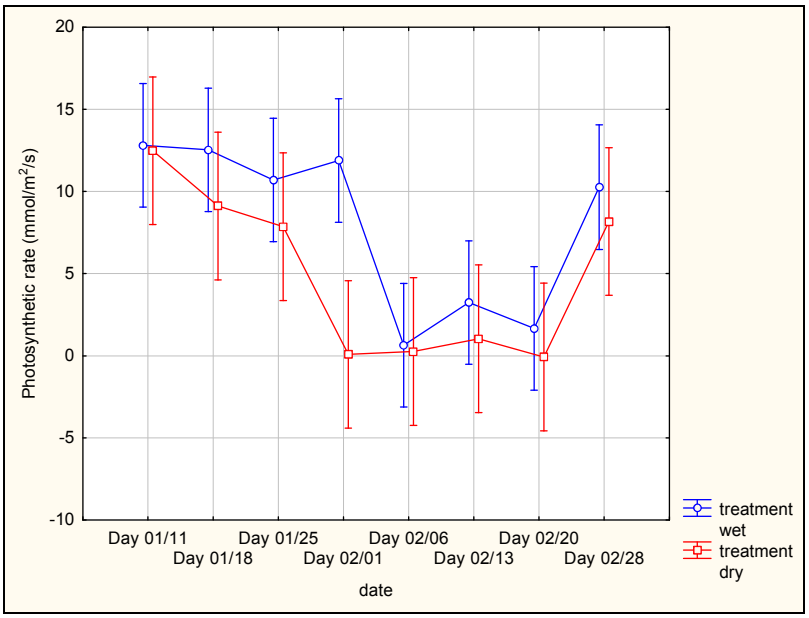

Figure 4.40 ANOVA computed for covariates at their means of the seasonal evolution of leaf photosynthetic rates for the wet and dry treatments (2008) (vertical bars denote 0.95 confidence intervals).

The difference between evapotranspiration for the wet and the dry treatments for the 2008 season was also significant ($P < 0.05$) (figure 4.41), with higher rates for the vines from the wet treatment. This should follow because of the decreased stomatal aperture of the vines from the dry treatment, causing a decrease in transpiration in order for the plant to increase its water-use efficiency during increased water deficits. There were no differences in evapotranspiration from leaves in the midday sun and midday shade sides during the season ($P > 0.1$).

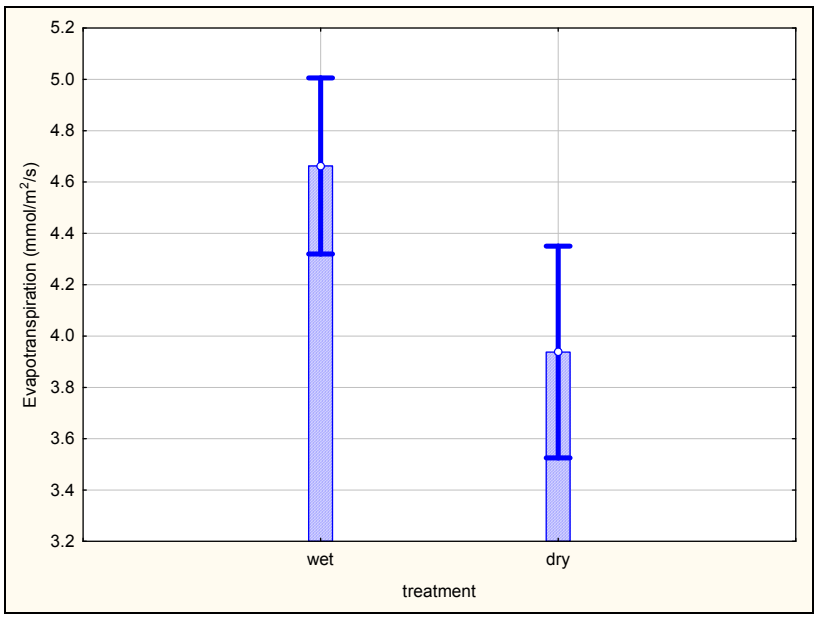

Figure 4.41 ANOVA computed for covariates at their means of leaf evapotranspiration rates for the wet and dry treatments (2008) ($P < 0.05$) (vertical bars denote 0.95 confidence intervals).

Evapotranspiration rates for both treatments were the highest around the start of the measuring season, at about a week before véraison until véraison (figure 4.42). They then decreased until about five weeks before harvest, as plant water stress measured by plant water potential increased during this period. The rates then increased slightly and stabilised until harvest. The reason again might be the high levels of rain recorded in this period.

The covariate had no significant effect on evapotranspiration ($P > 0.1$), although a weak correlation was found between pruning mass and evapotranspiration ($r^2=0.1845$; $P < 0.05$), with higher evapotranspiration rates for vines with higher pruning masses, where significant differences were found. This should be because of the slightly higher stomatal conductance in those leaves, resulting in increased transpiration from the leaf stomata. Higher leaf areas for these vines should also contribute to higher transpiration rates.

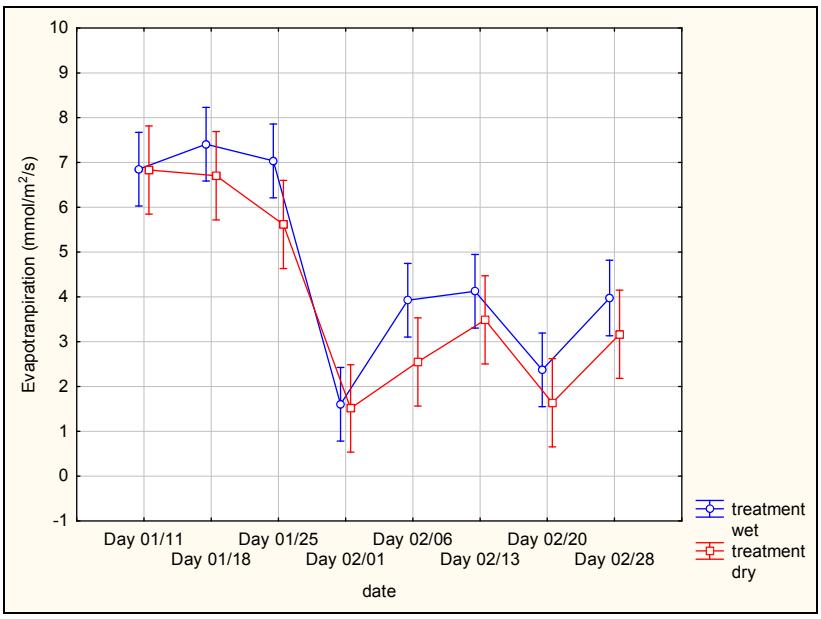

Figure 4.42 ANOVA computed for covariates at their means of the seasonal evolution of leaf evapotranspiration rates for the wet and dry treatments (2008) (vertical bars denote 0.95 confidence intervals).

Leaf temperature measurements showed significant differences at the 10% confidence level between the wet and dry treatments, with leaves from the dry-treatment vines having higher leaf temperatures than those of the wet-treatment vines ($P < 0.1$) (figure 4.43). This follows due to decreased stomatal closure for the dry-treatment vines, reducing the transpiration of those leaves and therefore their evaporative cooling.

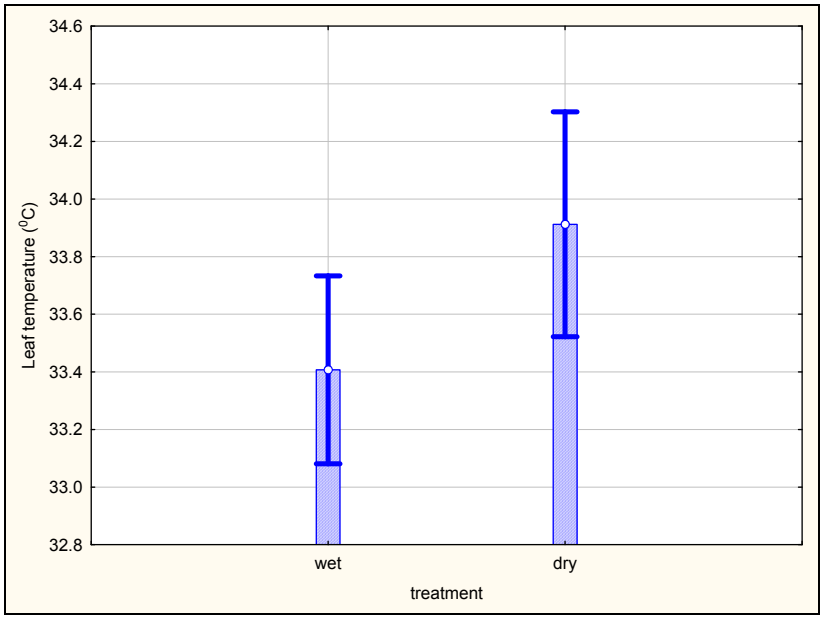

Figure 4.43 ANOVA computed for covariates at their means of leaf temperature for the wet and dry treatments (2008) ($P < 0.1$) (vertical bars denote 0.95 confidence intervals).

At the beginning of the measurement season, about a week before véraison, the leaf temperature showed low water deficit stress for both treatments (figure 4.44). This temperature then increased rapidly until the middle of the season, at a month before harvest, corresponding to the high environmental temperatures and low relative humidity recorded in this period. The environment might have a big influence on the measurement and it might be an idea to use the

instruments leaf and canopy temperature as a ratio in future studies. The leaf temperature then decreased rapidly towards harvest, to be at its lowest around harvest time.

No significant differences were found between the leaf temperatures of the vines exposed to midday sun and midday shade, although there was a slight trend ($P > 0.1$) for the sun-exposed vines to have slightly higher leaf temperatures than the shade-exposed vines, as expected.

The covariate did not significantly affect leaf temperatures ($P > 0.1$), although there was a weak correlation showing higher leaf temperatures for vines with lower pruning masses ($r^2=0.1191$; $P > 0.1$), although this was not significant. The less dense canopies might therefore have caused slightly more direct sunlight on the leaves, increasing their temperature.

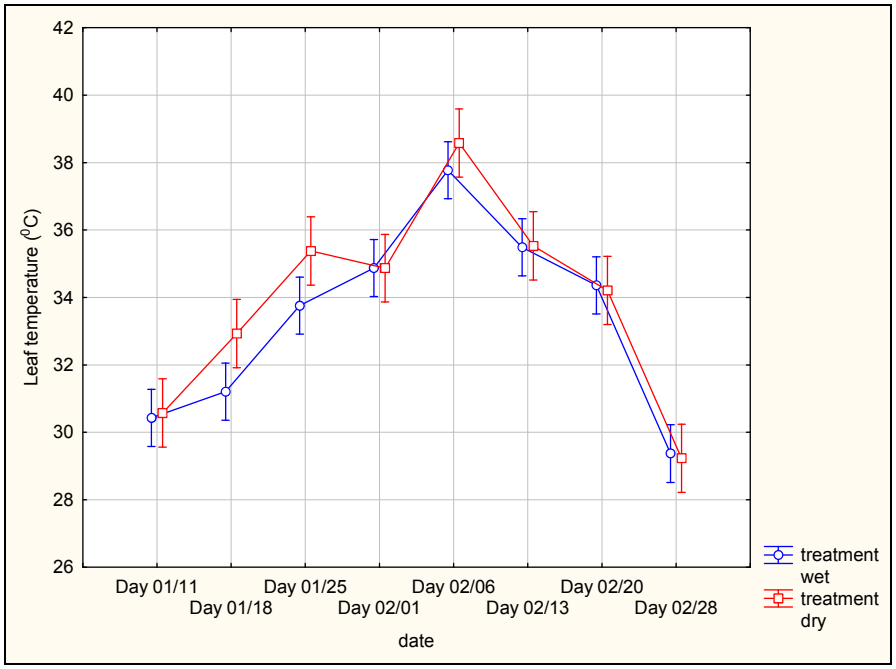

Figure 4.44 ANOVA computed for covariates at their means of the seasonal evolution of leaf temperatures for the wet and dry treatments (2008) (vertical bars denote 0.95 confidence intervals).

4.3.6 REPRODUCTIVE MEASUREMENTS

4.3.6.1 BERRY DEVELOPMENT

Significant differences could be found for berry size between the wet and dry treatments for both seasons ($P < 0.05$) (figure 4.45 and 4.46). The vines from the wet treatment had bigger berries than those from the dry treatment, as expected. This should follow because more water is available to the plant organs, expanding cells in the berries and increasing the berry mass, especially during the pre-véraison period (Alexander, 1965; McCarthy, 1997). The berries seem to have been smaller in the 2008 season, but this should be because berry size measurements were started much earlier in the season, when the berries were still very small.

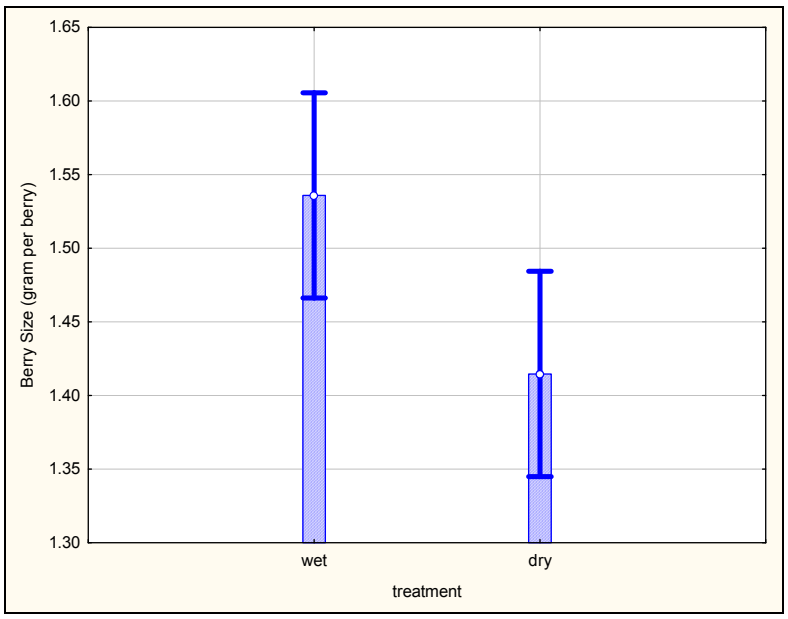

Figure 4.45 ANOVA computed for covariates at their means of berry size for the wet and dry treatments (2007) (P < 0.05) (vertical bars denote 0.95 confidence intervals).

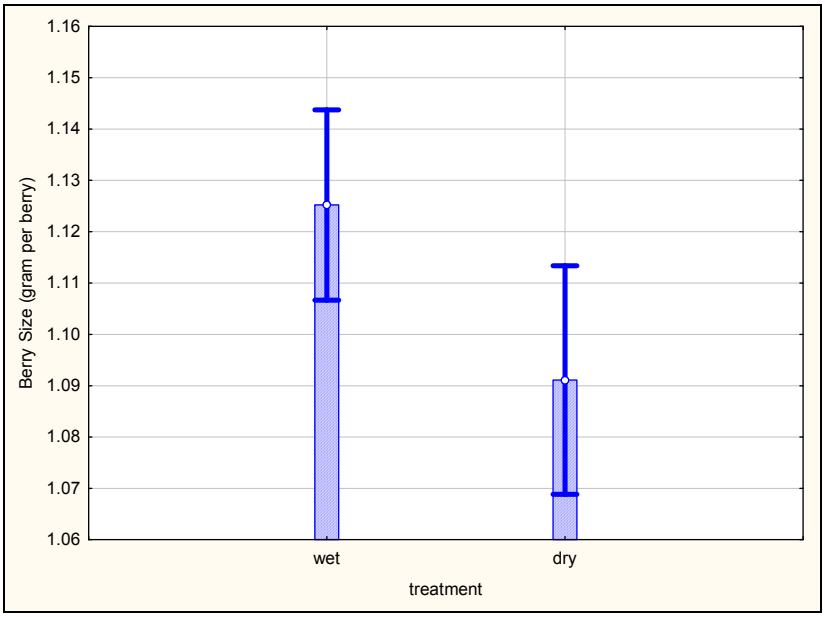

Figure 4.46 ANOVA computed for covariates at their means of berry size for the wet and dry treatments (2008) (P < 0.05) (vertical bars denote 0.95 confidence intervals).

The pattern of berry size development can be seen as a logarithmic growth pattern for the 2008 season (figure 4.48), while the pattern is not so visible for 2007 (figure 4.47) because of the short length of the measuring period in that season.

In both seasons there was a tendency for the berries from vines receiving midday shade to have higher masses than those from vines receiving midday sun, with weakly significant differences between the row sides (P < 0.1). The reason for this might be microclimate differences, with lower temperatures for shade-sided berries, resulting in less transpiration (Morrison and Noble, 1990).

Berry growth in the 2007 season was only measured in the last part of the season, when it seems that berry growth had already stabilised. The increase in berry size for the wet-treatment

vines between the first two measuring dates might be due to the high rainfall recorded in that period.

From about two weeks after véraison to three weeks after véraison in the 2008 season, the significance in the difference between the size of berries from the wet and dry treatments reduced. The size of berries from the wet-treatment vines did not increase, while it increased for the dry-treatment vines. Changes in plant water status during this period can explain this effect, as SWP and LWP measurements during this period showed increases in stress for the wet treatments and slight decreases in stress for the dry treatments. Berry growth also seems to have stabilised at about five weeks before harvest. During this stage there also was a decrease in relative humidity, which could have caused increased evapotranspiration from the berries. This could have impacted especially on the vines from the wet treatment, as transpiration should be greater when compared to the more stressed vines with smaller berries. This should then lead to the slight decrease in berry size for the wet treatments. After about three weeks before harvest it was again observed that the differentiation between the berry sizes of the wet and dry treatments increased, with those from the wet treatment showing increased berry size until harvest, while there was no increase in the size of berries from the dry treatment. SWP and LWP measurements during this stage can again help explain this effect, as there were decreases in stress for the wet treatment and no real decreases in stress for the dry treatment. Decreased temperatures and higher relative humidity could also have contributed to these changes in plant water potential, leading to decreased evapotranspiration. The berries of the wet treatment therefore increased in size, while the more stressed vines showed no increase in berry size.

Changes in plant water status therefore contributed to changes in berry growth. PDWP measurements did not show this effect on berry growth throughout the season like SWP and LWP did, and this might be because of the rehydration of the grapevine during the night.

The effect of the covariate on berry size in 2007 was non-significant ($P > 0.1$), while in 2008 there was a weakly significant effect ($P < 0.1$). Vines with higher pruning masses had berries with slightly higher masses in both seasons, with weak correlations being found in the 2007 season, with no significant differences ($r^2=0.2295$; $P > 0.1$), and a moderate correlation and significant differences being found in 2008 ($r^2=0.2580$; $P < 0.05$). This was again expected, as higher vigour should promote berry growth. The higher vigour during the 2008 season might have led to an increased influence of vigour on berry size in comparison to the 2007 season.

Berry size development by it self does not seem to have an influence of CID during the season (Gaudillere, personal communication), but the change of berry composition over the season does have an influence as discussed.

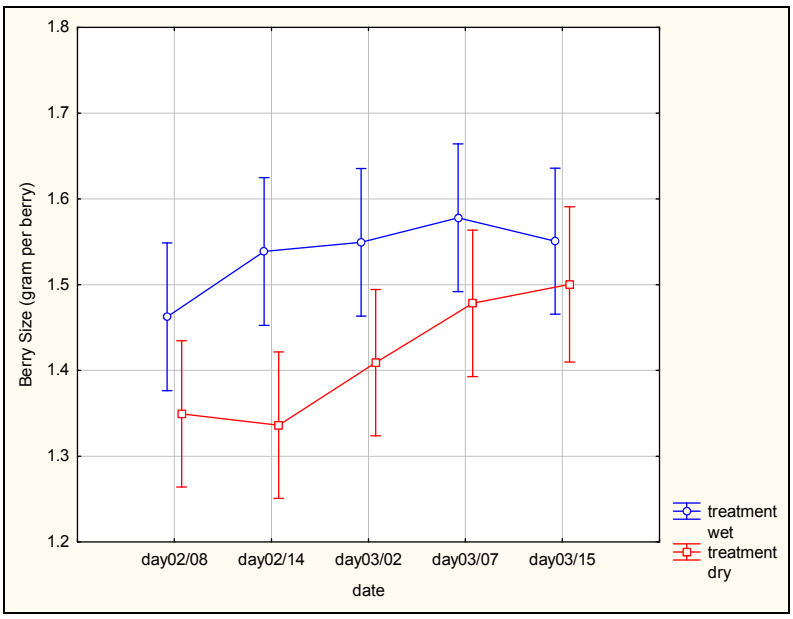

Figure 4.47 ANOVA computed for covariates at their means of the seasonal evolution of berry size for the wet and dry treatments (2007) (vertical bars denote 0.95 confidence intervals).

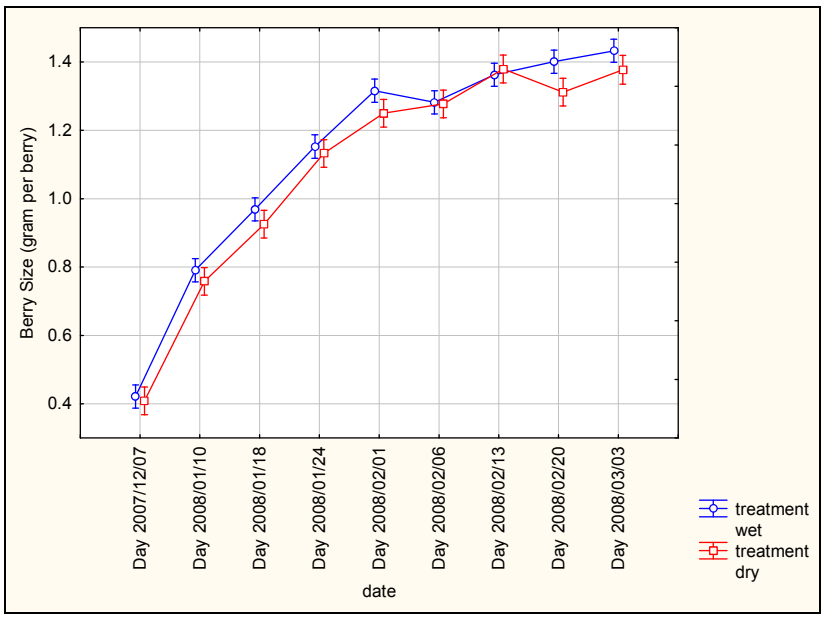

Figure 4.48 ANOVA computed for covariates at their means of the seasonal evolution of berry size for the wet and dry treatments (2008) (vertical bars denote 0.95 confidence intervals).

4.3.6.2 DEVELOPMENT OF CHEMICAL COMPOSITION OF BERRIES

No significant differences were found between the juice pH measurements of the treatments ($P > 0.1$) in both seasons (figures 4.51 and 4.52), indicating that the sensitivity of juice pH was not high. However, this might have been due to differences in berry sizes and therefore a dilution effect. There was however a slight trend in both seasons (data not shown), showing slightly higher pH for the berries of the wet treatments, meaning that increased shading of the canopies for the vines of these treatments might have led to increased berry potassium, causing this slight increase in pH (Smart, 1987).

The seasonal pattern for berry juice pH can again be seen better for the 2008 season due to the longer measurement period. The increase in pH was slow at the start of the season until about a week after véraison. It then increased rapidly for a while, as a strong reduction in berry acids should take place after véraison (Watson, 2003), and then increased steadily until harvest, as there is an increase in the ratio of acid salts and free acids when acid synthesis reduces and acids are metabolised during ripening.

For 2007, pH measurements were only conducted for the last part of the season, and it can be seen that pH increased at the beginning and then started to decrease towards harvest.

The effect of the covariate on juice pH in both seasons was not significant ($P > 0.1$). However, there was a moderate correlation between pH and pruning mass in 2007, and this effect was significant ($r^2=0.4171$; $P < 0.01$), with a higher pH for vines with higher vigour. A similar trend was found in 2008, with a weak correlation between pruning mass and juice pH, but the effect was not significant ($r^2=0.0906$; $P > 0.1$). Increased canopy shading in the higher-vigour vines could therefore have led to the increased pH of the berry juice because of microclimate differences between the higher and lower vigour. This would increase the potassium content of the berries, resulting in a higher pH.

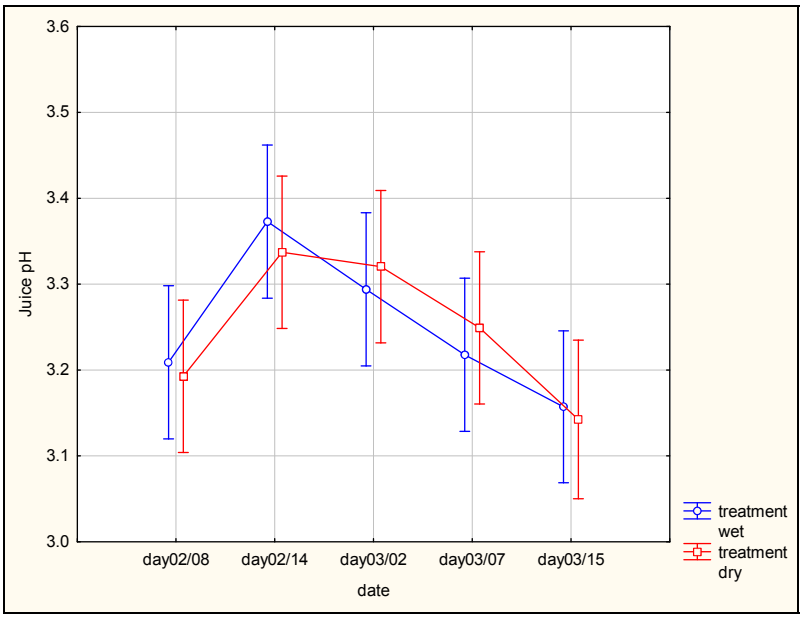

Figure 4.51 ANOVA computed for covariates at their means of the seasonal evolution of berry juice pH for the wet and dry treatments (2007) (vertical bars denote 0.95 confidence intervals).

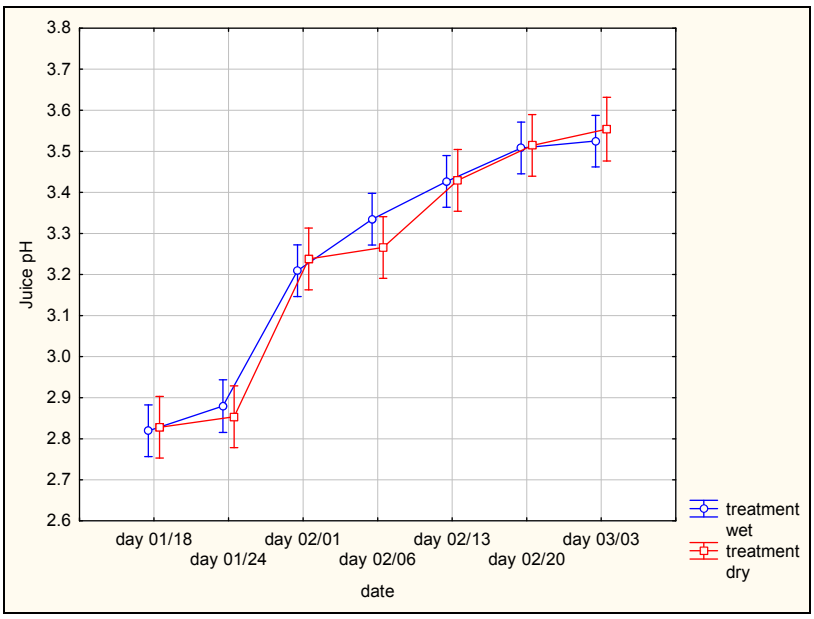

Figure 4.52 ANOVA computed for covariates at their means of the seasonal evolution of berry juice pH for the wet and dry treatments (2008) (vertical bars denote 0.95 confidence intervals).

There was no significant effect of treatment on the total soluble solids of the berry juice for both seasons (P > 0.1) (figures 4.53 and 4.54). Midday row side did not have significant effects on total soluble solids of the juice in both seasons (P > 0.1).

When berry size was accounted for and total soluble solid content was determined, the wet treatment had a significantly higher total soluble solid content in the berry juice, with strong significant differences in 2007 (figure 4.53) and significant differences in 2008 (figure 4.54) (2007: P < 0.01 and 2008: P < 0.05). Increased photosynthesis in the vines from the wet treatment could have led to increased sugar production and therefore a higher content of total soluble solids.

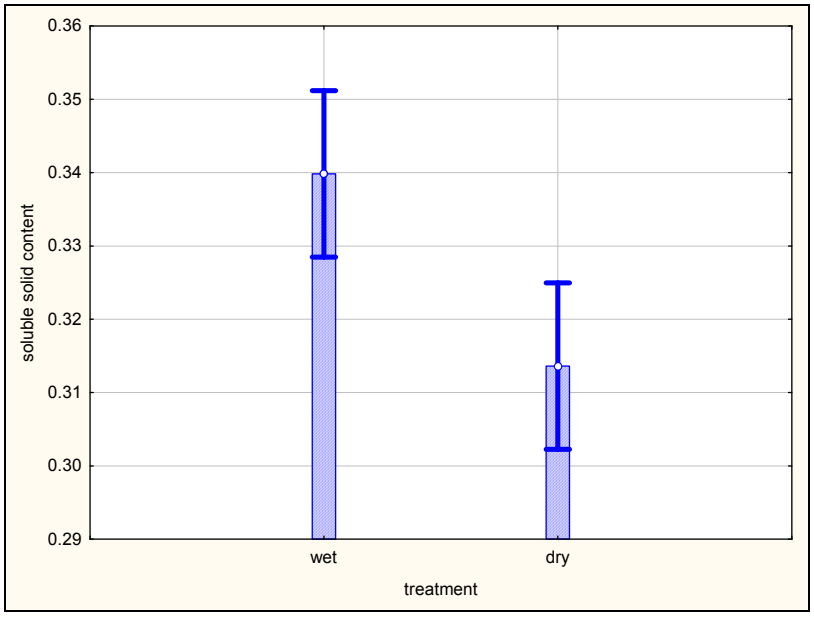

Figure 4.53 ANOVA computed for covariates at their means of the total soluble solid content of the berry juice for the wet and dry treatments (2007) (P < 0.01) (vertical bars denote 0.95 confidence intervals).

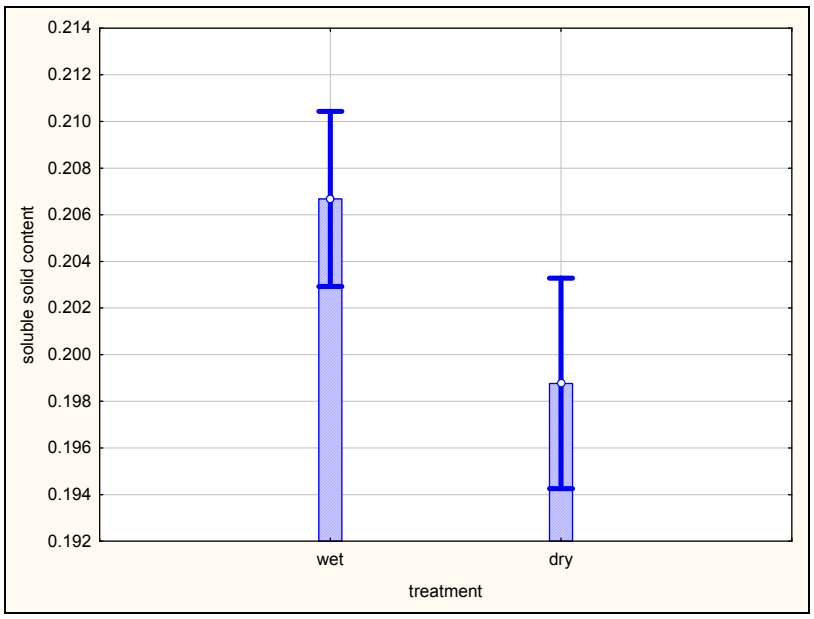

Figure 4.54 ANOVA computed for covariates at their means of the total soluble solid content of the berry juice for the wet and dry treatments (2008) ($P < 0.05$) (vertical bars denote 0.95 confidence intervals).

The seasonal pattern of the development of juice total soluble solids (figures 4.55 and 4.56) showed that sugar development was slow at first and then increased rapidly, stabilising towards ripening. There were no significant differences between the treatments throughout both seasons, although there was a tendency in both seasons for the wet treatment to have higher total soluble solids at the start of the season when compared to the dry treatment. However, the total soluble solids of the dry treatment tended to be higher in the last couple of weeks of the season. This might be due to a dilution effect, where the wet treatment had larger berries and therefore experienced increased dilution of the berry components such as sugars, while the stabilised or reduced plant stress during this period could also have contributed to higher sugar production for the vines from the dry treatment just before harvest.

The effect of the covariate on juice total soluble solids was not significant in 2007 ($P > 0.1$), but it had a significant effect on total soluble solid content in 2008 ($P < 0.05$). One possible explanation might be due to canopy shading, resulting in a reduced content of total soluble solids in the higher-vigour vines, as the microclimate due to increased shading could have caused a reduction in sugar production, which was more likely to occur in 2008 than 2007 because of the higher vigour in 2008. Microclimate differences could also have been responsible for the significant effect found in 2008 where vigour influenced CID as mentioned, where higher vigour vines showed lower stress and also had bigger berries than lower vigour vines, which could have led to dilution effects on berry sugars.

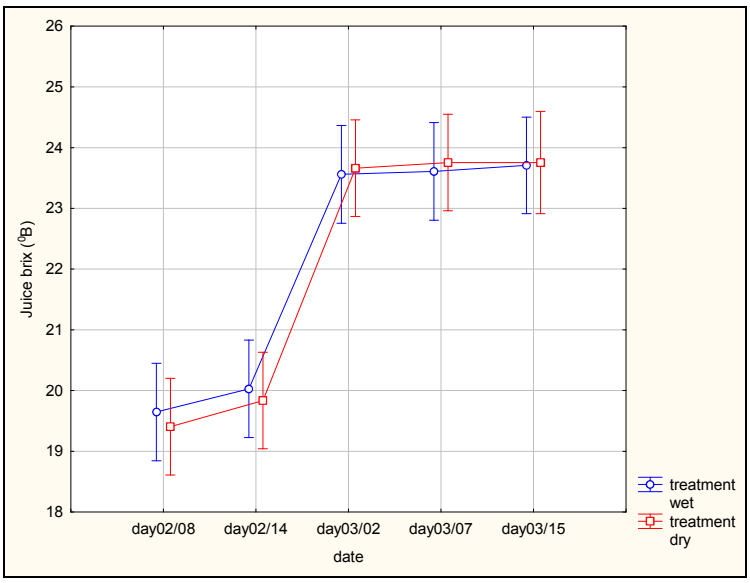

Figure 4.55 ANOVA computed for covariates at their means of the seasonal evolution of berry juice total soluble solids for the wet and dry treatments (2007) (vertical bars denote 0.95 confidence intervals).

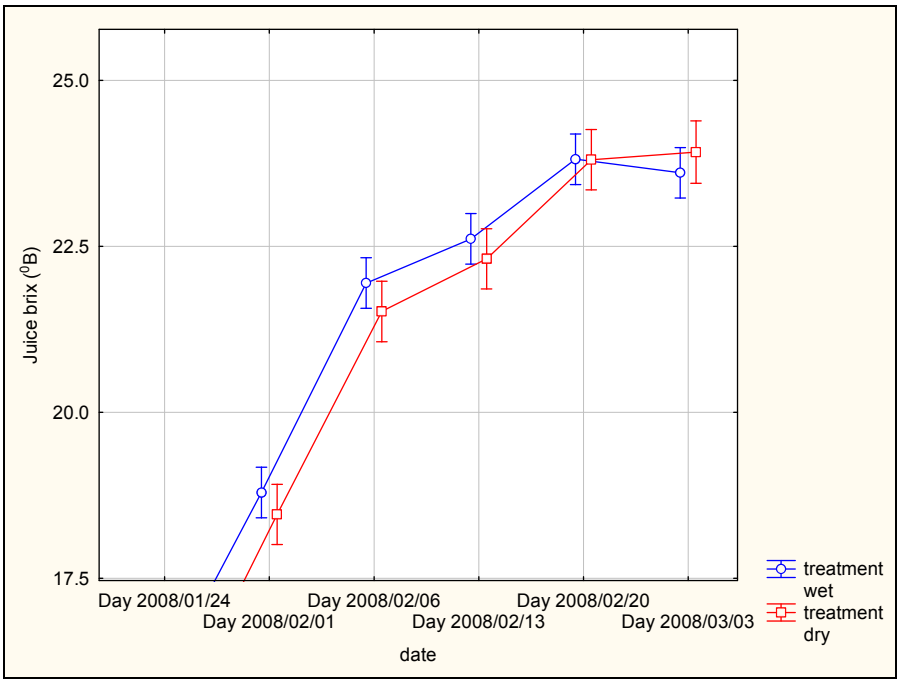

Figure 4.56 ANOVA computed for covariates at their means of the seasonal evolution of berry juice total soluble solids for the wet and dry treatments (2008) (vertical bars denote 0.95 confidence intervals).

4.3.6.3 MEASUREMENT OF BERRY COMPONENTS AT HARVEST

No significant differences in titratable acidity were found between the wet and dry treatments in both seasons ($P > 0.1$). The same was found for the content of titratable acid when the effect of berry size was accounted for. This corresponds to the literature, which states that irrigation sometimes does not greatly affect TA (Hardie and Considine, 1976; Reynolds and Naylor, 1994; Ginestar *et al.*, 1998). Vigour also had no effect on berry TA content, as the effect of the covariate on TA was non-significant ($P > 0.1$) in both seasons and no correlations were found between titratable acidity and pruning mass.

The effect of water status on total berry acids was also not significant, but there was a slight trend for the total acid of the wet treatments to be higher than that of dry treatments in the 2008 season (P > 0.1) (figure 4.57). The effect of the treatments on total acid content when berry mass was accounted for, was also not significant in 2007, but in 2008 there was a weakly significant effect (P < 0.1). The increased water availability could have caused lower temperatures in the vines and less degradation of malic acids. The effect of the covariate on total acids was not significant in both seasons (P > 0.1) and no correlations were found between pruning mass and total acids.

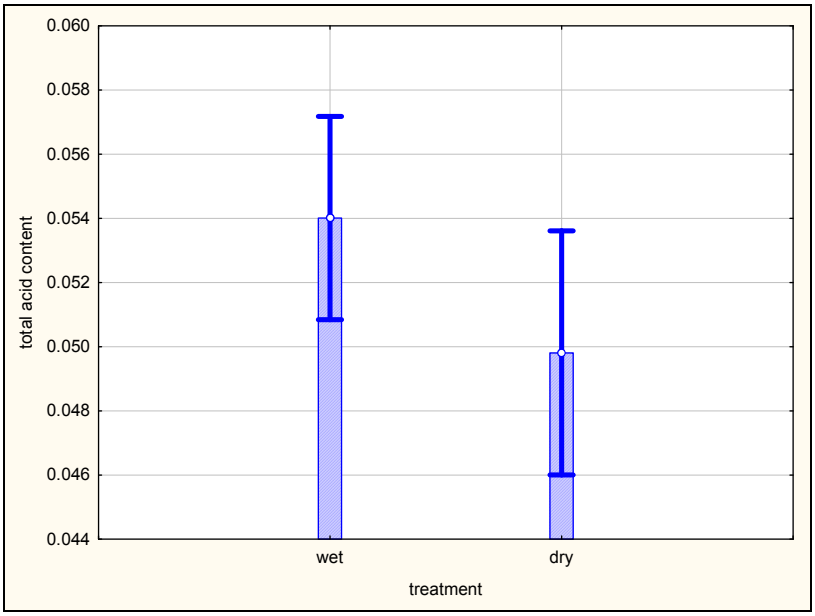

Figure 4.57 ANOVA computed for covariates at their means of the berry juice total acid content for the wet and dry treatments (2008), with berry mass taken out of the equation (P < 0.1) (vertical bars denote 0.95 confidence intervals).

Variation in grapevine water status or grapevine vigour did not have an effect on berry tartaric acid content. Vines from the wet treatment showed a slight trend to have a higher malic acid content that vines from the dry treatment, although it was not significant during the 2007 (P > 0.1) and 2008 (P > 0.1) seasons. Differences in grapevine canopy microclimate, vegetative growth and yield between the treatments might have caused the slightly higher malic acid concentrations for the wet treatment, as malic acid degradation slows down under wetter conditions (Esteban *et al*., 1999).

The effect of the covariate on malic and tartaric acids was not significant (P > 0.1). However, there were weak correlations between pruning mass and malic acid concentration, although no significant effects were found (2007, r^2=0.0872; P > 0.1 and 2008, r^2=0.0678; P > 0.1). This could be ascribed to the fact that vegetative growth can influence malic acid concentrations, and the vines with higher pruning masses had higher malic acid concentrations. Malic acid degradation should therefore have been slower for vines with higher vigour, although the effect was very small, with no significant differences. The effect of the covariate on berry acids was also non-significant (P > 0.1).

Anthocyanin concentration and colour intensity differences between the wet and dry treatments showed a trend for higher anthocyanin content and colour intensity in the berries of

the vines from the dry treatment in both seasons, although in both seasons it was not significant for both of these measurements (P > 0.1). This trend should follow the differences in berry sizes between the treatments, and the bigger berries of the wet-treatment vines should have led to a bigger dilution of anthocyanin in the dermal cell content, while the dry-treatment vines might have had more open canopies with more light penetration, stimulating colour synthesis. In both seasons no effect of vigour on berry anthocyanin content and colour intensity was found when berry size was taken out of the equation, as the effect of the covariant was not significant (P > 0.1) and no correlation existed with grapevine pruning mass.

Differences between grapevine yield of the wet- and dry-treated vines showed no significant differences in 2008 (P > 0.1), while weakly significant differences could be seen in 2007 (P < 0.1) (figure 4.58), when the vines from the wet treatment had higher yields than those from the dry treatment during harvest time. In the 2008 season there also was a non-significant tendency showing this effect. The effect was perhaps more significant in the 2007 season, as there were larger differences in soil water content between the treatments. The higher yields of the wet treatment follow a reduction in stomatal conductance in the leaves of the dry-treatment vines when compared to the wet-treatment vines. The increased berry mass of the wet-treatment vines will ultimately lead to higher yields.

The effect of the covariate was not significant in both seasons (P > 0.1). Although there were weak correlations between pruning mass and yield in both seasons (2007, r^2=0.2292; P < 0.05 and 2008, r^2=0.2068; P < 0.05), higher-vigour areas led to slightly bigger yields, as expected.

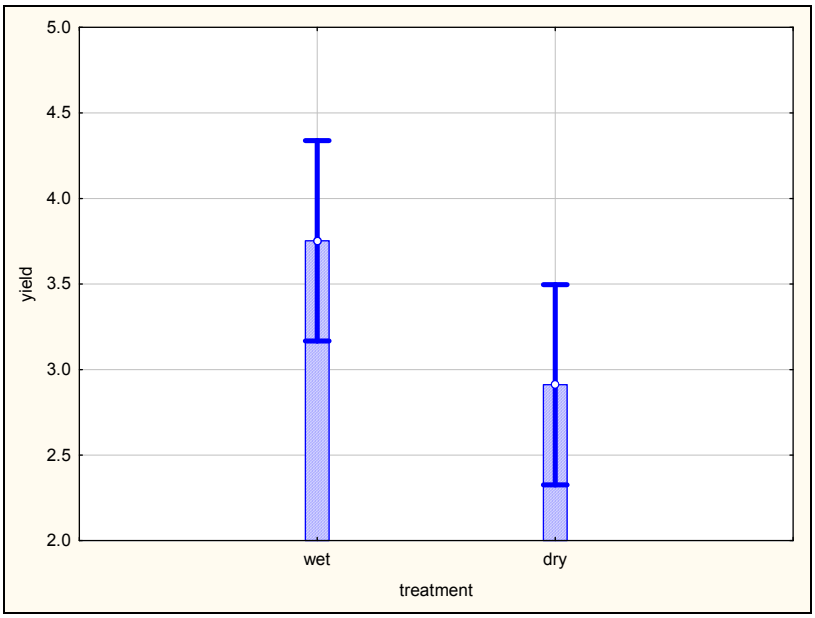

Figure 4.58 ANOVA computed for covariates at their means of the grapevine yield for the wet and dry treatments (2007) (P < 0.1) (vertical bars denote 0.95 confidence intervals).

4.4 DIURNAL MEASUREMENTS

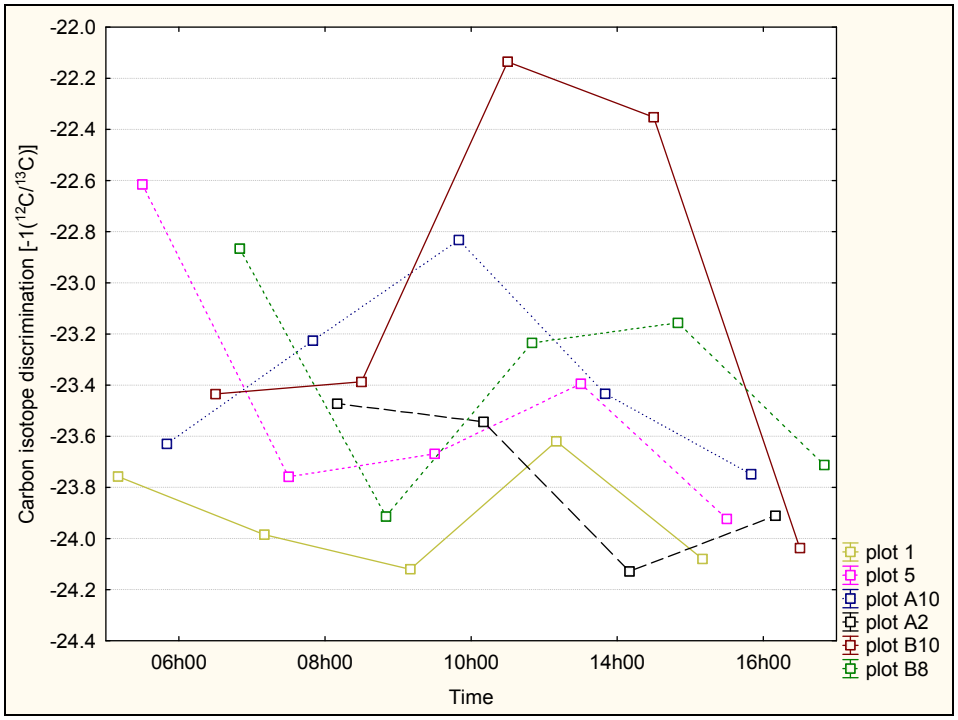

Figure 4.59 Diurnal CID measurements for the plots used for measurement on 20/11/2007.

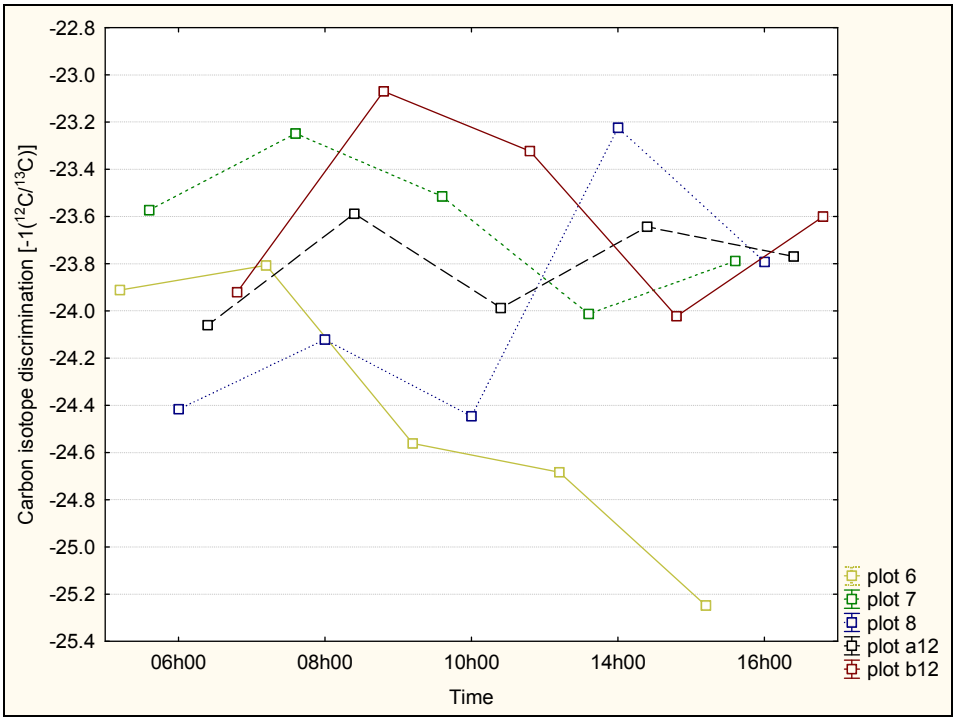

Figure 4.60 Diurnal CID measurements for the plots used for measurement on 06/12/2007.

It is important to note that the CID measurements during these two days of diurnal measurements were done on photosynthetic products in the petiole sap. These values should therefore differ from the values measured from berry juice samples, as $\delta^{13}C$ differs between plant parts (Brugnoli and Farquhar, 2000). Stress indexes therefore cannot be the same as those of juice-derived CID measurements and should indicate weak to absent water deficits

when looking at the plant water potential measurements (SWP and LWP) taken during these cycles, which indicated weak/absent water deficits (data not shown). The accuracy and significance of the CID measurement from xylem sap must be questioned as little sucrose is present.

At this stage of the season, petiolar sap should contain high levels of organic acids and some sugars, and organic acid transportation through the petioles should have an important impact on CID values. Organic acids and sugars have different biosynthetic pathways, leading to differences in CID between these compounds (Gaudillere, personal communication). It seems that when CID is measured from predominantly organic acids (like during these two days), the values are less negative than when it is measured from sugars.

It is likely that berry development could have had a big impact on CID measurements and this might explain why, for most of the plots, the CID values taken on 20/11/2007 (figure 4.59) indicated higher stress values than those taken on 6/12/2007 (figure 4.60). Berry development may have been more advanced by the second date, meaning that the sugars could have made a bigger contribution to CID measurements. This might be why the CID measurements at this stage of the season did not correlate with water potential and stomatal conductance (gs) measurements (data not shown), and berry development might have made more of an impact on CID than grapevine water status. The slightly higher daily temperature on 20/11/2007 (data not shown) could have contributed to the CID values indicating slightly higher stress levels than on 6/12/2007, but there were no real effects on grapevine water potential and stomatal conductance from this (data not shown).

Some of the plots, however, did show correspondences between gs and CID, for example, on 20/11/2007 plot B10 had low gs values and CID measurements, showing the highest stress for most of the day, while plots A2 and 1 had relatively high gs values on 6/12/2007, whereas the values for CID measurement on this day showed some of the lowest stress levels. However, it is necessary to place a question mark on how influential water status can be on CID measurements at such an early stage of the season because of the impact of berry development.

4.5 STELLENBOSCH TRIAL CONCLUSION

After the reclassification of plots into treatments in order to get a picture of the true nature of the plant water status of the different plots, these “new” treatment classes exhibited clear differences in soil water content.

Differences in soil water content led to differences in soil water potential and the gradient between root water potential and soil water potential. Vines from the wet treatment therefore had increased water uptake by the roots when compared to the vines from the dry treatment, as more water was available. This was also reflected in the measurements of SWP, LWP and pre-dawn LWP, which showed less negative values than for the dry-treatment vines.

Soil water content also influenced vigour, as grapevine vigour can be decreased by limitation of soil water. This leads to a smaller grapevine framework, which can in turn conserve water.

The differences in grapevine water potential led to physiological differences between the treatments (Smart *et al*., 1983). This may have been caused by differences in grapevine chemical signalling, as increased root drying in the vines of the dry treatment could have led to increased ABA synthesis and transport through the xylem to the leaves, and decreased cytokinin synthesis and transport (Davies *et al*., 1994). These factors influenced stomatal conductance, leading to higher gs for the wet-treatment vines and lower gs for the dry-treatment vines. This can be seen as a direct effect of grapevine water status on plant physiology.

Apart from these direct effects of water status on physiology, it is known that vigour variation might have indirect effects. In this study, there were vigour differences between different parts of the vineyard, as defined by differences in pruning mass between the plots. Higher pruning mass may lead to increased canopy density, as there is increased shoot and leaf growth. Canopy shading and microclimate therefore could have been affected, which means that stomatal control differed between the plots, as gs is influenced by microclimate conditions and sunlight interception. Vigour thereby influenced plant physiology in an indirect way by the affect it had on gs due to canopy microclimate.

Diurnal measurements also show that different climatic conditions on different days can influence gs and that changes in plant water status can affect gs in a direct way throughout the day.

Differences in stomatal aperture led to differences in transpiration, photosynthesis and leaf gas exchange. The results of this study show that water status influences stomatal control more than vigour, probably because of the more direct effect of water status, and that it leads to a faster response in stomatal control. It is also important to remember that water status influences vigour, and this contributes to the effects of vigour on physiology. Vigour differences can also influence plant water status during the season, and this could be seen more easily by looking at PDWP, while vigour only showed a slight effect on SWP and LWP. The reason why PDWP reacted more to vigour variability could be because of the rehydration of vines during the night, with the higher-vigour vines possibly struggling more to rehydrate than the lower-vigour vines. The reason for this is that, because of their increased leaf area, the higher-vigour vines would have lost more water through transpiration during the day. This effect was also more visible in the 2007 season, probably because of the greater variation in vigour in that season than in the 2008 season.

The CID measurements were also influenced more by water availability than by vigour variability, but the small effect of vigour variation on gs caused variations in carbon discrimination because it influenced leaf gas exchange. Higher-vigour vines had increased gas exchange, causing more intercellular CO_2 and therefore increased discrimination against ^{13}C, which led to reduced absorption of the isotope by the plant material. Water status also influenced CO_2 gas exchange and discrimination by Rubisco against ^{13}C. In this trial, CID measurements were done on raw juice and therefore influenced by berry development throughout the season, as juice organic compounds changed from mostly organic acids before véraison to mostly sugars from after véraison to ripening. Apart from this, it seems that CID was influenced greatly by grapevine water status, again showing how this measurement can indicate the effect of irrigation on physiology and water-use efficiency though the season. Extremely

stressed conditions did not show significant effects on plant water potential, but SWP measurements indicated slightly higher stress for the extremely stressed vines and LWP showed slightly less stressed conditions for these vines (data not shown). Measurements of gs showed slightly lower values for the extremely stressed vines, while measurements of CID showed large significant differences, with the extremely stressed vines having measurements showing high stress (data not shown). The measurement therefore indicated highly stressed conditions accurately, while other physiological measurements, such as photosynthetic rate, evapotranspiration and leaf temperatures, only showed trends and no significant differences (data not shown). Diurnal cycle measurements of CID showed that it changed during the day and corresponded somewhat to changes in gs, although CID is integrative and therefore shows discrimination taking place over time, thus reflecting gs measurements over time. CID measurements showed accumulative seasonal results and reflected long term effects of water status on plant physiology, while water potential measurements like SWP reflected short term water status' effects on the grapevine.

Other physiological measurements also reflected the influences of vigour and water status on gs, with large influences of water status on photosynthesis, evapotranspiration and leaf temperatures, while vigour only slightly influenced evapotranspiration and leaf temperature, and did not show effects on photosynthesis.

The physiological changes induced by differences in water status and vigour in different parts of the vineyard also led to differences in berry development. Because of the larger influence of water status on physiology, there were larger effects on berry development than on vigour. The vines from the wet treatment had bigger berries than those from the dry treatment due to more water being available for cell expansion, while the higher-vigour vines had slightly larger berries than the lower-vigour vines. Berry size influenced the dilution of berry compounds, and water status therefore had a larger influence on berry compounds than vigour. Berry sugar content, acid concentration and colour were largely influenced by water status and only slightly by vigour.

Within-vineyard variability in vigour and water status therefore caused changes in physiological grapevine performance, and this led to differences in juice quality. It seems that the measurement of CID integrated this effectively.

4.6 REFERENCES

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Chapter 5

General discussion and conclusions

5.1 GENERAL DISCUSSION AND CONCLUSIONS

In this study, measurements of plant water potential were important to differentiate between the treatments in order to indicate different plant water statuses. It was found that dryer treatments had less negative plant water potentials, which indicated more water deficit stress than in the wetter treatments. Stem water potential was a better indicator of grapevine water status than leaf water potential, as it showed better discrimination between treatments.

These differences in plant water potential led to differences in plant physiology, as they influenced stomatal conductance (gs). This effect on physiology seems to be a direct effect, as water potential should influence guard cell turgidity as a result of chemical signals from the roots. Wetter treatments had increased gs and dryer treatments had lower gs.

Differences in gs will lead to differences in leaf gas exchange, and this will influence CO_2 movement over the stomata and the ratio of leaf internal and environmental CO_2. The increased gas exchange for the vines with higher available water led to more CO_2 being available for discrimination and therefore increased discrimination against ^{13}C, as well as more negative carbon isotope discrimination (CID) measurements, indicating less stress.

CID corresponded with seasonal plant water potential and therefore grapevine water status, but berry development also had an influence on this measurement. It also seems that vigour affects CID due to microclimate effects, influencing gs and therefore gas exchange and CO_2 movement over the stomata. In this study it seemed that higher vigour led to increased discrimination against ^{13}C. Water status influenced CID more than did vigour.

Seasonal CID corresponded with grapevine water potential in that both indicated low water stress around the period of véraison, and an increase just afterwards. CID measurements were also accumulative towards water status through the seasons and showed good correspondences with gs.

Berry development through the season causes berry composition to change from having organic acids as the main organic material early in the season before véraison, to sugars becoming the main organic material in berry juice from véraison onwards. These molecules have different biosynthesises and CID, and berry development therefore influenced CID measurements. CID measurements early in the season therefore showed values that perhaps were not so representative of grapevine water potential due to berry composition, as it seems that organic acids lead to CID measurements indicating higher stress than what really exists in the grapevine. Measurements of CID after véraison might therefore be more representative of grapevine water potential, but measurements at harvest should be the most accurate, as they are cumulative of the whole season and should not be influenced by berry development.

Carbon isotope discrimination furthermore seemed to differentiate well between the treatments and even seemed to be a good indicator of extremely stressed conditions, even when water potential measurements did not correspond to show how extreme these stress conditions were.

It would be advisable to remove berry juice acids in future studies when CID is measured in raw grape juice, as this might eliminate the effect of seasonal berry development on the measurement.

Berry composition was also influenced by differences in grapevine physiology induced by differences in plant water potential. Wetter treatments had bigger berries, influencing the dilution and concentration of berry quality parameters and therefore influencing fruit composition.

This study showed that CID measurements may contribute to the difficult task of managing irrigation in an environment with differing grapevine water potential and vigour. Furthermore, they add a “tool” to the available toolbox for analysing plant responses that is superior to only monitoring the soil and environment in the effort to improve fruit composition and quality, with the ultimate goal of producing wine of an excellent quality. There are some negative aspects of the measurement however; including the difficulty of sampling sugars that recently assembled and the removal of organic acids out of the samples. The cost is also high, while there is normally a delay when waiting for CID results. These aspects might contribute to the difficulty of incorporating CID measurements into grape production.