IMPACT OF CHANGING CLIMATIC FACTORS ON PHYSIOLOGICAL AND VEGETATIVE GROWTH PARAMETERS OF YOUNG GRAFTED GRAPEVINES

by

Hanlé Theron

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Supervisor: Dr. Jacobus Johannes Hunter

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Summary

The establishment of a new vineyard is expensive and a high survival rate of the young vines is important to prevent re-planting and ensure that the vines come into full production as early as possible. The initial growth of the young vines is very sensitive to the environment and this has a direct effect on the performance and longevity of the vineyard. It is expected that future climatic scenarios will put additional strain on young vine growth. In this study the physiological functioning and growth of the vine during the first few months after planting were measured in simulated future conditions.

Newly potted vines were investigated during their first 12 weeks of growth in a glasshouse. The same rootstock (101-14 Mgt) was used throughout with Shiraz (SH 470) and Merlot noir (MO 348) as scion cultivars. The treatment factors comprised three climatic variables with two levels each: temperature, CO_2 and water. Measurements were taken at 4, 8 and 12 weeks after planting. The physiological activity, vegetative growth response, mineral uptake and translocation as well as the synthesis and allocation of metabolites to the various vine parts were investigated.

High CO_2 levels increased the photosynthetic activity of the young vines and improved the efficiency of water and nitrogen use, provided that water stress did not increase to severe levels. The negative effect of water deficit on physiological activity was to a certain extent mitigated by elevated CO_2. Inherent phenology-linked patterns in the grapevine pertaining to shoot and root growth, nutrient uptake, metabolite synthesis, translocation and accumulation, and reserve storage were similar in the various treatments. Merlot performance and growth seemed more sensitive to water deficit than Shiraz, but Merlot was more stimulated by elevated CO_2 levels. The effects of the treatment factors on macro- and micro-nutrient levels in vine tissues depended on the particular nutrient, the tissue type concerned, as well as the scion/rootstock genotype. Stronger vegetative growth was associated with lower nutrient concentrations in the tissues, but a similar (or higher) content.

The results showed that the choice of the scion-rootstock combination per terroir would become increasingly important. Soil preparation depth should be maximised to enhance depth penetration of roots and improve the buffer capacity of the vines against unfavourable conditions. Irrigation strategies for young vines should be aimed at increased root growth and distribution. Any cultivation should be done with circumspection in young vineyards and restrictive growth environments to avoid competition for water and nutrients. Higher CO_2 levels increased (and sustained) physiological activity and metabolism and induced stronger vegetative growth. These positive effects were further enhanced by water supply. It is suggested that irrigation and fertilisation programmes be re-evaluated, especially for young vines in the context of a changing climate where water would become less available and vegetative growth would increase as result of a higher atmospheric CO_2.

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Opsomming

Insetkoste vir die vestiging van 'n nuwe wingerd is hoog en dus is dit belangrik om hervestiging te beperk en die wingerd so gou moontlik in volproduksie te kry. Die aanvanklike groei van 'n nuut aangeplante wingerd het 'n direkte effek op langtermyn wingerdprestasie en lewensduur van die blok. Jong stokkies is uiters sensitief vir omgewingstoestande en daar word verwag dat toekomstige klimaatsomstandighede vegetatiewe groei in die eerste groeiseisoen sal beïnvloed. Hierdie studie het gefokus op fisiologiese aktiwiteit en mate van vegetatiewe groei van stokkies gedurende die eerste maande ná plant wanneer hulle aan verwagte veranderde klimaatsomstandighede blootgestel mag word. Shiraz (SH 470) en Merlot (MO 348) stokkies (met 101-14 Mgt as onderstok) is in potte geplant en vir 12 weke in 'n glashuis gemonitor. Behandelingsfaktore was drie klimaatsveranderlikes, nl. temperatuur, CO_2 en water, wat op twee vlakke elk toegepas is. Metings is 4, 8 en 12 weke na plant gedoen en daar is op fisiologiese aktiwiteit van stokkies, vegetatiewe groei, opname en vervoer van minerale, asook vervaardiging en interne verspreiding van metaboliete gefokus.

Fotosintese en die doeltreffendheid van water- en stikstofverbruik het verbeter waar die stokkies aan hoër CO_2 vlakke blootgestel is, mits watertekorte nie té straf was nie. Watertekort se nadelige effek op fisiologiese aktiwiteit is tot 'n mate deur verhoogde CO_2 vlakke teengewerk. Inherente patrone vir loot- en wortelgroei; mineraalopname; vervaardiging, vervoer en opbouing van metaboliete; asook reserwe opbouing in wingerdstokkies is nie deur die onderskeie behandelings beïnvloed nie. Merlot stokkies was meer sensitief vir watertekort as Shiraz stokkies, maar het sterker positief op 'n verhoging in CO_2 vlakke gereageer. Die effek van die behandelings op makro- en mikro-voedingstofvlakke in die onderskeie plantorgane het afgehang van die spesifieke mineraal, orgaan van toepassing en genotipe van die bo-/onderstok. Sterker vegetatiewe groei het gepaard gegaan met laer konsentrasies van minerale in die onderskeie weefsels, maar met vergelykbare (of hoër) totale minerale inhoud.

Die resultate dui daarop dat die keuse van bo-/onderstok kombinasie vir 'n spesifieke terroir in belangrikheid gaan toeneem. Gronde behoort tot maksimum diepte voorberei te word om dieptepenetrasie van wortels te verseker en die bufferkapasiteit van die wingerd teen ongunstige toestande te verhoog. Besproeiïngspraktyke moet daarop gemik wees om wortelgroei- en verspreiding by jong stokkies te bevorder. Alle verbouingspraktyke in jong wingerde (en wingerde wat blootgestel word aan beperkende groeitoestande) moet met omsigtigheid toegepas word sodat kompetisie vir water en voedingstowwe beperk word. Verhoogde CO_2 vlakke het fisiologiese aktiwiteit en metabolisme van jong stokkies gestimuleer en onderhou, en het ook sterkter vegetatiewe groei tot gevolg gehad. Hierdie positiewe uitwerking op die stokkies is verder deur voldoende watervoorsiening bevorder.

Dit word voorgestel dat besproeiïngs- en bemestingsriglyne herevalueer word, veral met betrekking tot jong stokkies in die konteks van klimaatsverandering, waar die beskikbaarheid van water na verwagting sal verminder en vegetatiewe groei deur die hoër atmosferiese CO_2 konsentrasie gestimuleer mag word.

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Biographical sketch

Hanlé Theron (née Cloete) was born on 19 September 1979 in Oudtshoorn and grew up in Paarl. She matriculated from Paarl Gymnasium High in 1997 and obtained her BScAgric degree in Viticulture & Oenology (*cum laude*) in 2001 from Stellenbosch University. She completed her MScAgric degree (*cum laude*) in 2004 at the same university with the title "The effect of shoot heterogeneity on the physiology and grape composition of Shiraz/Richter 99 grapevines". The results of this study were presented at an international conference on viticultural zoning in Cape Town held in 2004 and three peer-reviewed scientific articles were published in the South African Journal of Enology and Viticulture.

She started her teaching career in 2004 as part-time lecturer at the (then) Cape Technikon and was permanently appointed in 2006 at Cape Peninsula University of Technology, where she is still employed.

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Preface

This dissertation is presented as a compilation of seven chapters. Each chapter is introduced separately and is written according to the style of the South African Journal of Enology and Viticulture.

Chapter 1	**INTRODUCTION AND PROJECT AIMS**
Chapter 2	**LITERATURE REVIEW**
Chapter 3	**INTEGRATIVE EFFECTS OF CLIMATE CHANGE FACTORS ON SOME PHYSIOLOGICAL PROCESSES IN YOUNG, GRAFTED GRAPEVINES**
Chapter 4	**INTEGRATIVE EFFECTS OF CLIMATE CHANGE FACTORS ON VEGETATIVE GROWTH OF YOUNG, GRAFTED GRAPEVINES**
Chapter 5	**INTEGRATIVE EFFECTS OF CLIMATE CHANGE FACTORS ON THE NUTRIENT CONTENT IN YOUNG, GRAFTED GRAPEVINES**
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Chapter 7	**GENERAL DISCUSSION AND CONCLUSIONS**

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CHAPTER 1: INTRODUCTION AND PROJECT AIMS

1.1 INTRODUCTION

"Warming of the climate system is unequivocal,…" (IPCC, 2007). The climate directly affects physiological processes as well as parameters important in sustainable viticulture and oenology (Hunter et al. 2010; Hunter & Bonnardot, 2011; Hunter et al. 2011). A change in climate may necessitate changes in vineyard management strategies (Jones, 2008). However, scientific information on grapevine response to predicted levels of climate parameters is scarce and not sufficient to properly position the Wine Industry for the future.

Although the South African wine industry was not believed to be at great risk as a result of climate change (Jones et al. 2005; Carter, 2006), some recent models indicate that the total area suitable for wine grape production will probably decrease in future (Hannah et al. 2013). Wine grape production will be affected by the increased atmospheric CO_2, rising minimum and maximum ambient temperatures and especially the decreased rainfall that is expected. Water availability is considered to be the most limiting factor for agricultural production in South Africa (Benhin, 2006). Most of the viticulture regions are already experiencing water shortages and rainfall is likely to become even less reliable as a water source. The amount of precipitation in especially these regions is not sufficient to meet the water demands of the grapevine (Hunter & Myburgh, 2001).

It is very difficult to define clear relationships between climate conditions and grapevine performance (Schultz, 2011), due to the large natural adaptive physiological capacity (plasticity) of the grapevine (Jones & Alves, 2013; Seguin & Garcia de Cortazar, 2015). Interaction among various climate variables is highly likely (Bindi et al. 2001). Research on the combined effects of increased CO_2 and temperature combined with decreased water availability on the plant is therefore critical (Hunter et al. 2010) to expand knowledge of the mechanisms that may regulate physiological adaptation of the grapevine to the changing environment. Better understanding of how plants would react morphologically and physiologically (at leaf, root and whole-plant level) to climatic stress factors would benefit decision-making regarding adaptation and mitigation measures to ensure sustainable/profitable production of good quality grapes under future climate conditions.

1.2 PURPOSE STATEMENT AND PROJECT AIMS

The purpose of this study is to quantify the effects of predicted changes in climatic parameters on the physiological functioning and vegetative growth of young grafted grapevines under controlled conditions, simulating projected future climate conditions. This study will enable the wine industry to better position itself in preparation for future cultivation conditions by providing much-needed

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information required for terroir selection, scion and rootstock selection, and making adaptations to current cultivation practices. It is expected that the results of the study will benefit decision-making regarding adaptation and mitigation measures to ensure sustainable and profitable production of good quality grapes.

By working with young grafted grapevines in glasshouses, the reaction of the grapevine during the very early growth stages will be investigated. The initial growth (and functioning) of the vine during the first year after planting is pivotal to the optimal functioning, production and longevity of the mature grapevine. This study would provide the means to better understand the reaction of the grapevine during this very climate-sensitive stage.

1.2.1 Brief concept and substantiation of the study

It was decided to use glasshouse compartments for this study in order to take advantage of the fact that climatic factors can be easier monitored and controlled, in spite of Photosynthetic Active Radiation (PAR) being lower than ambient levels. Grafted vines from commercial vine nurseries were used (to better investigate the interaction between the rootstock and scion cultivars) and planted in soil taken from a wine producing region, rather than using an artificial growth medium.

Vines were planted in 7 L pots, which is comparable with pot sizes used in similar studies, while still being small enough to be manageable. Control temperature levels were long-term minimum and maximum monthly averages of a warm wine region in South Africa, with the treatment being 3 ^{o}C warmer, which is in line with IPCC projections. To improve the accuracy of the simulated climatic conditions, both minimum and maximum temperatures were increased every four weeks with 2 ^{o}C to match the natural temperature increases during the first three months of the growth season. An ambient CO_2 level of 400 ppm was used, while the elevated CO_2 level was set at 800 ppm. Water deficit plants received 50 % of each irrigation volume supplied to the well-watered vines, the latter being irrigated twice per week to water holding capacity. Treatments were applied from the day of planting, so that any new growth or acclimation would occur within the simulated environments.

To improve the validity of the results, the population size, sample size, and the number of repetitions were larger than those of most studies in literature. The study comprised of five growth periods of 12 weeks each. The same rootstock (101-14 Mgt) was used throughout with Shiraz (SH 470) as scion cultivar for the first three plantings and Merlot noir (MO 348) for the remaining two. Shiraz was chosen based on its proven record in warm wine producing areas with water scarcity, while it was expected of Merlot to provide better insights into more stress-sensitive scion cultivars. The rootstock 101-14 Mgt was selected due to its perceived sensitivity to water stress conditions. The effects of different combinations of ambient temperatures [maximum ranges 27-31 ^{o}C (T0) compared to 30-34 ^{o}C (T1)], CO_2 [400 ppm (C0)

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vs 800 ppm (C1)], soil water [irrigation to water holding capacity (wet) and 50 % thereof (dry)], phenological stage (4, 8 and 12 weeks after planting) and scion cultivar (Shiraz and Merlot) on the physiological activity (Chapter 3), vegetative growth response (Chapter 4), mineral uptake and translocation (Chapter 5) as well as the synthesis and allocation of metabolites to the various vine parts (Chapter 6) were investigated.

The study was laid out as a complete randomised block design, with 108 vines per each of the four CO_2/temperature combinations. Within each combination, water supply treatments were allocated in pairs, resulting in a sample population of 54 vines per CO_2/temperature/water treatment combination. Measurements and analyses (three replicates comprising of 6 vines each) were done at 4, 8 and 12 weeks after planting.

Since the establishment of a new vineyard is an expensive endeavour, a high survival rate of the young vines is very important for the producer to prevent re-planting and ensure that the vines come into full production as early as possible. It is expected that future climate scenarios will put additional strain on the first year of vine growth. It was decided to study the physiological functioning and growth of the vine during its first few months after planting, since initial growth of the young vines is very sensitive to the environment and has a direct effect on the performance and longevity of the vine during maturity.

The results of the study should contribute to the pool of knowledge required to:

- Contribute to criteria for terroir selection under envisioned climatic stress conditions
- Facilitate cultivar selection under envisioned climatic stress conditions
- Recommend vineyard management practices to accommodate predicted changes in environmental growth conditions to restrict possible detrimental effects due to climatic stress conditions.

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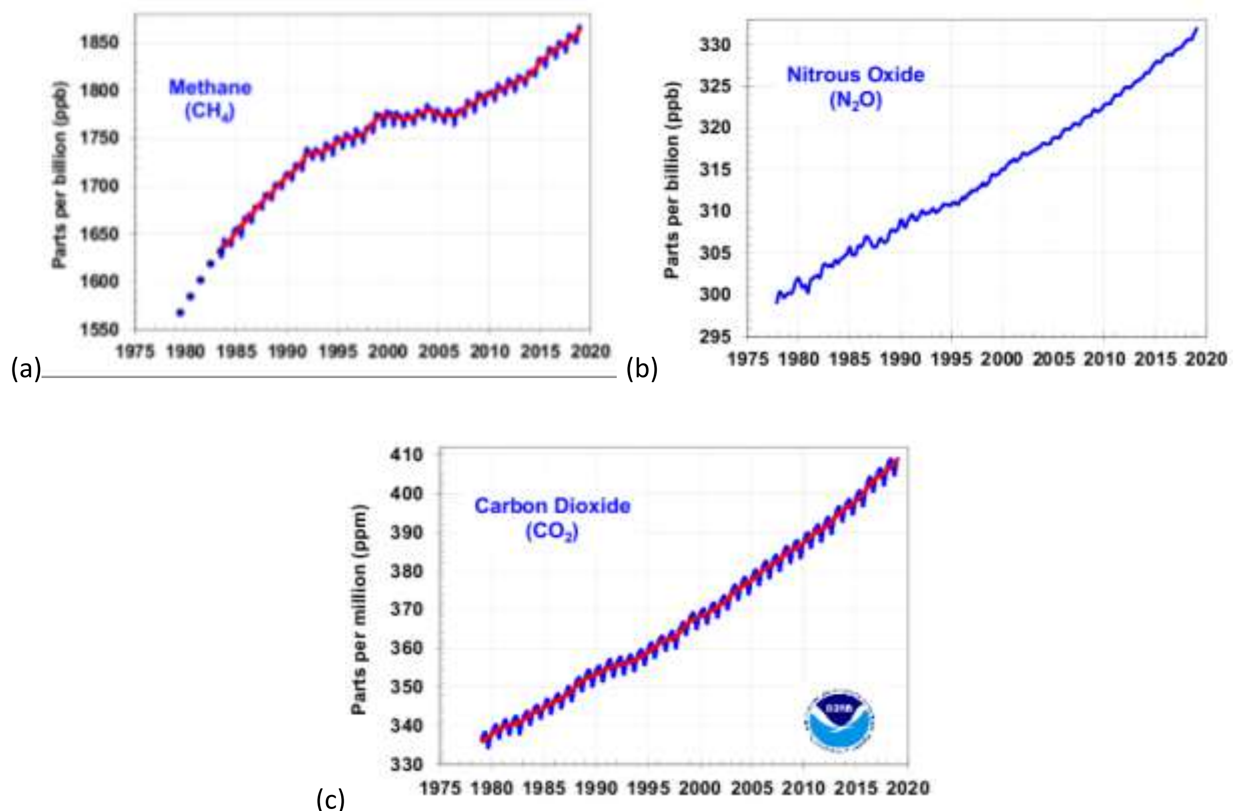
CHAPTER 2: LITERATURE REVIEW

2.1 GLOBAL CLIMATE CHANGE PREDICTIONS AND STRATEGIES

The current concept of climate change does not refer to the naturally occurring warming and cooling cycles over extremely long periods of time, but rather to "a change of climate that is attributed directly or indirectly to human activity that alters the composition of the global atmosphere and… is in addition to natural climate variability…" (UNFCCC, 2011). "Warming of the climate system is unequivocal,…, [with] many of the observed changes…unprecedented…" (IPCC, 2014). The increase in anthropogenic greenhouse gas (GHG) concentrations [expressed as carbon dioxide (CO_2) equivalents] is considered to be the main cause of warming and results from emissions of mostly CO_2, methane (CH_4) and nitrous oxide (N_2O) into the atmosphere (Figs 2.1a-c).



Figs 2.1a-c Increase in respective atmospheric GHG concentrations over the last 45 years (Butler & Montzka, 2019).

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The rate of total GHG emissions has increased between 1970 and 2010 (especially over the final ten years), mainly because of fossil fuel combustion and industrial processes that account for 78 % of this increase (Fig. 2.2).

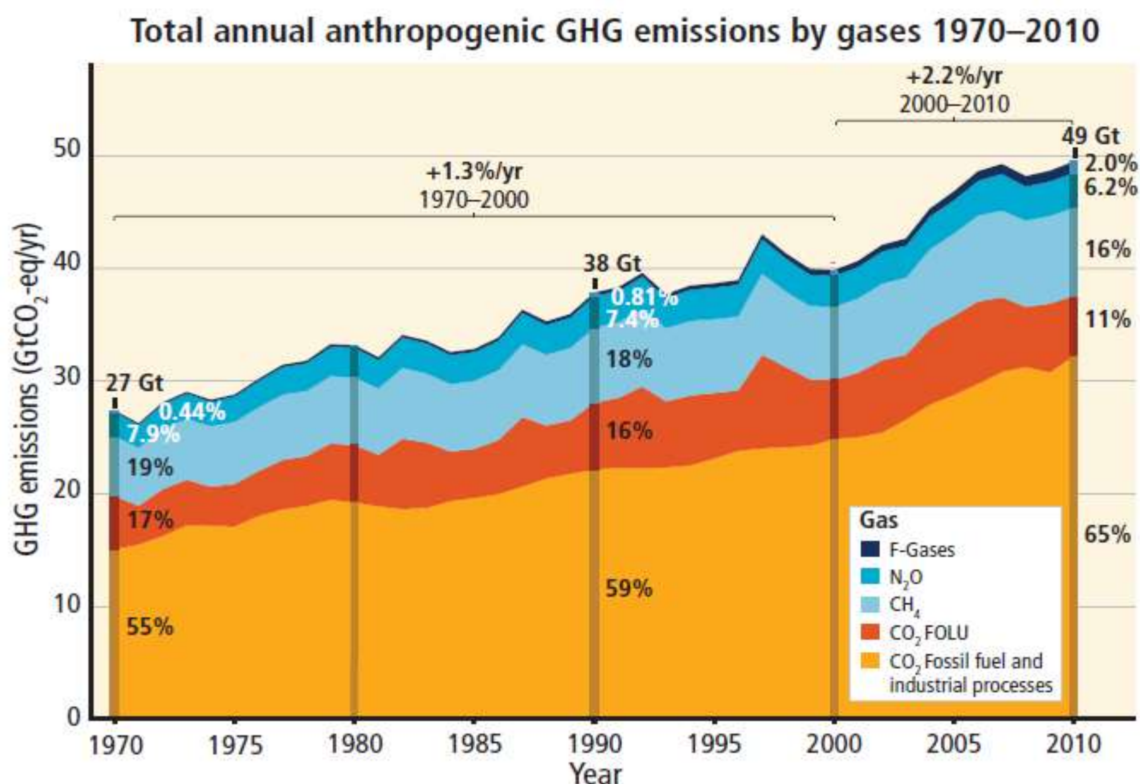

Fig. 2.2 Total annual anthropogenic greenhouse gas (GHG) emissions [gigatonne of CO_2-equivalent per year, ($GtCO_2$-eq/yr)] for the period 1970 to 2010 by gases: CO_2 from fossil fuel combustion and industrial processes; CO_2 from Forestry and Other Land Use (FOLU); methane (CH_4); nitrous oxide (N_2O) and fluorinated gases (F-Gases). (IPCC, 2014).

Higher GHG levels in the atmosphere increase the capture of radiated heat from the Earth (Mozell & Thach, 2014), resulting in warming of the air and land-ocean surface (Fig. 2.3). Changes in the climate occurred over the last few decades. The number of cold nights and days as well as the frequency of extreme cold spells decreased (IPCC, 2014). There were more warm days and nights, with more frequent heat waves, especially in Europe, Asia and Australia. Precipitation patterns changed and more frequent, localised flooding occurred due to heavy precipitation. The sea level rose, glaciers retreated and the amount of ice in the Arctic sea and surface ice in Greenland decreased. Due to the higher temperatures, more CO_2 was absorbed by the ocean, which resulted in its acidification.

The IPCC Reports make use of various Representative Concentration Pathways (RCP) to make projections of future climatic conditions based on various levels of future CO_2-eq emissions: RCP2.6 refers to a scenario where stringent mitigation practices will be enforced; RCP4.5 and 6.0 refer to intermediate emission scenarios, while RCP8.5 indicate the projections should current emissions continue to increase

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at the same rate without any further effort to limit future emissions (Fig. 2.4). Continued emission of GHG will result in further warming and the concomitant higher risk of causing irreversible damage to ecosystems and the quality of life of humans. The close relationship between the level of GHG emission and projected temperature increase is clearly shown in Fig. 2.4. Global mean surface temperature at the end of the 21st century will largely be determined by the CO_2-equivalent units (CO_2-eq) that have already been emitted in the past as well as the amount that will be emitted in future. Certain facets of the climate system (such as ocean temperatures and acidification, sea level rise, soil carbon cycles, etc.) will continue to change for centuries to come, even if GHG emissions stop immediately (IPCC, 2014).

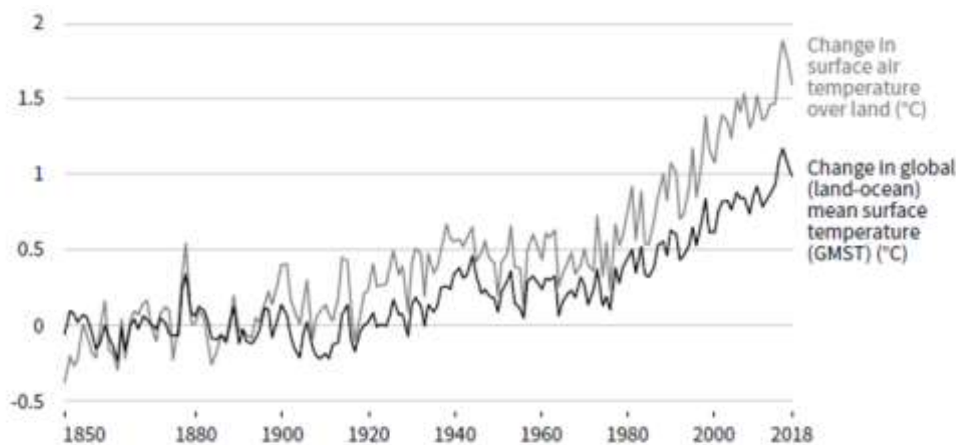

Fig. 2.3 Observed changes in air and surface temperature (both land and ocean) (in °C) relative to 1850-1900 (IPCC, 2019).

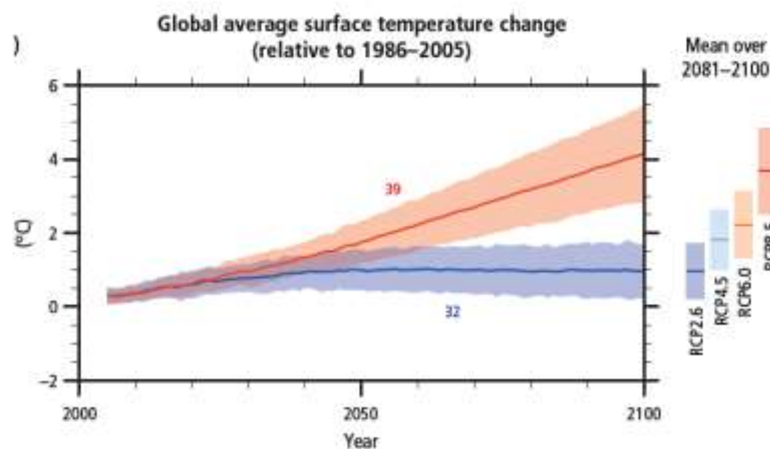

Fig. 2.4 Global average surface temperature change from 2006 to 2100 as determined by multi-model simulations. Time series of projections and a measure of uncertainty (shading) are shown for scenarios RCP2.6 (blue) and RCP8.5 (red). RCP2.6 refers to a scenario where stringent mitigation practices will be enforced; RCP8.5 indicate the projections should current emissions continue to increase at the same rate without any further effort to limit future emissions (IPCC, 2014).

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Based on the RCP's, the following changes in the world climate are projected for the period 2081-2100 (IPCC, 2014): the global mean surface temperature will increase with 0.3-4.8 °C from the 1986-2005 mean (depending on the RCP used) (Fig. 2.5a). Most land areas will experience more frequent hot and fewer cold temperature extremes, on a daily and/or seasonal basis. There will also be more and longer heat waves. Changes in precipitation patterns will be heterogeneous – more annual precipitation is expected in higher latitudes, while less is projected for mid-latitude and subtropical dry regions (Fig. 2.5b).

However, the frequency and intensity of extreme precipitations will increase in mid-latitudes, with a higher risk of regional flooding. The reduction in global glacier volume, permafrost and Arctic sea ice will continue and the sea level is expected to rise with between 0.26 m and 0.82 m (depending on the RCP used). Warming of the ocean (especially in the tropical regions and subtropical regions of the Northern Hemisphere) as well as its acidification will also continue (IPCC, 2014).

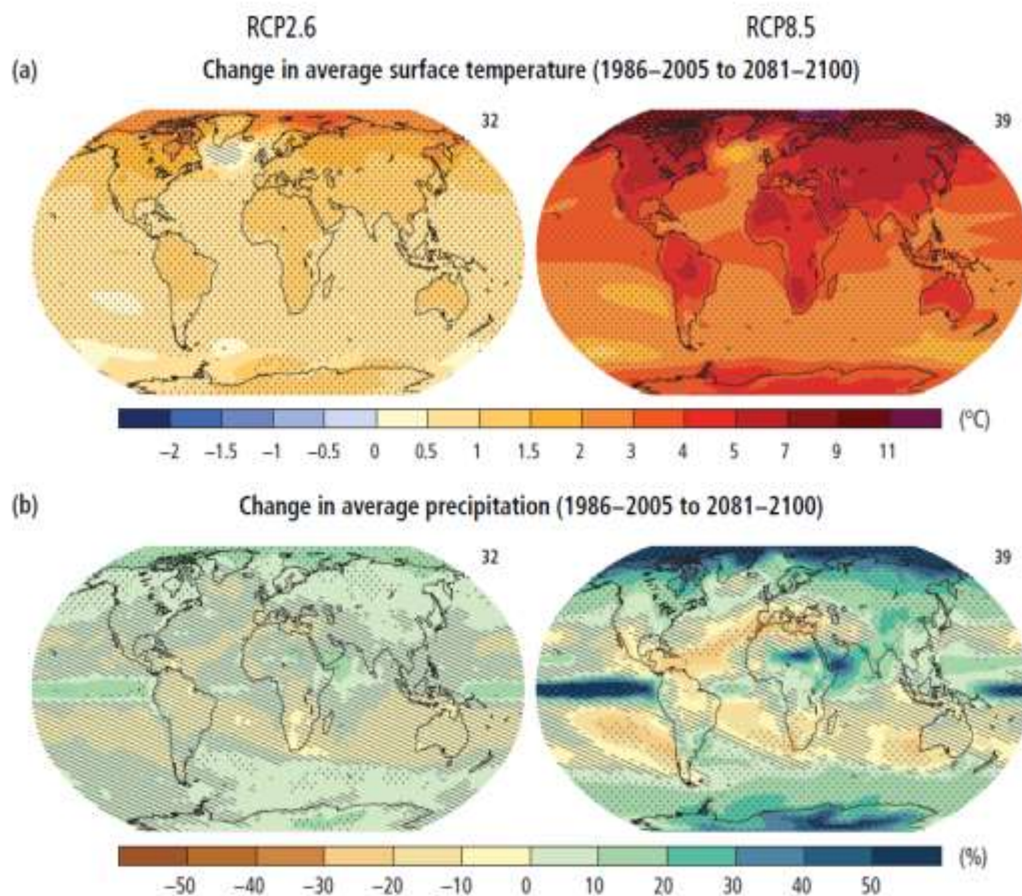

Fig. 2.5 Change in average surface temperature (a) and change in average precipitation (b) based on multi-model mean projections for 2081-2100 relative to 1986-2005 under the RCP2.6 (left) and RCP8.5 (right) scenarios. RCP2.6 refers to a scenario where stringent mitigation practices will be enforced; RCP8.5 indicate the projections should current emissions continue to increase at the same rate without any further effort to limit future emissions (IPCC, 2014).

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Changes in the climate will increase existing risks while also creating new ones for both natural and human systems. The geographical ranges and migration patterns of many terrestrial, freshwater and marine species have shifted in response to the changing climate, but most plant species, some aquatic species (such as freshwater molluscs) and small mammals are not able to shift their habitats fast enough to keep up with the projected rate at which the climate changes. There is thus an increased risk of extinction for many species, especially in coral reefs and the polar ecosystems (IPCC, 2014). Food security for humans is also expected to decrease with climate change, especially in the context of a fast-growing global population expected to reach 9.7 billion in 2050 and nearly 11 billion around 2100 (UN, 2019). Fisheries will be hard-pressed to sustain their provision of fish. Should no adaptations be made, the production of wheat, rice and maize will be negatively impacted in tropical and temperate regions (IPCC, 2014). It is also expected that the importance of crop pest and disease management will increase with climate change. New geographical areas with sufficiently high temperatures for the survival of plant pests and disease causing species will emerge and changes in their migration/distribution patterns are likely (Mira de Orduña, 2010). In most dry subtropical regions the renewable water resources (both surface and ground water) will decrease, which will intensify competition among water users (IPCC, 2014).

Poorer, developing countries are especially vulnerable should economic growth and food security decrease with climate change, while general health will likewise be negatively impacted. It is clear that risks pertaining to climate change are not evenly distributed and are generally greater for disadvantaged people and communities with limited resources to adapt to the changing environment. This is ironic, since the developing countries contribute very little to global GHG emissions.

Anthropogenic GHG emissions depend on factors such as the size of the population, the general lifestyle maintained, land and energy use patterns of the country, and its economic activity (IPCC, 2014). China, the United States, the European Union, India and the Russian Federation together account for 60 % of global GHG emissions (Fig. 2.6), but it should also be kept in mind that they contribute to 65 % of the global gross domestic product (GDP) (Olivier et al. 2017).

The risks associated with climate change should therefore be reduced as far as possible and managed in such a way that sustainable development, economic and social well-being, and effective natural resource and biodiversity conservation are ensured (IPCC, 2014). Adaptation and mitigation are the two types of action that could be taken against climate change (CCC, 2009). Adaptation is generally focused on how to remedy the current effects that the climate has on natural, biological and socio-economic environments. The impact of these practices is visible within a relatively short period of time. Mitigation is directed at the longer term and addresses the causes of climate change by attempting to reduce or

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eliminate sources of GHG emissions. These two strategies are complementary (IPCC, 2014). Adaptation can reduce the impact of climate change, but it will not be effective in the long term without supporting mitigating actions.

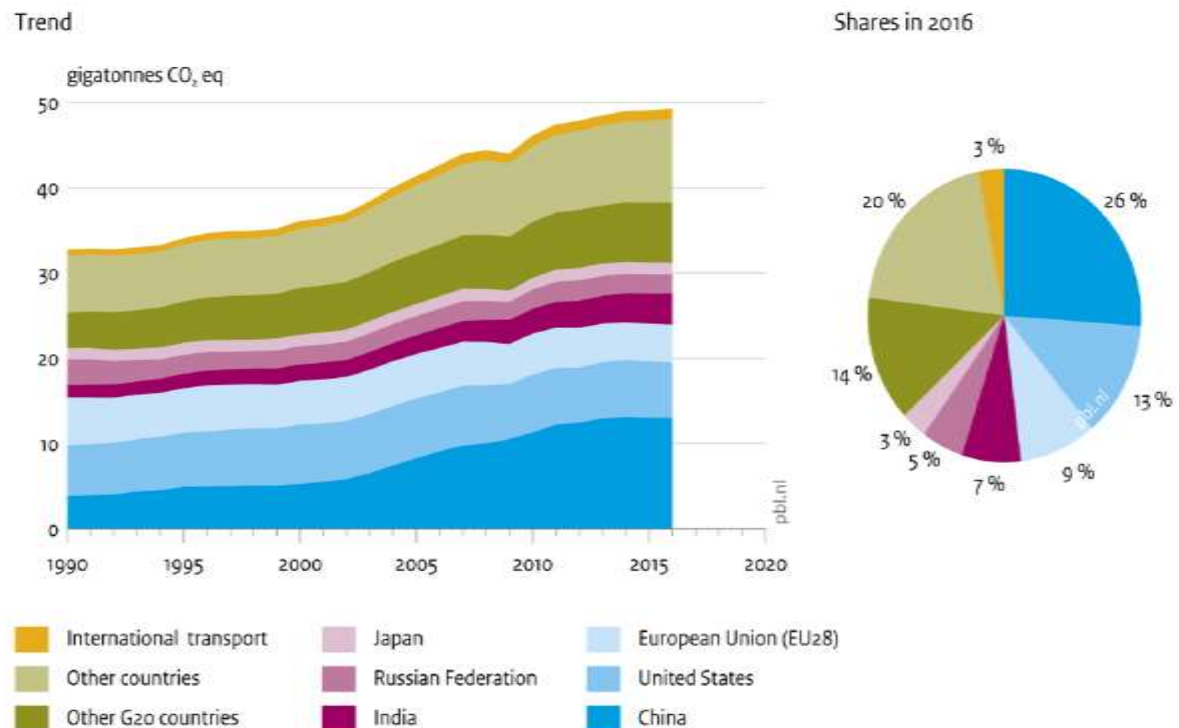

Fig. 2.6 Global greenhouse gas emissions, per country and region (Olivier et al. 2017). These values exclude emissions from land-use, land-use change and fires (forestry; forest and peat) (LULUCF); CH_4 and N_2O.

Emissions should be substantially reduced over the next few decades (mitigation) to increase the success of concurrent adaptation measures and limit the cost and difficulty of future actions that may be required. This will need a complementary strategy by individuals, industries and government (IPCC, 2014) that is based on both climate and socio-economic data (UNFCCC, 2007).

Countries vary substantially in their capacity to enforce adaptation and mitigation strategies, since a substantial (and sustainable) decrease in GHG emissions will be challenging at technological, social, economic and industrial levels. International cooperation is therefore required to effectively address GHG emission and its reduction (IPCC, 2014). The Paris Agreement was signed by 196 states and the European Union on 12 December 2015 (PCACP, 2019). It confirms a mutual undertaking to "combat climate change and to accelerate and intensify the actions and investments needed for a sustainable low carbon future" (UNFCCC, n.d.). The commitments that the various countries made are expressed as Nationally Determined Contributions (NDCs). The main aim of the Paris agreement is to limit the average

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global temperature increase to less than 2 ^oC (compared to pre-industrial levels[1]), the ideal being 1.5 ^oC. The significance of this 0.5 ^oC difference is detailed in the IPCC Special Report on Global Warming (IPCC, 2018). This report also states that, should current emission trends continue, the average increase of 1.5 ^oC will already be reached as early as 2030. The timelines in the 2014 IPCC report were also forwarded in the Special Report - in order to meet the temperature increase limitation contained in the Paris Agreement, global CO_2 emissions must now show a sharp decline by 2030 and reach net zero around 2075. Interestingly, global GHG emission was relatively constant around the time of the Paris Agreement meeting (2014-2016) (Olivier et al. 2017; UNEP, 2018), but increased again in 2017 to reach a record high of 53.5 $GtCO_2$-eq.

The United Nations Emissions Gap Report of 2018 conveyed the urgency for immediate action. According to the report, commitments expressed in the NDCs are insufficient in scale and pace to meet the target of the Paris Agreement. Even if all the current unconditional NDCs are successfully reached, the total global GHG emission is expected to be 56 $GtCO_2$-eq in 2030. If that growth line (between now and 2030) is extrapolated, the projected global warming will be about 3 ^oC at the end of the century. In order to limit the temperature increase to less than 2^oC, the GHG emissions of 2017 should decrease by 25 % to 40 $GtCO_2$-eq by the year 2030 (UNEP, 2018). For the target of 1.5 ^oC, a decrease of 55 % to 24 $GtCO_2$-eq is required for the same time period (Fig. 2.7).

The South African government legislated the implementation of carbon tax (Carbon Tax Policy Paper, May 2013) and limitation of GHG emissions to levels that are 34 % lower by 2020 (Simeonova-UNFCCC, n.d.) and 42 % lower than the “business as usual trajectory” by 2025 according to the country’s Cancun pledge made in 2009. Given that the national total GHG emissions for 2008 was reported as 530 $MtCO_2$-eq (Olivier et al. 2017), this pledge translates into emission levels of 350 $MtCO_2$ by 2020 and 307 $MtCO_2$ by 2025. According to the Western Cape climate change response strategy of 2014 (WCG, 2014), the pledge that was made is very ambitious.

[1]“Pre-industrial” is not specifically defined by the UN or the IPCC (Hawkins, 2017). Historically the period 1850-1900 was used as baseline, while the IPCC reports use 1986-2005 (about 0.6 ^oC warmer than pre-industrial levels) as point of reference. Hawkins further suggests that the period 1720-1800 should be used as baseline for this concept. This point is still much debated.

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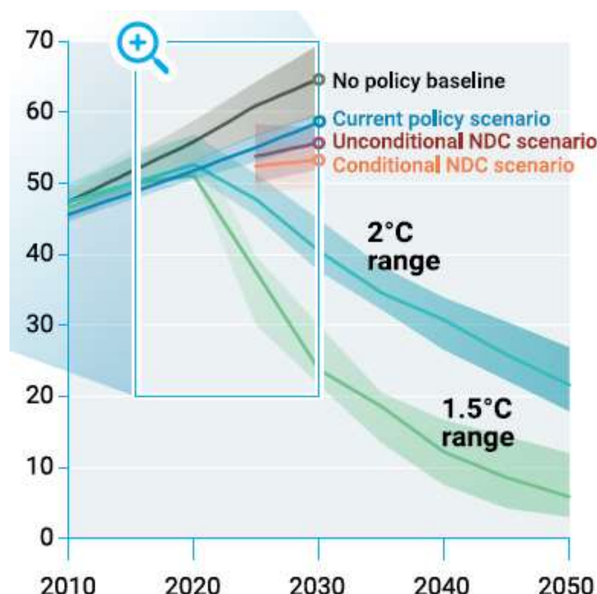

Fig. 2.7 Global greenhouse gas emissions under different scenarios with the emissions gap in 2030 (coloured areas indicate the median estimate line and the tenth to ninetieth percentile range). (UNEP, 2018).

South Africa has managed to keep its GHG emissions within a constant range of 490-510 $MtCO_2$-eq between 2008 and 2016 (Olivier et al. 2017). However, UNEP (2018) mentioned South Africa among a group of six G20 members (the others are Canada, Indonesia, Mexico, the Republic of Korea and the United States of America) that are either not projected to achieve their Cancun pledges or there is uncertainty whether they will be able to achieve them. The report also indicated that about 50 % of G20 members (Argentina, Australia, Canada, EU28, the Republic of Korea, Saudi Arabia, South Africa and the United States of America) are lagging behind in their trajectories to meet their unconditional NDCs of the Paris Agreement.

Therefore "unprecedented and urgent action is required by all nations" (UNEP, 2018). Countries were urged to revise and strengthen their policies and compile more ambitious actions by 2020 while making sure that their current NDCs are implemented. It is crucial that emissions peak by or before 2020 and sharply decrease thereafter in order to comply with the target set in Paris.

2.2 FOOD SECURITY AND CLIMATE CHANGE

2.2.1 Impact of Agriculture on global GHG emissions

Agriculture, Forestry and Other Land Use (AFOLU) activities contributed 23 % to the net global GHG emission during 2007-2016, which comprised mainly of CO_2 (13 % of global emission); CH_4 (44 % of global emission) and N_2O (86 % of global emission). It is not possible to quantify the total GHG emission of agriculture, since global data for agriculture-specific CO_2 emissions is not available (IPCC, 2019.)

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According to Olivier et al. (2017), global methane (CH_4) emissions in 2016 were stable compared to 2015 and amounted to 9.2 $GtCO_2$-eq. Cattle farming (both dairy and non-dairy) and rice production contributed 23 % and 10 % respectively to that total, which amounts to 58.8-75.6 $MtCH_4$ for cattle and 25.6-32.9 $MtCH_4$ for rice [1 ton CH_4 equals 28-36 tCO_2-eq; EPA, (2017)]. Total nitrous oxide (N_2O) emissions in 2016 increased with 1.3 % from 2015 to 2.9 $GtCO_2$-eq, with the global agriculture sector emission accounting for 75 %. This may only amount to 7.3-8.2 MtN_2O, but 1 ton N_2O has the same GHG effect as 265-298 ton CO_2 (EPA, 2017). The N_2O emissions from the agricultural sector increased the fastest of all the sectors monitored between 2014 and 2016 with an average of 1.7 % per year. The main sources of agricultural N_2O emissions were the increased manure deposition in managed pastures, rangeland and paddocks (22 % of emissions in 2016), and the incorrect timing and volume of inorganic N fertiliser application (18 % of emissions in 2016) (Oliver et al. 2017; IPCC, 2019). The agricultural sector (as part of AFOLU) therefore makes a significant contribution to global GHG emissions and should put mitigation strategies in place to prevent or decrease that (Tubiello et al. 2014).

2.2.2 Linking climate change and food security

"Land provides the principle basis for human livelihoods and well-being" (IPCC, 2019) and it is therefore critical that land degradation is limited (or, ideally, prevented) in order to limit loss of natural ecosystems and biodiversity, while simultaneously sustaining human health and well-being as well as food security.

Land degradation is the decrease in land condition and is directly or indirectly caused by human-induced processes such as unsustainable land management and anthropogenic climate change. It results in vegetation loss, soil erosion and an overall decrease in the productive capacity of the land for commercial purposes (IPCC, 2019).

The expected increases in rainfall intensity and flooding, and the increase in frequency, intensity and duration of droughts and heat waves will exacerbate land degradation. Droughts have already started to make an impact in especially Mediterranean and Southern African regions. The increase in air temperature with concomitant higher evapotranspiration, and the lower amounts of precipitation will all contribute to desertification (IPCC, 2019).

Climate change (and desertification) has already started to affect sustainable food production. Decreased yields (such as maize and wheat) are experienced in lower-latitude regions and in the pastoral systems in Africa lower animal growth rates and productivity occur. It is expected that climate change would especially decrease food security in drylands (parts of the world defined as dry sub-humid, semi-arid, arid or hyper-arid), due to reduced crop and livestock productivity. These regions are home to approximately 38 % of the global population (IPCC, 2019).

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The demand for agricultural produce will increase together with the increase in global population. The 2019 IPCC report shows a 200 % increase in cereal yields, 100 % increase in irrigated water used and an 800 % increase in the use of inorganic N fertiliser in the agricultural sector for the period 1961-2017. However, humans are already using about 70 % of the global ice-free land surface (Fig. 2.8) and there is therefore limited room for further expansion of agricultural land. The higher yields that will be required in future should therefore mainly come from existing agricultural land. This clearly indicates the urgent need to avoid, reduce and reverse land degradation and desertification.

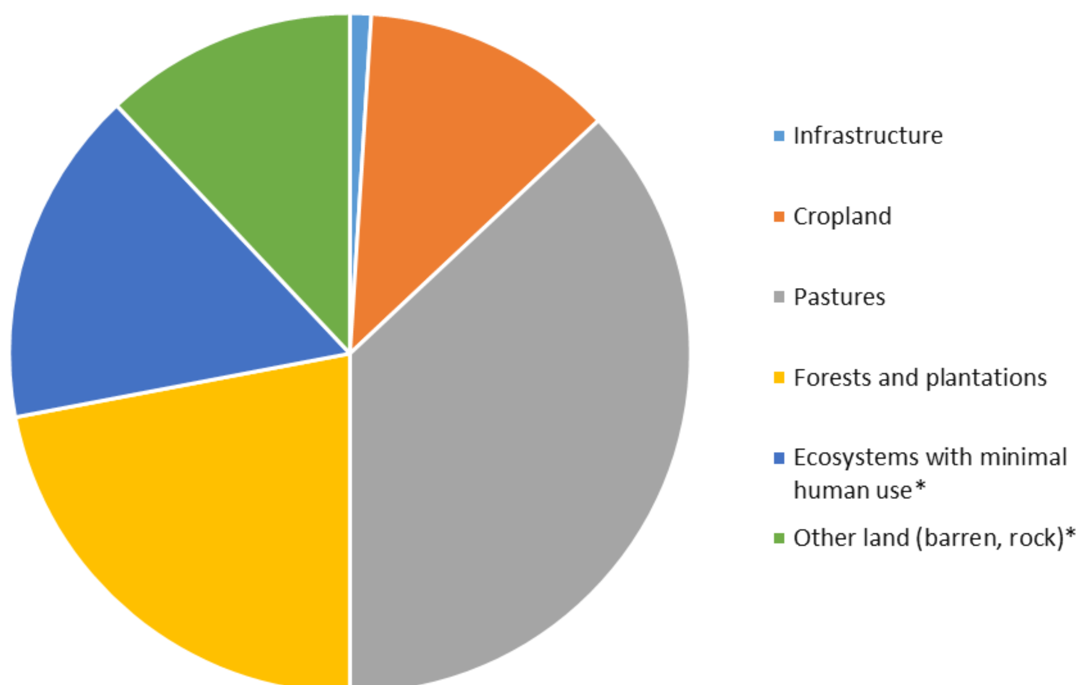

Fig. 2.8 Representation of the use of the global ice-free land surface of 130 Mkm^2 (IPCC, 2019).
* indicate groupings not used by humans

2.2.3 Adaptation and mitigation options for sustainable land management and food security

The climate is not changing uniformly over the world and various regions will therefore face different challenges with regard to type and severity. Developing countries have limited resources to adapt to changing climate conditions and are considered to be vulnerable with low adaptive capacity (Carter, 2006; Schulze, 2016). Each region should be individually analysed to determine which adaptation and mitigation measures are needed or even possible (Schultz, 2017), since the success of any strategy addressing climate change depend on both local environment and socio-economic conditions (UNFCCC, 2007; IPCC, 2019). The adaptation capacity of a region is strongly influenced by factors such as lifestyles and culture (IPCC, 2014). The implementation of adaptive measures may be constrained by people having different perceptions of the risks involved regarding climate change and how any adaptation will

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benefit them directly. Food producers are not willing to invest a lot when land tenure is uncertain, while they may also lack the knowledge and experience (or access to technological and financial resources as well as agricultural advisory services) to successfully implement and monitor the effects of adaptation practices (UNFCCC, 2007; IPCC, 2014; Schulze, 2016; IPCC, 2019).

If land and climate policies (on all levels of government) are well-aligned, planning and implementation of adaptive measures will be enhanced, while resources are saved and collaboration among stakeholders on different levels (e.g. individuals; local, regional and national government; private sector) are improved. Other sectors such as transport, energy and infrastructure, environment, water and public health should also be incorporated to increase the level of engagement and create opportunities for obtaining co-benefits (IPCC, 2014, 2019). Integrative programmes that simultaneously address poverty alleviation, improve water availability and food security, limit/prevent land degradation and loss of biodiversity are generally more successful than narrowly focused objectives (UNFCCC, 2007).

Any mitigation strategy aimed at the agro-ecosystem (farms and surrounding landscapes which provide the environmental, social and economic context) should focus on an integrated approach. It should combine measures to decrease energy use and net GHG emission, to decarbonise energy supply (fossil fuel) with its replacement by cleaner sources (biological, wind, solar), and to increase extraction and sequestration of CO_2 from the atmosphere by enhancing carbon sinks (IPCC, 2014; Tubiello et al. 2014) such as the cultivation of cover crops (Tezza et al. 2019). The expected increase in water demand and scarcity will force the agricultural sector, which is currently accountable for 70 % of global freshwater use (IPCC, 2019), to improve water retention in the soil, adapt irrigation strategies to limit water use and consider alternative water sources such as recycled wastewater for irrigation. Climate Smart Agriculture (CSA) is one such approach aimed at "securing sustainability and resilience [of production systems] while providing economic, ecological and social benefits" (WCG, 2015).

Various options are available to manage the current and future potential risks of climate change and its effect on sustainable land management (Table 2.1). The success of these options will depend on how promptly they are implemented. Any postponement in response to the climate threat will increase the risk of "irreversible loss in land ecosystem functions and services required for food, health, habitable settlements and production" (IPCC, 2019). This will deprive millions of people, especially in the more vulnerable regions, of food and livelihood security.

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Table 2.1 Approaches to limit climate change-associated risks through adaptation options to be used in agro-ecosystems. This is not an exhaustive list and these examples are overlapping (could be relevant to more than one category) and are often applied simultaneously (UNFCCC, 2007; Pott et al. 2009; IPCC, 2014, 2019; Midgley et al. 2016; Montmasson-Clair & Zwane, 2016; Schulze, 2016).

CATEGORY	EXAMPLES
Ecosystem and biodiversity management	Measure and monitor land use change; introduce payment for ecosystem services; establish drought resilient and ecologically appropriate plants; maintain genetic diversity; ecological corridors; ecological restoration; soil conservation
Agricultural production	Avoid de-forestation; harvest rainwater; decrease over-extraction of groundwater; diversify water resources; improve irrigation efficiency; increase soil water retention; increase soil organic matter and improve soil carbon management; use cover crops and retain crop residue to limit erosion; reduce tillage; improve fertiliser management; choose animal breeds/crop varieties more tolerant to heat and drought; improve manure management; increase systematic monitoring using permanent weather stations and remote sensing; establish early warning systems for impeding climate events
Production/supply chain and marketing management	Decrease annual food loss of 25-30 % by improving harvesting, storage, transport and packaging technology; promote educated consumption focused on waste prevention; increase agricultural diversification; expand market access
Human/social development	Accelerate knowledge transfer; enhance extension services and possible mentoring programmes; include indigenous knowledge in practices; conduct participatory action research and social learning; establish knowledge sharing and learning platforms
Economic sustainability	Enable financial support mechanisms; increase incentives for sustainable production; set up disaster contingency funds

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2.2.4 Addressing climate change in South Africa

South Africa made considerable effort to include climatic change in national, provincial and local government policies, but further policy alignment is still needed (WCG, 2015). Montmasson-Clair & Zwane (2016) found misalignment between various critical policies at national level. The apparent lack of a strong national political commitment regarding climate change translate into inadequate political and financial support at provincial and local government level and therefore lack of implementation of proposed adaptation response strategies to climate change.

The Action Plan of 2008 (WCG, 2008) focused on adaptation practices to minimise potential detrimental effects as a result of climate change. Research focus on the impacts of climate change and the development of renewable energy options was prioritised, as well as the reduction of the provincial carbon footprint. In response, the Confronting Climate Change programme was developed "to support the South African fruit and wine sectors through identifying and responding to the risks and opportunities associated with carbon emissions" (CCC, n.d.). This initiative is developing an encompassing database to serve as benchmark for energy use and carbon emissions on fruit and wine farms, as has been adopted by the grain industry of the Western Cape. Further plans are to form partnerships with the Sustainable Initiative of South Africa (SIZA) and the World Wide Fund for Nature South Africa (WWF-SA) (Midgley et al. 2016).

The Smart Agriculture for Climate Resilience (SmartAgri) project commenced in 2014 with the purpose of developing "a practical and relevant climate change response framework and implementation plan specifically for the agricultural sector of the Western Cape" (WCG, 2014). This project is directed by the African Climate and Development Initiative (ACDI) of the University of Cape Town in collaboration with the Western Cape Department of Agriculture, the Western Cape Department of Environmental Affairs and Development and the agricultural sector. In May 2016, the SmartAgri climate change response strategy and action plan was launched in which six priorities (conservation agriculture; restoring degraded landscapes; improved catchment management for water security and job creation; energy efficiency; "climate-proofing" the Western Cape's agri-processing sector and integrated knowledge system for climate smart practices) were highlighted to be focused on by both government and industry (WCG, 2016).

The formulation and implementation of a comprehensive national strategy and action plan to address climate change in South Africa was a multi-disciplinary and multi-sectorial challenge that required effective collaboration at national, regional and local levels and includes contributions from various disciplines. It was launched on 8 March 2019 by the Council for Scientific and Industrial Research (CSIR). The overarching aim of the on-line tool (so-called "Green Book") is to "contribute to resilient, sustainable and liveable human settlements through climate change adaptation" (Moodley, 2019) by facilitating "the

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mainstreaming of climate change adaptation into local government planning instruments…" (CSIR, 2019).

Since actively logging weather stations are sparsely distributed and food producers have limited access to high resolution climate and terrain data, a study was done to investigate remote sensing as alternative technology to supplement weather station data (Southey, 2017). Integrated platforms already exist that provide information to the agricultural sector on climate, terrain and soils to better understand the topographical and climatic complexity of the Western Cape and to aid producers with long and short term decision making.

2.3 IMPACT OF CLIMATE CHANGE ON GLOBAL WINE PRODUCING REGIONS WITH SPECIAL REFERENCE TO THE SOUTH AFRICAN WINE INDUSTRY AND FOCUS ON THE WESTERN CAPE

Originally the grapevine as agricultural crop was considered to be very sensitive to any change in climate, both in the short and long term. Since the grapevine is indigenous to the Mediterranean region and was mostly cultivated over narrow climatic and geographical ranges (mid-latitude regions that often experience large climate variability), Jones and Webb (2010) are often cited in subsequent publications to support this assumption. Since then, new wine-making regions in tropical and meso-tropical climates started to emerge (Mira de Orduña, 2010). Currently, grapevines are cultivated on six of the seven continents across a wide climate range (Schultz, 2016). It is clear that the grapevine has a natural ability to adapt to the environmental conditions in which it is grown. This is shown by a study where the climates of well-known wine producing areas were compared (Schultz, 2011). The climate differences between the regions were larger than any change predicted by climate models. Due to the ecophysiological adaptation capacity (plasticity) of the grapevine, it is thus not sufficient to use only bioclimatic indices when evaluating a region for quality wine production (Schultz, 2011; Seguin & Garcia de Cortazar, 2015). Furthermore, the large physiological and morphological differences between grape cultivars allow successful wine grape production over a wide range of climates (Anderson et al. 2008; Keller, 2010).

2.3.1 Changes in climate already experienced and projected

Over the last few decades, the average temperature during the grapevine growing season has increased in most of the global wine producing regions. This warming was not uniform, with higher warming rates in the Northern than in the Southern Hemisphere (Jones et al. 2005; Webb et al. 2013) and a higher increase at higher than at lower latitudes. A higher frequency of temperature extremes was measured (Jones, 2007). Koch & Oehl (2018) reported higher day and night temperatures (especially during spring) in Southwest Germany. The response of plant growth to increased temperatures will depend on the background environment (Sadras & Moran, 2013). In cool regions, warming increases growth and

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improves grape and wine quality, as found in the Mosel and Rhine Valleys (Jones et al. 2005). Although higher temperatures may further enhance growth and yield in warmer regions (should water availability be constant), the quality will decrease due to unbalanced ripening profiles (Jones, 2007) and fruit composition (Van Leeuwen et al. 2008). Fraga et al. (2016) reported an altered wine style under higher ripening temperatures, while Jones et al. (2005) stated that the higher temperatures may exceed the optimum for certain cultivars in some regions (Jones et al. 2005).

Changes in the patterns of rainfall and other forms of precipitation are not as consistent as that of temperature, but generally climatic models indicate a wetter climate for higher latitude regions (such as New Zealand, the Mosel Valley and the north of Oregon) and a drier climate for Southern Europe, Australia and South Africa (Webb et al. 2013). The higher winter temperatures could result in increased rainfall and less snowfall. In regions where the flow of rivers depends on melting snow during summer, the availability of water for irrigation will decrease during the critical, hotter part of the ripening season (Keller, 2010.)

The water requirement of vineyards (300-700 mm) is higher than the annual mean precipitation in many winegrowing regions (Medrano et al. 2015a). Higher environmental temperature will increase evapotranspiration, which may accelerate salination of the root zone in semi-arid and arid regions (Wooldridge, 2007; Keller, 2010). Limited availability of good quality irrigation water may also have this effect (Anderson et al. 2008). This may result in wines being described as "brackish", "seawater like" or "soapy" (Mira de Orduña, 2010). Higher evapotranspiration may also increase water stress in the vines, which will have a negative impact on yield (Fraga et al. 2016).

Atmospheric CO_2 continues to rise, with current levels at 410 ppm (NOAA-ESRL, 2019) compared to about 340 ppm in 1980. This is considered to be the main cause of warming (IPCC, 2014) and increased CO_2 and increased temperature would therefore be an inseparable combination in future climates. When this is combined with the expected decrease in water availability, it is clear why multi-factorial research on the interactive effect of these climate factors on plant response (growth and physiological functioning) was identified as an important and unavoidable research question (Hunter et al. 2010; Salazar-Parra et al. 2012; Zinta et al. 2018) to meet the primary global challenge of climate change for the wine industry of the future (Schultz & Stoll, 2010).

2.3.2 Models to evaluate viticultural sites

Climate models are often used to determine the suitability of a region for a specific purpose. Average temperature as single factor is commonly used (Webb et al. 2007; Hall & Jones, 2009; Hannah et al. 2013), but these models are not able to discern between regions based on climate variability (Hunter & Bonnardot, 2011). Another method is to integrate climatic factors within one model, as was done by

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Webb et al. (2013) with temperature and precipitation, but this is also insufficient should the total effect of the complete climate system on wine production be the objective. Regions with similar mean temperature and precipitation may differ significantly in terms of the timing and frequency of the precipitation or the diurnal temperature range or the occurrence of extreme climatic events.

Hunter and Bonnardot (2011) combined temperature and potential photosynthetic activity to quantify the temperature impact on grapevine physiological behaviour at specific locations. They concluded that the use of mean indices is not sufficiently discriminatory and may lead to the zoning of only apparently homogeneous terroirs. It is therefore necessary to assess climatic suitability of a region at fine scale (regarding time and space) to better understand physiological activity at a specific location/terroir, especially in regions with a complex terrain (Hunter & Bonnardot, 2011; Quénol et al. 2017; Sturman et al. 2017). Fraga et al. (2016) coupled dynamic crop models, which simulate plant growth and development, with high-resolution climate model simulations to generate future projections of yield, phenology and possible stress indicators for grapevines. Even sophisticated methods such as these have their limitations, since certain assumptions and generalisations are always required in the programming.

2.3.3 The concept of "terroir"

The OIV resolution (OIV/VITI 333/2010) defines the vitivinicultural terroir as "*a concept which refers to an area in which collective knowledge of the interactions between the identifiable physical and biological environment and applied vitivinicultural practices develops, providing distinctive characteristics for the products originating from this area*". Three important aspects may be extracted from this definition:

- knowledge of interaction between the physical and biological environment
- knowledge of applied vitivinicultural practices
- production of a distinctive characteristic

2.3.3.1 Interaction between the physical and biological environment

The purpose of investigating these interactions is to optimise the physiological activity of the grapevine (scion-rootstock genotype) under the site-specific growth conditions in order to produce satisfactory grape quality to ensure economic sustainability. The better the fit between the physical environment (climate and soil) and the grapevine, the less intervention *via* cultivation/management practices are required and the better the expected grape quality. This will reduce input costs and increase profitability while limiting detrimental effects to the environment.

An integrated research approach is required with multi-disciplinary focus areas. Improved and expanded knowledge on ecophysiological mechanisms in the plant in response to all environmental factors seems

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crucial (Schultz, 2011; Martínez-Lüscher et al. 2015), while it should ideally be combined with molecular, genetic and anatomical studies, in combination with plant physiology (Schultz & Stoll, 2010).

There are also more factors to consider in the physical environment than CO_2, temperature and water availability. The increase in sea level could potentially alter the mesoclimate of nearby vineyard sites, while lower lying regions might be exposed to an increased risk of flooding (Tate, 2001). Due to changed migration patterns, the occurrence of pests and diseases is increasing in low temperature areas thus far considered inhabitable (Tate, 2001; Anderson et al. 2008; Mira de Orduña, 2010).

Soil degradation and soil structure decline, as an indirect result of climate change, will have a major effect on viticulture (Anderson et al. 2008) since soil is one of the two main factors (the other being climate) that determine high quality wine production (Leibar et al. 2015). Soil water holding capacity is critical because of its direct effect on plant water status and thus on vine functioning and eventual grape composition (Van Leeuwen & Destrac-Irvine, 2017). The soil clay content generally determines the water holding and nutrient capacity of a soil and will impact nutrient absorption by the roots (Hunter & Myburgh, 2001).

The location of a specific soil will also determine its characteristics. Mountain sites are considered to be sensitive to climate change (Caffarra & Eccel, 2011) with cooler soils that dry out faster (Hunter & Myburgh, 2001). Soil management strategies to retain soil structure, water and nutrient content are thus critical for sustainable production, since increased effective soil depth, improved water holding capacity and good drainage will enhance deep root penetration that would help to buffer grapevines more effectively against adverse climate conditions.

Phenological events generally shifted backwards over the last few decades due to the changing climate, with earlier bud break, flowering, véraison and harvest (Koch & Oehl, 2018) and shorter time intervals between stages (Jones & Davis, 2000), a reduction in the optimum harvest window for quality wines (Jones, 2007), and compression of harvest dates (Anderson et al. 2008). Grape ripening now tends to occur during the warmer and drier months in summer (Mozell & Thach, 2014; Fraga et al. 2016), resulting in a faster ripening rate and sugar increase, with a lack of phenolic and flavour expression, lower acid levels (mainly due to higher respiration of malate), higher pH, and an overall unbalanced juice composition (Jones 2007; Van Leeuwen et al. 2008; Keller, 2010; Mira de Orduña, 2010; Koch & Oehl, 2018). The resultant wines normally contain higher alcohol (due to higher sugar levels) that affects the flavour profile and mouth-feel (Keller 2010). These effects strongly depend on the region and in previously cool climates the faster ripening and increased sugar levels may often result in improved wine quality (Jones et al. 2005).

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2.3.3.2 Applied vitivinicultural practices

Since the impact of climate change is highly heterogeneous across varieties and regions (Jones et al. 2005; Fraga et al. 2016), its effects on viticulture will depend on the cultivar and the cultivation strategies followed within a specific terroir. In order to protect the grapevine against detrimental effects caused by climate change and to improve its resilience, a total cultivation strategy should be adopted regarding both long term practices (starting at site selection and soil preparation) and short term practices performed seasonally (Hunter et al. 2010).

Soil: Good soil management practices should prevent soil degradation and erosion, while improving physical, chemical and biological properties. Excessive tillage would cause soil degradation (Keller, 2010; IPCC, 2019) and increase evaporation from the soil (Schultz, 2000). Open soil cultivation would increase CO_2 release from enhanced breakdown of organic matter and erosion where increased precipitation intensities are expected (Schultz & Stoll, 2010). Evaporation and the risk for erosion may be decreased by covering the soil surface with straw or organic mulch (Keller, 2010; Medrano et al. 2015a). Cover crops are also used for these purposes as well as to decrease water run-off and improve soil structure and fertility (Medrano et al. 2015a). In arid and semi-arid regions, care should be taken to avoid excessive vine stress due to competition with the cover crops for water and nutrients (Schultz & Stoll, 2010).

Water: Water scarcity is expected to become one of the main challenges in many viticultural areas and it is therefore important to improve the effectiveness of water use by the vine (Salazar-Parra et al. 2012; Mozell & Thach, 2014; Fraga et al. 2016) for long term sustainability. The amount of water required per irrigation depends on many factors, such as the soil texture (a lower frequency with larger volume per irrigation is advised for compact, silty and clayey soils), seasonal climatic conditions, the scion-rootstock combination, vigour of the growth, and viticulture practices (Hunter & Myburgh, 2001). The correct type of irrigation system may increase water supply efficiency and thereby improve water saving (Van Zyl & Van Huyssteen, 1988). Alternative sources of irrigation water (such as wastewater from wineries) should also be considered in order to reduce the impact of grapevine cultivation on natural water sources (Myburgh & Howell, 2014). Judicious deficit irrigation increases the water use efficiency (WUE) of the vineyard (Clingeleffer, 2010) while simultaneously saving water. The success of this method is strongly dependent on the interaction between the genotypes (scion cultivar/rootstock) and the environment in which it is grown (Medrano et al. 2015a).

Vineyard practices: Even before the relatively new concept of “climate change” was introduced, producers used to adapt their cultivation practices according to the prevailing climatic conditions to consistently produce a good quality product (Clingeleffer, 2010; Neethling et al. 2013).

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According to Keller (2010), producers are moving away from labour-intensive (and thus expensive) trellising systems with vertical, manually positioned shoots that may require further shoot thinning and/or leaf removal practices. This is however an over-simplification of grapevine growth and management. The orientation of the vine row affects the light and temperature regimes in the canopy and the optimal direction within the context of the region may protect vines against the impact of ambient warming (Hunter et al. 2010). The extent and timing of canopy management practices directly affect microclimatic conditions and thus eventual grape quality. Management neglect or injudicious execution of cultivation practices may lead to under-utilisation of the site potential (also determined by soil, climate, and scion-rootstock genotype) for grape growing and wine quality (Hunter & Bonnardot, 2011).

Cultivar replacement: In regions where climate change is occurring at a relatively fast rate and magnitude, the new temperature conditions might necessitate the establishment of cultivars that are new to the region. Due to the lifespan of plantings and the cost involved in vineyard replacement (Schwab & Maass, 2010; Edwards et al. 2017), a timely cost analysis (on vineyard or winery level) is advised (Mozell & Thach, 2014) to determine whether only cultivar replacement is required or whether the cultivation of other crops should be considered (Bonfante et al. 2010).

Regional changes in cultivar spectra are already occurring (Koch & Oehl, 2018) and it may be expected that lesser-known cultivars better suited to the regional environment (current and predicted) would increasingly be established (Keller, 2010). More than 4000 wine grape cultivars were listed by the OIV in 2013, which indicate the large genetic variability and plasticity of the grapevine genome (Medrano et al. 2015a; Bota et al. 2016). These cultivars should be evaluated under regional conditions to select new possibilities based on ideal traits, such as:

- adaptability to variable climate conditions (Clingeleffer 2010)
- high fruit: leaf area ratio and optimal berry composition (specifically colour and flavour) when ripening under high temperatures (Clingeleffer et al. 2013)
- late seasonal ripening to extend the harvest (Van Leeuwen et al. 2008; Duchêne et al. 2010; Schwab & Maass, 2010)
- production of high quality wines (Duchêne et al. 2010)
- efficient physiological usage of water (WUE), particularly under conditions of water stress (Clingeleffer et al. 2013; Bota et al. 2016)

A change in cultivars might be compelled by climate change, but it is prohibited in certain wine producing countries (e.g. France, Italy and Germany) by legislation where only approved cultivars may be established according to the regional cultivar/quality classification (Webb et al. 2013). In countries

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where such legislation does not exist, such as South Africa, Australia, the USA and China, cultivar replacement should pose fewer problems.

The sensitivity of the scion cultivar to climate change may be reduced by the rootstock choice. Under such circumstances, the most important characteristics for rootstocks seem to be moderate vigour (Clingeleffer, 2010); tolerance to soil salinity (Keller, 2010) and tolerance to low soil water conditions and drought (Serra et al. 2014; Hunter et al. 2016; Simonneau et al. 2017; Peccoux et al. 2018).

In the long term, genetic improvement of cultivars (scion and rootstock) is one of the better strategies to support sustainable wine production systems (Torregrosa et al. 2017) and it could be advantageous for wine industries to invest in breeding programmes (Jones, 2010), despite them being slow and expensive (Bota et al. 2016). Modification of the grapevine genotype to incorporate desirable traits is also possible, but its practical application is currently prevented by policies and legislation (Anderson et al. 2008).

Vineyard relocation: It is commonly predicted that vineyards will be relocated to higher latitudes and higher elevations in future where projected climates will be more conducive to high quality production (Jones et al. 2005; Duchêne et al. 2010; Keller, 2010; Hannah et al. 2013; Fraga et al. 2016, 2017). The current European regulations may prevent this expansion to other regions, since there are strict specifications for Origin Wines regarding the production area, vineyards, cultivars used and winemaking practices allowed (EFOW, 2019). Moving vineyards to higher elevations also have disadvantages, such as the increased difficulty of access and the higher risk of erosion and fire (Wooldridge, 2007).

According to Hannah et al. (2013), vineyards are known to have long-lasting effects on the environment and their relocation may lead to conversion of natural vegetation, with substantial implications for conservation of ecosystems. Furthermore, existing viticultural areas may not necessarily be abandoned in the process or if so, may be replaced by other crops or urban development. There is a growing preference of consumers for environmentally friendly produce (Hannah et al. 2013; Medrano et al. 2015b), which might serve as an incentive for the Wine Industry to investigate and quantify its GHG emissions (and therefore its impact on climate change) and improve the management and conservation of natural resources (Schultz, 2016). The carbon and water footprints are also becoming increasingly important in food and wine trades and concerns about the water footprint (WFP) of grape and wine production are raised (Medrano et al. 2015a).

Any decision made or action performed should be geared towards reaching the “ultimate goal of harmony between grape and wine production, the environment and social aspects, while still maintaining economic viability” (Hunter et al. 2011).

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2.3.3.3 Distinctive product character

Within any specific region, grape ripening dynamics are expected to change (even with adapted cultivation practices) with an increase in ambient temperature, resulting in a shorter overall harvesting period (Anderson et al. 2008; Clingeleffer et al. 2013). The altered grape and juice composition and balance may require adaptation of practices in wineries. Temperature control may become more important (with additional infrastructure costs involved) (Anderson et al. 2008), while the addition of acid, dilution of juice and/or alcohol removal from wine may be applied increasingly (Mira de Orduña, 2010). Potential higher grape sugar content may lead to sluggish or stuck fermentations (with associated risks of the development of off-flavours) that might require the development of new wine yeasts that are better adapted to the new conditions (Anderson et al. 2008). It is thus expected that more interventions in the cellar will be required to support desired aroma and wine styles, but within the boundaries of local winemaking regulations (Mira de Orduña, 2010).

Climate change would affect grapevine growth and ripening, which would necessitate adjustment in long and short term cultivation practices to manipulate the immediate surroundings of the leaves and ripening bunches. Any change in microclimate would alter energy dynamics within the canopy and inside the vine itself, which would affect grape ripening, composition and the eventual wine style (Hunter et al. 2010). Within limits, the wine quality and style may also be influenced by changing the harvest date to meet certain ripeness criteria (Hunter et al. 2004; Hunter & Bonnardot, 2011; Terblanche, 2019). A gradual change in wine styles across estates (within production regions) seems unavoidable (Wooldridge, 2007; Jones, 2010; Koch & Oehl, 2018), but it would not be a major problem should the market accept changes in the typicity of wines (Duchêne et al. 2010) and the taste of the consumer evolves with the changes in wine style (Tate, 2001).

Current winemaking regions are associated with their distinctive wine style or cultivar, be it as result of official legislation (Anderson et al. 2008; Webb et al. 2013) or from more informal means, such as experience, local culture and tradition (Trombi et al. 2011), or the production of consistently outstanding quality (Davis et al. 2019). Wine regions are often major socio-economic role players that contribute significantly to national exports and tourism (Fraga et al. 2017). Trombi et al. (2011) recommended that an integrated assessment of the effect of climate change is done (in Tuscany). The rural tourism sector depends to a large extent on the surrounding vineyards and the abandonment of traditional cultivars and/or changes in the crops cultivated may have serious repercussions on local tourism, economy and culture. It is imperative that these concerns are considered at various levels, since socio-economic issues, politics and regulations also have significant impact on the Wine Industry (Mozell & Thach, 2014).

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2.3.4 The South African wine industry

2.3.4.1 Size and structure

South Africa is currently (2018) the 8th largest wine producing country (by volume) in the world, with a production of 960.2 million litres of which 63 % is white and 37 % red (SAWIS, 2018). About half (51 %) of the total wine produced is exported of which 59.8 % is in bulk (which is a decrease from 66 % in 2013). The reasons for this reversed trend are partly because of the strengthened performance of packaged wines and also because of the EU Trade Agreement that increased the duty-free quota for South African wines from 50 to 110 million litres per year (DKC, 2015; SAWIS, 2015). The five largest international markets (per volume) are the United Kingdom, Germany, France, the Netherlands and Denmark (SAWIS, 2018).

The wine industry accounted for 1.2 % of the national GDP in 2013 and contributed R36.1 billion to the economy – 53 % of which originated in the Western Cape (DKC, 2015; SAWIS, 2015). A further amount of R6 billion was generated through tourism. Apart from the domestic tourist trade, 43 % of overseas visitors to the Western Cape also visit the Cape Winelands.

There are 2873 wine grape producers in the country of which 76 % produce less than 500 tons of grapes per year (Table 2.2) (SAWIS, 2018). Only four producers are producing more than 10 000 tons of grapes per year. The 542 wine cellars comprise of 468 private wine cellars, 47 producer cellars and 27 wine producing wholesalers. Once again the smaller producers are in the majority, with 72 % processing 500 tons of grapes or less per vintage.

Table 2.2 Structure of the South African wine industry in 2018 (SAWIS, 2018).

Production Category (tons of grapes)	Number of primary grape producers
1 – 100	1 157
> 100 - 500	1 035
> 500 – 1 000	346
> 1 000 – 5 000	323
>5 000 – 10 000	8
> 10 000	4
	2873 (total)
Production Category (tons of grapes)	**Number of wine cellars**
1 – 100	222
>100 – 500	166
>500 – 1 000	45
>1 000 – 5 000	58
>5 000 – 10 000	13
>10 000	38
	542 (total)

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The South African wine industry provides 289 151 employment opportunities (including tourism) of which 58 % are situated in the Western Cape (DKC, 2015; SAWIS, 2015). Of the total number, 55.6 % are classified as unskilled; 29.3 % semi-skilled; and 15 % skilled.

Ten regions are demarcated under the Wine of Origin scheme (Fig. 2.9) of which Stellenbosch and Paarl are the largest (in terms of hectares) and the Cape South Coast and Klein Karoo the smallest (SAWIS, 2018). In total there are 93 021 hectares of wine grape vineyards of which 55.3 % are planted with white cultivars and 44.7 % red.

A variety of cultivars are planted, but 82 % of all the vineyards basically comprise of eight cultivars – Chenin blanc, Colombar, Cabernet Sauvignon, Sauvignon blanc, Shiraz, Pinotage, Chardonnay and Merlot noir (Fig. 2.10) (SAWIS, 2018).

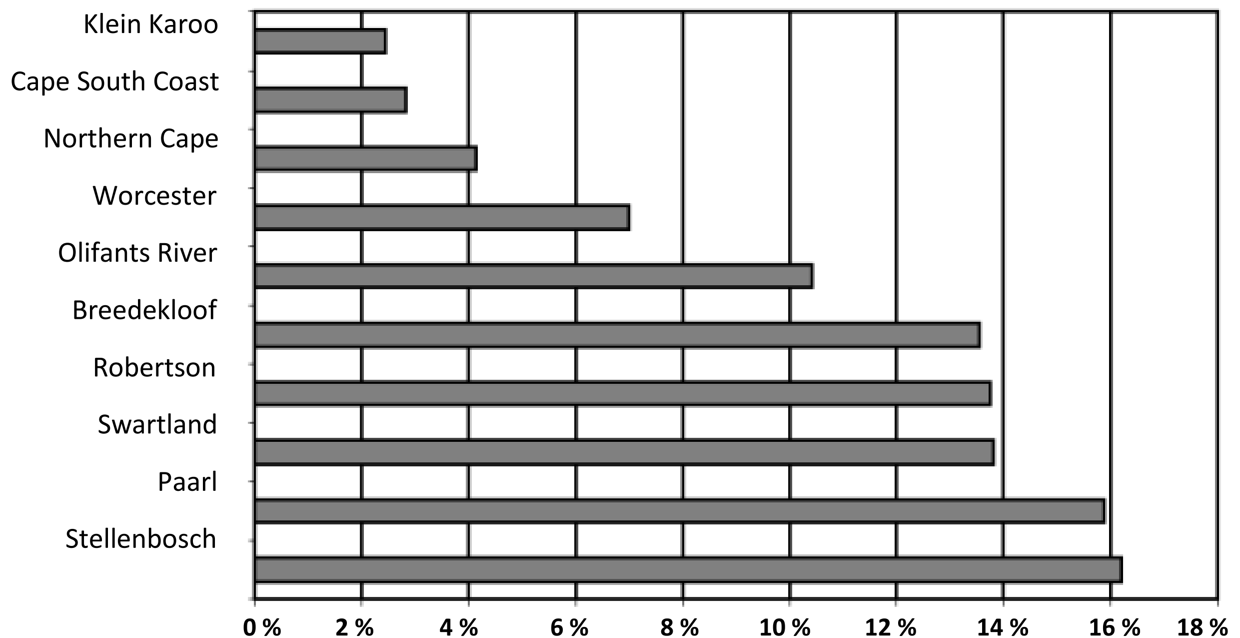

Fig. 2.9 Distribution of wine grape vineyards (ha) per wine region, 2018 (excluding Sultanina) (SAWIS, 2018).

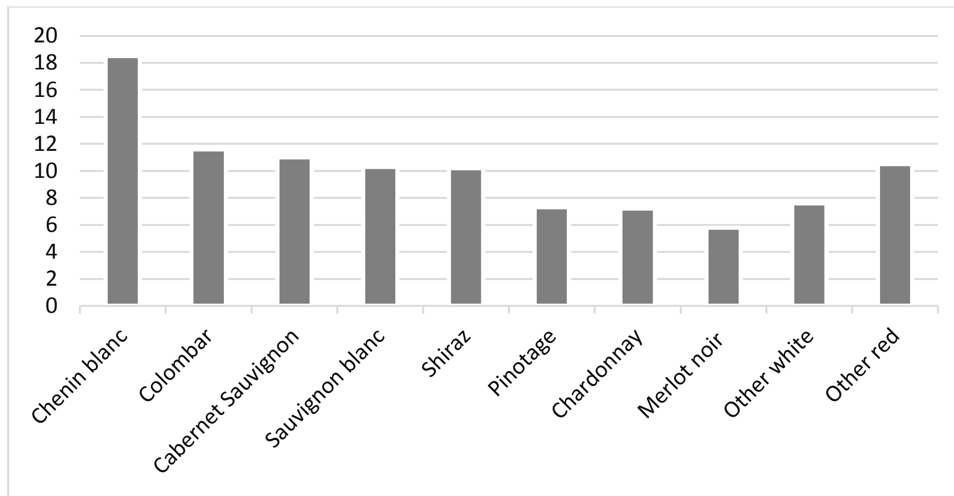

Fig. 2.10 Distribution of main wine grape cultivars as percentage of total area, 2018 (SAWIS, 2018).

2.3.4.2 Effect on climate change

In the Confronting Climate Change report of 2019 for South African Wine Grapes, it was reported (based on combined data from 2011-2018) that wine grape production emits 3-4 tCO_2-eq/ha (gross value) into the atmosphere, which is relatively low compared to table grapes (9-10 tCO_2-eq/ha), stone and pome fruit (9 tCO_2-eq/ha) and citrus (7 tCO_2-eq/ha) (CCC, 2019). These values were obtained from normalised, graded data from the current database (representing less than 10 % of the wine industry (in hectares) (Blignaut, 2019). However, when expressed as kg CO_2/kg fruit, the emission was higher for wine grapes (0.41 kg/kg) than for pome fruit, stone fruit or citrus (Fig. 2.11), because of the relative lower yield per hectare of wine grapes (Blignaut, 2019).

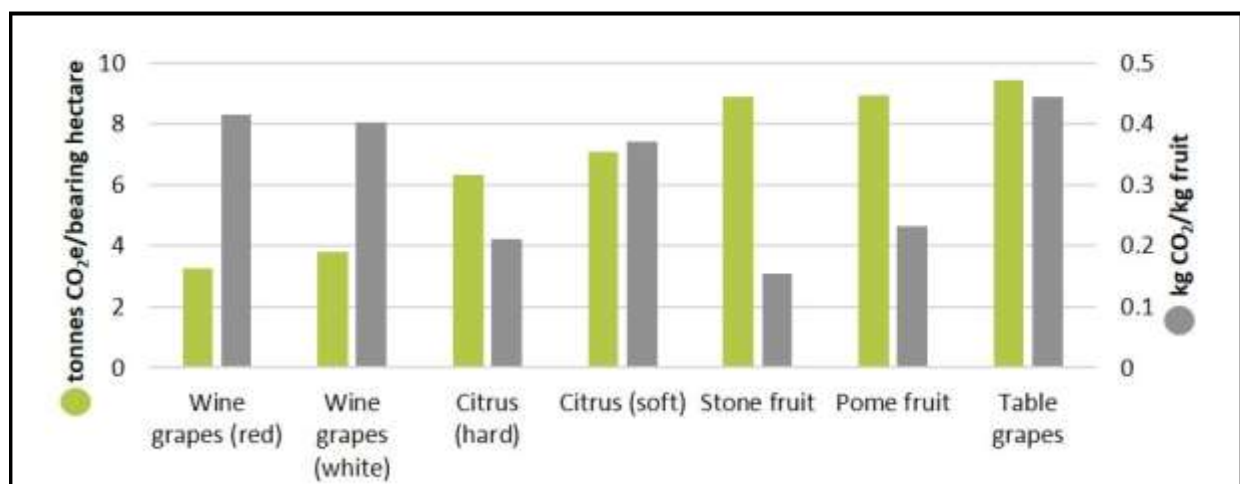

Fig. 2.11 Comparison of GHG emissions of respective fruit industries in South Africa (CCC, 2019).

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The main contributors to GHG emissions on wine grape farms are electricity (46-48 %), fuel consumption (28 %) and the use of fertilisers (20 %) (Fig. 2.12). The electrical use is primarily related to irrigation, while fuel consumption comprises all traffic during the production process (spraying of pesticides and herbicides, fertilisation, pruning, harvesting, etc.). Emission values would most probably differ between regions, cultivars used and cultivation practices, but due to the small representing sample it is too early to make any deductions. However, it is clear that these three aspects should receive special attention when developing strategies to decrease GHG emissions on the farm. Greenhouse gas emissions as result of the winemaking process is calculated separately and does not form part of this data.

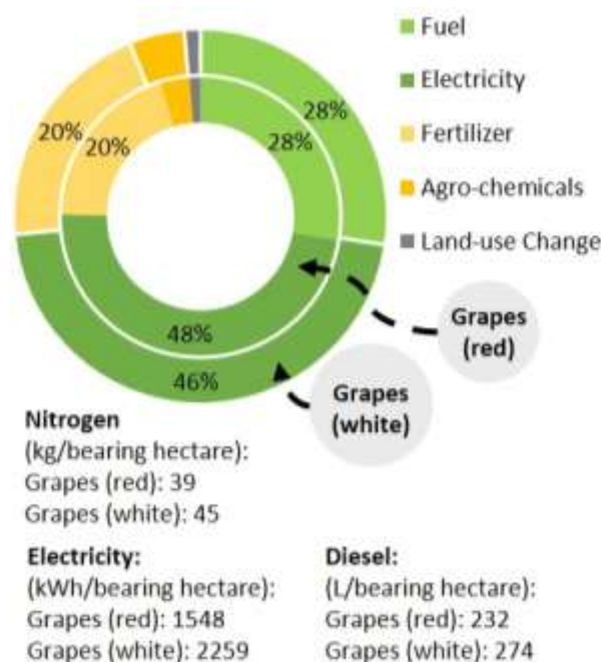

Fig. 2.12 Sources of GHG emissions in wine grape production (CCC, 2019).

2.3.4.3 Focus on sustainable production

The wine industry is entirely dependent on natural resources and initiatives to promote grape and wine production in an environmentally friendly and sustainable way (Hunter et al. 2011). Sustainable Wine South Africa (SWSA, n.d.) is an alliance between the Wine of Origin (WO) and Integrated Production of Wine (IPW) schemes of the Wine and Spirits Board (WSB). A certification seal was implemented in 2010 that was the first of its kind in the world (Fig. 2.13). It is a guarantee of integrity and sustainability. “Integrity” confirms the origin (100 % of content), vintage (at least 85 % of content) and cultivar(s) (at least 85 % of content) information on the bottle label. “Sustainability” declares that the grapes were produced and the wine was made according to strict environmental guidelines (originally drafted in 1998

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and updated annually), while biodiversity was protected and waste water treated. Producers complete their own evaluation forms, but are frequently audited to verify the accuracy of their mark allocation.

Fig. 2.13 Certification seal of Sustainable Wine South Africa (SWSA, n.d.)

2.3.4.4 Topography and climate

The Western Cape province in South Africa is characterised by a complex topography with large variation in elevation (Fig. 2.14), slope gradients and aspects as well as a long coastline with various degrees of exposure to the sea breeze effects from both the Indian and Atlantic oceans, but which is intensified by the cold Benguela current of the latter (Carey, 2001; Hunter & Myburgh, 2001; Bonnardot et al. 2002). There is also significant variation in soil type regarding texture, depth and water and nutrient holding capacity (Hunter & Myburgh, 2001).

All of these result in a variety of meso climates within very short distances (Hunter & Bonnardot, 2011; WCG, 2015; Midgley et al. 2016) that often require small scale (spatial) adaptation of agricultural practices to accommodate the respective local growth conditions. This diversity of terroir units enables South Africa to produce a wide range of wine types and styles from different cultivars (Vink et al. 2012).

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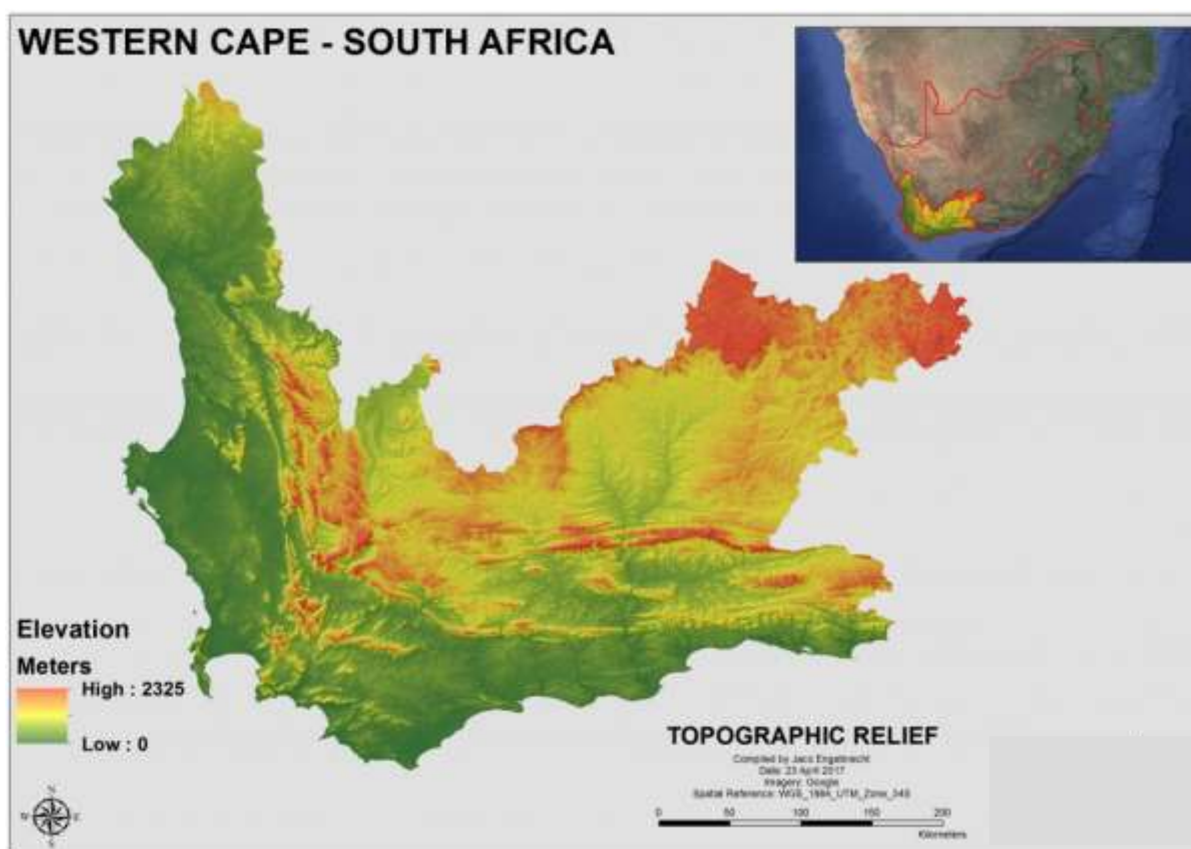

Fig. 2.14 Topographic relief map indicating the elevation variation in the Western Cape of South Africa (Visualviticulture, 2017).

Water availability is considered to be the most limiting factor for agricultural production in South Africa (Benhin, 2006). In most of the viticulture regions, the amount of precipitation is not sufficient to meet the water demands of the grapevine (Hunter & Myburgh, 2001). According to Van Zyl & Van Huyssteen (1984), producing grapevines cultivated in the coastal region of the Western Cape require approximately 500 mm of water between September and April of which an average of 300 mm is normally contributed by rainfall. The industry therefore depends strongly on irrigation during the dry summer months – in the Berg River catchment area 89 % of agricultural irrigation water is allocated to table and wine grape cultivation (Midgley et al. 2016).

The Western Cape has experienced water shortages over the last decade, with strong (and increasing) competition between the agricultural industry, urbanised regions and environmental reserves (Schulze, 2016). The rainfall in the province is projected to decrease with a shorter core-season and lower average precipitation (Midgley et al. 2005). The reliability of the rainfall will also be less with regards to amount, intensity, timing, geographical distribution and annual variability (Wooldridge, 2007). Although the Western Cape is the province with the most registered dams in South Africa (1592 out of 5592 – RSA, 2019), the majority are small and mainly used for irrigation (Midgley et al. 2016). Sufficient water storage capacity will always be a critically important factor, especially within the context of climate change.

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Montmasson-Clair & Zwane (2016) found that South Africa is currently underprepared for the occurrence of future droughts.

Both minimum and maximum temperatures in the Western Cape are expected to increase in future, with an average increase of more than the global mean prediction (Midgley et al. 2016) and more hot and fewer cold/frost days (WCG, 2015). Generally, the future will be warmer and drier for the main viticulture regions of South Africa (Vink et al. 2012).

The challenges faced by the wine industry in South Africa are similar to those in the rest of the world. Dedicated research and knowledge transfer would make it possible to put timeous strategies in place to increase the adaptive capacity of the industry and convert challenges to opportunities.

2.4 EFFECT OF CHANGING CLIMATIC FACTORS ON THE PHYSIOLOGY AND VEGETATIVE GROWTH RESPONSE OF *VITIS VINIFERA*.

It is very difficult to define clear connections between climate conditions and grapevine performance (Schultz, 2011), due to the large natural adaptive physiological capacity (plasticity) of the grapevine (Jones & Alves, 2013; Seguin & Garcia de Cortazar, 2015). Interaction among various climate variables is very possible. Research on the combined effects of increased CO_2 and temperature, and decreased water availability on the plant is therefore critical (Hunter et al. 2010) to expand knowledge of the mechanisms that may regulate physiological adaptation of the grapevine to the changing environment. Better understanding of how plants would react morphologically and physiologically (at leaf, root and whole-plant level) on climatic stress factors would benefit decision-making regarding adaptation and mitigation measures to ensure sustainable/profitable production of good quality grapes under future climatic conditions.

2.4.1 Ambient CO_2

Photosynthetic rates of plants are not limited by current ambient CO_2 levels, since an increase in CO_2 resulted in elevated assimilation rates (Curtis & Wang, 1998; Long et al. 2004; Robredo et al. 2007; Edwards et al. 2017). Higher CO_2 levels decreased stomatal conductance by inducing partial closure of stomata (Long et al. 2004), while the rate of transpiration also decreased (Kriedemann et al. 1976; Edwards et al. 2017). Due to the higher assimilation:transpiration ratio (Kriedemann et al. 1976), the water use efficiency of the plant improves (Long et al. 2004; Robredo et al. 2007) and could possibly decrease the rate of soil water depletion and prolong physiological activity under water-limited conditions (Robredo et al. 2007; Salazar-Parra et al. 2012). This stress-mitigation effect of plants in reaction to CO_2 was also reported by Aranjuelo et al. (2008) and Zinta et al. (2014).

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There are many reports of decreased respiration with an increase in atmospheric CO_2 discussed in the review compiled by Drake et al. (1997). A meta-analysis of research done on the effect of elevated CO_2 on woody plants indicated a significant decrease in leaf dark respiration (Curtis & Wang, 1998). However, Davey et al. (2004) used an alternative method to determine respiration rates by measuring the rate of respiratory O_2 uptake instead of CO_2 release. They reported no decrease in respiration for various plant species, even when the CO_2 concentration was increased to 2 000 ppm. In fact, a significant increase in respiration was found. Their findings were supported by Alonso et al. (2009), who ascribed the increased respiration rates to the higher leaf carbohydrate content (and thus availability of respiratory substrate) under elevated CO_2 conditions. The latter was also found by Moore et al. (1999), Leakey et al. (2009) and Edwards et al. (2017).

Elevated CO_2 levels significantly increased grapevine growth rate (Kriedemann et al. 1976) as well as total vegetative and reproductive growth (Bindi et al. 2001; Long et al. 2004). Leaf tissues contained less nitrogen (and protein), P, K, Ca, Mg, S, Fe, Mn, B and Mo (Morales et al. 2016) and more starch (Curtis & Wang, 1998; Long et al. 2004). According to Bindi et al. (2001) no significant difference in berry composition and wine quality was found between grapes that ripened under elevated compared to ambient CO_2 conditions, while Duchêne et al. (2010) reported a change in the wine aromatic profile directly as a result of higher CO_2 levels. Long-term free-air CO_2 enrichment (FACE) studies indicated that the stimulation of crop yields was much smaller than originally expected (Leakey et al. 2009).

There are various mechanisms by which higher ambient CO_2 may increase net carbon uptake and assimilation. C_3 plants basically respond to high ambient CO_2 by decreasing the opening of stomata (and thus stomatal conductance) and increasing RubisCO carboxylation efficiency and RuBP regeneration capacity (Leibar et al. 2015; Pan et al. 2018). Increased CO_2 concentration may also enhance the competitive inhibition of RuBP oxygenation by RubisCO. This results in a decrease in the rate of photorespiration under elevated CO_2 (Kriedemann et al. 1976; Flexas et al. 2002; Zinta et al. 2014; 2018) and an increase in net production *via* photosynthesis. This last reason might be more important, since its effect is unrelated to whether photosynthesis is limited by RubisCO and/or RuBP, while it also does not require any additional light, water or nitrogen resources (Drake et al. 1997) to increase the efficiency of net photosynthesis (Long et al. 2004).

However, it seems as if the stimulatory effect of CO_2 on photosynthesis is only temporary, since acclimation of photosynthesis is often mentioned with longer term plant exposure to high CO_2 levels (Salazar-Parra et al. 2012, 2015; Leibar et al. 2015; Martínez-Lüscher et al. 2015; Morales et al. 2016), which might give the impression that an initial stimulation in photosynthetic rate would decrease again over the long term. Statements such as "there is abundant evidence that in the long term, photosynthesis acclimates to elevated ambient CO_2" (Drake et al. 1997) and "in the longer term this

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increase [in net photosynthesis] is often offset by down-regulation of photosynthetic capacity" (Long et al. 2004) are often cited to support this. A cessation in photosynthetic stimulation in high CO_2 environments is normally associated with the inability of plants to obtain sufficient N (Alonso et al. 2009; Morales et al. 2016) to support and sustain growth. It is important to note that Long et al. (2004) did mention that most of the information (at that stage) on elevated CO_2 concentrations was gleaned from enclosure studies with limited capacity for root development. According to Curtis & Wang (1998), acclimation of photosynthesis occurred due to limited root development in pots smaller than 0.5 L, but Ainsworth et al. (2002) found that even when large containers (>9 L) are used, the physical restriction on root growth affected yield. Based on the results of these chamber studies, it was predicted that CO_2 stimulation of photosynthesis and growth would only be transient. For this reason, acclimation of photosynthesis is not expected in field studies, because of the larger, relatively unrestricted root growth, and fertilisation practices. This conclusion is supported by a 10-year FACE study on rye grass in Switzerland - no evidence of a change in assimilation rate was found for the duration of the study (Ainsworth et al. 2003).

Drake et al. (1997) explained acclimation as follows: "the photosynthetic properties of leaves developed at elevated ambient CO_2 differ from those developed at the current ambient CO_2". This means that leaves that developed and grew in high CO_2 concentration environments would not assimilate carbon at the same rate under ambient CO_2 than plants that grew in ambient CO_2 from the beginning. High CO_2 levels may cause plants to grow thicker leaves (higher leaf mass per unit area) due to larger mesophyll cells (Moutinho-Pereira et al. 2009), although Robertson & Leech (1995) found no major changes in chloroplast structure or leaf anatomy of wheat plants when grown under elevated CO_2. Acclimation of photosynthesis is indicated by a higher leaf sucrose and starch content, higher C/N ratio (mainly due to a decrease in N), lower protein and RubisCO content and decreased photosynthetic capacity (Drake et al. 1997; Moore et al. 1999; Long et al. 2004; Aranjuelo et al. 2008).

Higher CO_2 levels result in increased mesophyll CO_2 concentrations when stomata are open. Since photosynthesis is then limited by RuBP regeneration (and not RubisCO activity), high photosynthetic rates under these conditions can be maintained with lower total RubisCO content that enables the plant to utilise N for other purposes (Drake et al. 1997; Leakey et al. 2009). In future environments with higher atmospheric CO_2, acclimation of photosynthesis would mean a lower N requirement for RubisCO synthesis and thus lower leaf N concentrations without a decrease in photosynthetic production (Drake et al. 1997).

Moore et al. (1999) proposed a model in which increased hexose flux rate is responsible for the repressed transcription of RubisCO genes in plants (Fig. 2.15). The higher leaf sucrose and starch levels normally found in elevated CO_2 conditions indicate that photosynthetic production probably exceeds

carbohydrate export and utilisation, even though elevated CO_2 stimulates dark respiration (Leakey et al. 2009). This would then cause a build-up of carbohydrates in the mesophyll of the source leaves. When this accumulated sucrose is split by invertase, the resulting hexoses may be perceived by a hexokinase enzyme sensing system, which subsequently may generate a signal to decrease the gene transcription of RubisCO.

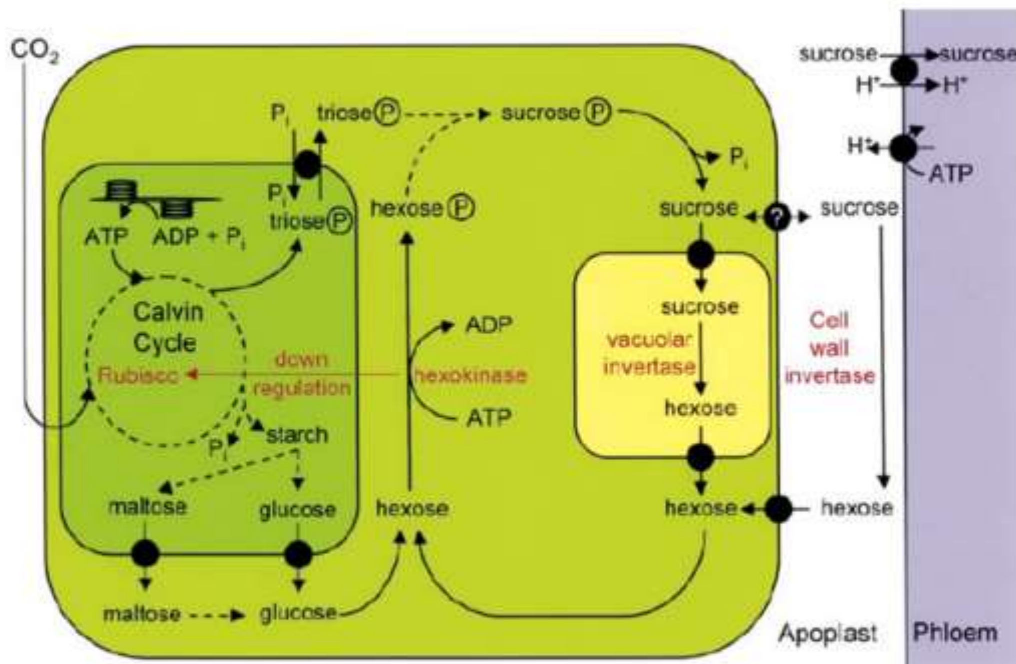

Fig. 2.15 Simplified diagrammatic representation of the model suggested by Moore et al. 1999 (Long et al. 2004).

Aranjuelo et al. (2008) expected, since the light energy received is in excess of that required for C assimilation for plants grown under elevated CO_2, that the superfluous photo-energy would cause an increased generation of reactive oxygen species (ROS) and thus cause oxidative damage to the photosystem. However, no photo-oxidative damage was found under elevated CO_2 conditions in nodulated alfalfa plants. They ascribed it to the enhanced de-epoxidation of violaxanthin to zeaxanthin and antheraxanthin that facilitates thermal dissipation and thus protects the photosystem from the excess photo-energy. Zinta et al. (2014, 2018) also found mitigation of heat and drought stress under high CO_2 levels in *Arabidopsis thaliana*, but they attributed it to an increased synthesis of lipophilic anti-oxidants, combined with reduced photorespiration (and thus less ROS, especially H_2O_2 levels) in the plants under high CO_2 conditions.

2.4.2 Temperature

Higher temperature causes a shorter growth season with earlier budding (Webb et al. 2007), flowering, véraison (Duchêne et al. 2010) and ripening, occurring in warmer conditions during midsummer (instead of late summer to early autumn) within a shorter time interval (Caffara & Eccel, 2011). Torregrosa et al.

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(2017) however found delayed berry ripening (regarding accumulation of sugars and phenolic compounds) with heat stress applied at the green growth stage of the berry. It seems as if the duration and intensity of the heat stress, as well as the grapevine phenological stage when it occurs, would affect berry ripening processes. De Rosas et al. (2017) reported reduced anthocyanin content in red cultivars under high temperatures (ambient + 3 ^{o}C; with ambient temperatures reaching maximum levels of > 40 ^{o}C), while Davis et al. (2019) stated that temperature in Burgundy was the most important climatic factor determining good vintages and that large diurnal-nocturnal temperature differences enhanced anthocyanin production and are important for producing quality red wines.

Higher ambient temperature increased shoot growth rate (Galat Giorgi et al. 2013) and weight per shoot (Sadras & Moran, 2013; Torregrosa et al. 2017), while the effect on yield varied from a 46 % reduction to a 177 % increase in [ambient + (0.7-1.6 ^{o}C)] compared to ambient temperatures (Sadras & Moran, 2013). These authors linked the increase in yield with mainly higher bud fertility and also higher number of berries per bunch and concluded that the effect of warming on grapevine yield can only successfully be assessed within the context of the background temperature and seasonal temperature variations in the particular region. Soil temperature also affects vegetative growth. Between dormancy and flowering, higher soil temperature (23 ^{o}C) resulted in higher shoot biomass and leaf area and relatively lower root growth compared to vines grown in cooler (13 ^{o}C) soils (Field et al. 2009). This was ascribed to the faster remobilisation of carbohydrate reserves from the roots at the end of dormancy in the warmer soils. Xylem sap flow rate (and thus translocation of root-derived cytokinins) was also higher in the latter treatments, causing enhanced shoot and suppressed root growth (Field et al. 2009). The importance of the specific temperature range investigated is illustrated by Hochberg et al. (2015) who found reduced leaf growth as well as a lower growth rate under day/night temperatures of 35/30 ^{o}C. There is thus an optimal temperature range for vegetative growth, which was probably exceeded in the Hochberg study, but not in the others.

The relative long period of time between harvest and leaf fall in warmer climates should be beneficial to reserve accumulation, especially since a linear relationship was found between leaf N content and the CO_2 assimilation rate at this stage (Hunter & Ruffner, 1997). The remobilisation and export of leaf nutrients such as C and N out of old leaves just before leaf fall may further contribute to the total reserve pool (Conradie, 1986; Hunter et al. 1995). However, Torregrosa et al. (2017) found a decrease in carbon reserve storage in woody parts with higher temperatures, while Sadras & Moran (2013) also found reduced starch concentration in trunks, but not in the roots. High temperatures seemed to activate stress related metabolism in leaves, since secondary sugars (ribulose, raffinose, fucose, erythronate) increased while primary sugars (glucose, fructose and sucrose) decreased, irrespective of the cultivars investigated (Hochberg et al. 2015). It seems that the effect of warmer temperatures will depend on the

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specific temperature readings. The "high" temperatures in the latter studies were 25 ^{o}C and 30 ^{o}C (Torregrosa et al. 2017); ambient + 2 ^{o}C (Sadras & Moran, 2013) and 35 ^{o}C (Hochberg et al. 2015).

Photosynthetic activity is temperature dependent with optimum temperature at 25 ^{o}C (Alleweldt et al. 1982), but with a wider optimum range of 25-30 ^{o}C under field conditions (Hunter & Bonnardot 2011). A marked decrease in photosynthesis occurred between 30 ^{o}C and 45 ^{o}C, of which only 15-30 % was attributed to stomatal closure despite there being a linear decrease in stomatal conductance between 20 ^{o}C and 45 ^{o}C. The dominant mechanism of reduced photosynthesis under high temperatures is therefore not directly linked with gas exchange, but is biochemically limited by the rate and efficiency of RuBP carboxylation by RubisCO (Greer & Weedon, 2012).

The heat stress-induced reduction in RubisCO activity was also mentioned by Pan et al. (2018), but they associated it with decreased stomatal conductance (limiting CO_2 supply) and damage to PSII, which is very temperature-sensitive. Higher temperatures decreased stomatal conductance, which resulted in an increased O_2:CO_2 ratio within the chloroplasts (Flexas et al. 2002). Both increased temperatures and relatively lower CO_2 availability would increase photorespiration compared to photosynthesis (Long, 1991; Zufferey et al. 2000), also because of lower solubility of CO_2 than O_2 under high temperatures (Leibar et al. 2015).

The average temperature in wine producing countries is expected to increase in future and it is very likely that the daytime temperature profile is going to increase to levels outside the considered optimum range for photosynthesis in current warm regions (Hunter et al. 2011). The photosynthetic efficient temperature duration during the day would then be shorter and would occur earlier during the day (Hunter et al. 2010), which could very well affect the growth and berry ripening dynamics of the grapevine.

Respiration in plant tissues generally increases with increase in temperature (Zufferey, 2016), but the extent of the temperature effect may differ according to the type of tissue, the age of the organ/plant and the environmental conditions (such as light intensity). The rate of leaf respiration is more sensitive to temperature changes than root respiration (Loveys et al. 2003). Respiration rate in younger, actively growing leaves is higher than in mature leaves (Zufferey, 2016) and less sensitive to increases in temperature (Atkin et al. 2005). Plant respiration is especially high in actively growing tissues where respiration supports both growth and maintenance, while there is a general decline in respiration rate after véraison, which could be attributed to both leaf senescence and decreased respiratory requirements (less active growth) (Zufferey, 2016). Plant respiration at night exponentially increases between 15 ^{o}C and 30 ^{o}C (Torregrosa et al. 2017) that, together with a decrease in photosynthesis and increase in photorespiration during the day, would result in lower net carbon gain in the plant under high temperature conditions.

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Loveys et al. (2003) observed that temperature dependency of photosynthesis is affected by the temperature at which the specific leaves developed. Leaves developing and growing in adjusted (increased) temperature environments often differ anatomically from control leaves by having higher specific weight (Atkin et al. 2005) and/or altered bio-membrane structure (Zufferey et al. 2000). Galat Giorgi et al. (2013) found increased primary xylem vessel density and size under high temperatures in shoots, which might affect hydraulic conductivity and transpiration. Activity of photosynthetic enzymes in the leaves may also be different by adaptation of enzymes to iso-enzymes with higher optimum temperatures for functioning (Zufferey et al. 2000). This has important implications for research on temperature effects on plants. Treatments starting before budbreak with all new roots and leaves developing and growing in higher temperature conditions would provide different results than when a temperature increase occur at any specific stage during the growth season.

2.4.3 Water

Drought may be considered as the main environmental factor limiting the photosynthetic activity of plants (Martínez & Chacon, 2010). Three plant-based measurements are often used to determine the water status of grapevines, namely predawn leaf water potential (ψ_{PD}), midday leaf water potential (ψ_L) and midday stem water potential (ψ_S). Choné et al. (2001) considered ψ_S to be a more reliable indicator of vine water status than either leaf or pre-dawn water potential. However, Williams & Araujo (2002) found all three measurements to be equally viable to assess vine water status due to similar correlations with soil water content, leaf gas exchange and with one another. The choice of method used is often determined by practical considerations – the predawn time span for example is too short if the ψ_{PD} in a number of vineyards needs to be measured for irrigation scheduling (Myburgh, 2018). The level of water stress experienced by the vine may be classified according to certain threshold values for plant-based measurements (Table 2.3).

Table 2.3 Threshold values of ψ_{PD}, ψ_L, ψ_S (Myburgh, 2018) and stomatal conductance (g_s) (Lovisolo et al. 2010) used to indicate the level of grapevine water stress.

Class	**Level of water stress**	ψ_{PD} (MPa)	Ψ_L (MPa)	Ψ_S (MPa)	g_s ($mmol.m^{-2}.s^{-1}$)
I	None	≥ -0.2	≥ -1.0	≥ -0.6	200 - 500
II	Low/Mild	-0.2 to -0.4	-1.1 to -1.2	-0.6 to -0.9	≈ 150
III	Moderate	-0.4 to -0.6	-1.2 to -1.4	-0.9 to -1.1	50 - 150
IV	High	-0.6 to -0.8	-1.4 to -1.6	-1.1 to -1.4	
V	Severe	< -0.8	< -1.6	< -1.4	< 50

* These values are meant as guidelines only and absolute values may differ as result of cultivar, soil type and/or environmental conditions

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As soon as water supply starts to become limited, stomata start to close with the associated decrease in gas exchange and therefore photosynthesis (Flexas & Medrano, 2002; Palliotti et al. 2008; Martínez & Chacon, 2010; Zufferey, 2013a; Ping et al. 2015). The transpiration rate also decreases as well as the rate of sap flow in the plant (Chaves et al. 2010; Zufferey, 2013b).

Growth and vigour are limited in water stressed vines, with strong reductions in main and lateral shoot and internode length, number of lateral shoots per vine, number of main and lateral leaves as well as individual area of leaves on both main and lateral shoots (Palliotti et al. 2008). Simonneau et al. (2017) reasoned that the decrease in evaporative area (smaller total leaf area) would lower total transpiration in the plant. Folding or wilting of leaves are other possible mechanisms to minimise the effect of limited water by reducing the amount of light intercepted by the leaves and thus leaf temperature increase and water loss (Palliotti et al. 2008; Simonneau et al. 2017). Water stress decreases both leaf and root respiration (Atkin et al. 2005; Zufferey, 2016). This is possibly due to a decrease in substrate availability, a reduced demand for respiratory products for growth and maintenance, and/or feedback inhibition by increased starch concentrations in leaves as result of slower export during water stress (Ayub et al. 2011).

Root growth was found to be less limited by water stress than shoot growth (Sharp & Davies, 1989), which enables the vine to optimise water and nutrient absorption and transport to the shoots. Grapevines are generally considered to be well-adapted to semi-arid conditions because of their large and deep root system (Chaves et al. 2010). The rootstock used has a significant effect on the drought tolerance of the grafted vine – deeper root penetration allow access to the moister soil layers and would sustain stomatal conductance and transpiration rates for longer (Peccoux et al. 2018).

Mild water stress: The decrease in photosynthesis under mild water stress conditions is predominantly due to restriction of stomatal and mesophyll conductance of CO_2 (Lovisolo et al. 2010; Schultz & Stoll, 2010). This would decrease the CO_2 concentration in the mesophyll and therefore limit the availability of CO_2 for RubisCO (Salazar-Parra et al. 2012). The result is an increase in photorespiration since RubisCO activity is not affected by mild water stress (Flexas & Medrano, 2002).

It is clear that in instances where water conduction is reduced under water stress, the loss of water through transpiration (with the concomitant increase in tension and risk of cavitation) should be limited as far as possible. This would require strong control over stomatal aperture to prevent excessively negative stem water potentials (Vandeleur et al. 2009). However, rapid stomatal closure would decrease CO_2 uptake and therefore photosynthesis. This reaction of the vine to a dry environment is a compromise between sustained conductance and photosynthetic activity, and the protection of the soil-plant-air water continuum.

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Substantial variations in stomatal behaviour exist among scion cultivars (Bota et al. 2016), while rootstocks (inherently and depending on the soil conditions) may also show large diversity in their effect on scion response to water deficient conditions. Davies & Zhang (1991) suggested that the increased abscisic acid (ABA) levels in xylem sap as result of soil drying might play an important signalling role in stomatal aperture, the detail of which is discussed in more detail in a following section. Interactions found between rootstock and scion cultivars (compared to ungrafted vines) by Medrano et al. (2015a) highlighted the need for further studies on scion/rootstock combinations and their interaction for enhancing grapevine adaptability to challenging climatic conditions.

Evidence exists that hydraulic conductivity in all major tissues (roots, shoots, petioles) decline in grapevines subjected to water stress (Lovisolo et al. 2010 and references therein). This would increase the hydraulic tension inside the vascular tissues and thus the risk of embolism formation. Roots are highly vulnerable to cavitation compared to shoots (Alder et al. 1996; Lovisolo et al. 2008; Zufferey, 2013a), which indicates a capacity of the grapevine to develop hydraulic compartmentalisation among organs (Zufferey, 2013b). For example, xylem cavitation in the surface roots may cause the primary region for water uptake to shift downwards to the moister soil layers, provided that the root system has deeper roots in place.
Other studies have found an increase in root hydraulic conductance under mild to moderate water stress conditions (Chaves et al. 2010; Schultz & Stoll, 2010) that was associated with the upregulation of aquaporin formation by the increased level of VvPIP1;1 gene expression (Vandeleur et al. 2009) through the effect of ABA (Chaves et al. 2010). Aquaporins are membrane-imbedded proteins that act as water conduits in plants and are involved in the regulation of intercellular water movement across the plasma membrane (Maurel et al. 2015). The higher water conductance in the vessels enable plants to keep their stomata open for longer and maintain photosynthetic activity. This seems to be the ideal situation, provided that plants have mechanisms in place to induce stomatal closure and prevent permanent hydraulic failure and death (Sevanto et al. 2014), should the drought conditions become worse.

Grapevines seem to respond to limited water supply in various ways. Even with a specific cultivar, stomatal response to water deficit may vary according to the rootstock used, the climate and the intensity and duration of the water deficit (Chaves et al. 2010). The phenological stage at which the stress occurs may also play a role (Poni et al. 1993). Simonneau et al. (2017) challenged the genetic origin of a cultivar's response to water stress.

Cultivars seem to display a difference in behaviour, termed isohydric and anisohydric. Whether this behaviour is purely a response of (any) cultivar to circumstantial water deficit/temperature conditions or whether it is also genetically predisposed to such a response, is still debated. With isohydric behaviour, the leaf water potential is maintained above a certain threshold under conditions of

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decreased water availability (Lovisolo et al. 2010). Stomatal closure is then induced at relatively high plant water potential, thereby decreasing stomatal conductance, water loss through transpiration and the risk of developing embolisms. Gene expression for aquaporin formation in roots is not up-regulated and the rate of sap flow in the vessels decrease.

On the other hand, with anisohydric behaviour the leaf water potential decreases with decrease in soil water availability (Lovisolo et al. 2010). Aquaporin formation in roots as well as the hydraulic conductivity in the vessels are enhanced. According to Soar et al. (2006) stomatal conductance and transpiration are higher compared to isohydric behaviour (Soar et al. 2006), but Lovisolo et al. (2010) found no difference in stomatal conductance or assimilation rate between so-called isohydric and anisohydric cultivars. They based the classification of behavioural type on the decrease (or not) of leaf water potential with a decrease in soil water availability.

Most of the internal signals involved in drought-induced stomatal closure are closely related to ABA metabolism (and therefore also expression and activity of aquaporins) and hydraulic conductivity (Lovisolo et al. 2010). Both chemical and hydraulic signals seem to be important in grafted grapevines (Serra et al. 2014; Peccoux et al. 2018), but the interaction between them is still under debate (Simonneau et al. 2017).

Drought is normally associated with growth inhibition that may mainly be due to a decrease in cell volume, since Ojeda et al. (2001) found that water deficit did not affect cell division in grape berries, but limited cell enlargement by decreasing the extension ability of the cell walls. Bartels & Sunkar (2005) stated that drought stress leads to dehydration of plant cells by the outflow of water into the extracellular space. The resultant decrease in cell cytosol and vacuole volumes may therefore cause a decrease in cell size. Cell growth depends on maintaining turgor pressure in cells. This is obtained by adjusting the osmotic potential in the cell to retain water, and increasing the elasticity of cell walls (Patakas et al. 1997). An increase in cell wall elasticity would enable a cell to keep its turgor pressure with a lower cell water content, thereby increasing the drought tolerance of the plant (Alsina et al. 2007). The concept of cell wall elasticity and turgor is clearly illustrated by a study of Patakas et al. (1997) who compared immature, mature and old leaves with regards to their response to water deficit. They found a decrease in cell wall elasticity with increase in leaf age, with the result that positive turgor pressure could be maintained in immature and young leaves that, together with the high elasticity of their cell walls, sustained cell enlargement and thus leaf growth under mild water stress conditions.

<u>Moderate to high water stress:</u> With an increase in water stress severity (moderate to strong), both stomatal and (increasingly important) non-stomatal inhibition of photosynthesis occur (Flexas et al. 2004). The decrease in photosynthesis is due to an impaired rate of electron transport (decreased PSII activity) and lower resultant ATP production (Lovisolo et al. 2010). The latter would then cause a

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decrease in RuBP regeneration (Flexas & Medrano, 2002) and thereby limit the carboxylation substrate for RubisCO. Although the RubisCO content and activity may have been reduced (Salazar-Parra et al. 2015), the activity of the Calvin cycle enzymes and carboxylation rate are normally maintained under moderate water stress conditions (De Souza et al. 2005).

It was found that leaves in water-deficit vines contained higher sugar levels and lower starch levels during the day (Dayer et al. 2013), which may be linked to the increased activity of α-amylase and β-amylase in water (and temperature) stressed plants (Zinta et al. 2018). This could be an indication of reduced metabolic activity and carbon export as a strategy against severe water stress, while the sugar accumulation in the leaves could be explained by osmoregulation and the maintenance of leaf cell turgor (Wardlaw, 1990).

Severe water stress: When plants are experiencing severe water stress, photochemistry reactions are down-regulated with significant lower PSII efficiency and RubisCO activity (Flexas & Medrano, 2002; Ping et al. 2015; Salazar-Parra et al. 2015). Most of the time the metabolic down-regulation is reversible – it is only under extreme dry conditions for long periods (and basically complete stomatal closure) that permanent photo-damage and inhibition of photosynthesis occasionally occur (Flexas & Medrano, 2002).

The time it takes for the grapevine to recover after a period of water deficiency would depend on both the severity and the duration of the stress. Where the vine was only mildly stressed, full recovery of photosynthetic activity occurred within one day after irrigation (Flexas et al. 2004), while it took more than a week when vines were severely stressed.

In a glasshouse study on two-year-old potted Grenache vines, Lovisolo et al. (2008) investigated the connection between hydraulic conductance, xylem embolism and stomatal conductance when water deficit plants were rehydrated. Irrigation was withheld for a period of 10 days from previously regularly irrigated vines, whereafter they were irrigated again on the 11th day. When the dry roots were supplied with water, leaf water potential quickly recovered (85 % during the first two hours and all the leaves within five hours after irrigation), perhaps due to the strong increase in root hydraulic conductivity (Chaves et al. 2010). This could possibly be explained by the relatively large size of grapevine xylem vessels (Lovisolo & Schubert, 1998) or an increase in aquaporin activity. Vandeleur et al. (2009) found an up-regulation of aquaporin gene expression in certain cultivars after re-watering, which indicated a possible variation in drought recovery response time of grapevine cultivars after an irrigation/rainfall event.

The recovery of leaf transpiration was not directly dependent on the recovery of hydraulic conductance and was delayed until the following day (Lovisolo et al. 2008). Embolism repair occurred within several

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minutes to hours after re-watering (Zufferey, 2013b), while transpiration was still impaired by stomatal closure (Lovisolo et al. 2008) due to the remaining high ABA levels in the leaves. Leaf ABA is therefore not only important for limiting transpiration during water stress, but also during the early stages of recovery and embolism repair.

Water deficits over long periods of time cause adaptation in plants, making them more drought tolerant. Osmoregulation is one such strategy that maintain plant water status by increasing the cell turgor to enable stomatal opening at lower water potentials (Chaves et al. 2010). During & Dry (1995) also found that leaf gas exchange is enhanced by improved root water status *via* osmotic adjustment. There is limited evidence of osmoregulation in water-stressed roots (Vandeleur et al. 2009), which supports the findings of Davies & Zhang (1991) that the root cortex often dehydrates and dies in dry soil. However, the root tip remained turgid and connected to the plant by the stele. It is therefore possible that osmoregulation occurred only in the root tip to ensure growth potential of the root. Zhang & Davies (1989) suggested that secondary and tertiary roots might be less effective at maintaining their turgor than primary roots. This may indicate that primary roots would primarily focus on deeper penetration to reach possible moist soil layers. Vandeleur et al. (2009) found very high aquaporin expression signals in root tips that would enhance the capacity of the young, actively growing roots to absorb soil water under these conditions. Zhang & Davies (1989) further mentioned that water relations and metabolic activity may vary substantially from one part of the root system to the other, while certain classes of roots (sizes/diameter) may be better water sensors than others. It would therefore be beneficial in root studies to distinguish between the root classes when investigating the effects of water deficit on root growth and physiological activity.

Long-term water deficits may also lead to adaptation in leaf structure and anatomy as well as biochemical composition. Leaves that developed after the onset of water stress had smaller stomata and higher stomatal density (but still lower total leaf area covered by stomata), which should decrease total water loss *via* transpiration (Palliotti et al. 2008). Serra et al. (2014) found that the specific rootstock used might also affect stomatal density and pore diameter in scion leaves when vines are water constrained. In addition, chlorophyll *a*, *b* and carotenoid concentrations were significantly lower in new leaves that developed during water stress conditions. That would affect the optical properties of the leaves so that there is a substantial reduction in light absorbance and increase in transmittance. In contrast with Palliotti et al. (2008), Ping et al. (2015) and Salazar-Parra et al. (2015) found no difference in chlorophyll concentrations in leaves from well-watered and water stressed plants, while Leibar et al. (2015) found an increase in both chlorophyll *a* and *b* in water-stressed plants.

The cross-sectional area of primary shoots was reduced as well as the average diameter of the xylem vessels (Alsina et al. 2007; Palliotti et al. 2008) which may decrease vulnerability to embolisms and

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enhance recovery capacity after cavitation (Alsina et al. 2007). Higher amounts of suberin were found in root endo- and exodermal layers of water stressed compared to well-watered vines (Vandeleur et al. 2009). All of these anatomical adaptations of the roots may contribute to a reduced hydraulic conductivity in response to water stress.

It is clear that plants have many mechanisms on morphological, anatomical, physiological and biochemical levels to adapt to limiting environmental conditions. The responses are not exact and are dependent on various aspects, such as the genotype and phenological stage of the vine; the severity and duration of the climatic stress factors, as well as the way they interact with one another. Research results on a single climate factor may provide general indications of the expected response of the grapevine under future climatic conditions. However, multi-variable studies would provide a more complete picture of the net effect that increased temperature and CO_2 concentration with simultaneous decrease in water supply would have on grapevine behaviour.

2.4.4 Combined effects and interaction between climatic stress factors on photosynthetic activity

Plant response to stress conditions is ultimately aimed at sustaining growth and reproduction (Alsina et al. 2007), which can only be achieved by maintaining a balance in the plant between photosynthetic activity, growth and storage of reserves.

Diffusional limitation of photosynthesis would occur when there is resistance against the movement of ambient CO_2 into the leaf through the stomata and across the leaf into the chloroplasts through the mesophyll. Mesophyll conductance is a function of leaf anatomy (Tomás et al. 2013) and thus also the specific leaf area (SLA) (Salazar-Parra et al. 2012). Elevated CO_2 levels result in a decrease in mesophyll conductance (Aranjuelo et al. 2015), which may be due to the lower SLA (Long et al. 2004) and also thicker leaves (Moutinho-Pereira et al. 2009) found under these conditions. Mesophyll conductance is also reduced under water stress conditions (Flexas et al. 2002; Salazar-Parra et al. 2012), but increased exponentially between 10 ^{o}C and 40 ^{o}C although (especially at the higher temperatures) it remained limiting to photosynthesis (Bernacchi et al. 2002). Stomatal conductance generally follows the same pattern, with decreased rates during drought conditions (Flexas et al. 2002; Alsina et al. 2007; Palliotti et al. 2008) and elevated CO_2 levels (Long et al. 2004; Martínez-Lüscher et al. 2015). Stomatal conductance decreased with an increase in temperature (Greer & Weston, 2010; Pan et al. 2018), while a 2 ^{o}C higher increment in ambient temperature either did not affect stomatal conductance (Edwards et al. 2017) or increased it (Sadras & Moran, 2013).

The diffusional pathway of CO_2 from the leaf boundary layer into the chloroplast would determine the internal CO_2 concentration (C_i) and thus the CO_2:O_2 ratio. These two gasses compete for RuBP and RubisCO activity at the start of the Calvin cycle – carboxylation is part of the photosynthetic pathway

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and oxidation of RuBP results in photorespiration. Biochemical limitation of photosynthesis may occur due to decreased RuBP regeneration and a decrease in RubisCO content or carboxylation rate (Salazar-Parra et al. 2012).

Under conditions where water supply is not limited, photosynthesis is enhanced by increasing temperature up to 25 °C and starts to decrease at temperatures higher than 30 °C, due to the limited rate of carboxylation (Greer & Weedon, 2012). Elevated CO_2, on the other hand, increases photosynthetic rate both as a result of increased RubisCO carboxylation efficiency and capacity of RuBP regeneration (Long et al., 2004; Pan et al. 2018). When these two factors were combined, a synergistic effect was found with regards to the photosynthetic rate (Alonso et al. 2008; Edwards et al. 2017), while the optimal temperature range for photosynthesis increased with simultaneous increase in ambient CO_2 (Kriedemann et al. 1976; Long, 1991; Alonso et al. 2008; Greer & Weedon, 2012). Alonso et al. (2009) reported enhanced carboxylation, electron transport as well as net carbon assimilation rates when a high CO_2 level was combined with a high temperature (30-35 °C), but not with a low temperature (15-25 °C).

Transpiration rate in elevated CO_2 and higher temperature treatments was higher than that of the control, while the stomatal conductance was similar (Leibar et al. 2015; Edwards et al. 2017; Douthe et al. 2018). This caused a decrease in stem water potential, indicating lower hydraulic conductance on whole-plant level (Robredo et al. 2007; Leibar et al. 2015). The increase in transpiration rate was still smaller than the increase in photosynthesis, which therefore still resulted in a higher WUE (Douthe et al. 2018).

Given the above, it is expected that more carbon would be assimilated under higher temperature and CO_2 conditions where grapevines are adequately provided with water. A strong sink strength (and thus high export rate from the leaves to the receiving sink) is required to sustain high carbon assimilation rates (Salazar-Parra et al. 2015). Sugars tend to build up in leaves where export is limited (Hunter et al. 1994), resulting in down-regulation of photosynthesis (Keller, 2010; Salazar-Parra et al. 2012). Prevention of carbohydrate accumulation in leaves might be achieved by the development of new, strong sinks (e.g. new vegetative or reproductive structures), an increase in the growth rate or storage ability of existing sinks, or a higher respiration rate (Leibar et al. 2015; Morales et al. 2016). Elevated CO_2 could therefore alleviate the heat stress-induced limitations to photosynthesis (Pan et al. 2018) under expected future climatic conditions, provided that sink limitation of assimilate export and thus acclimation of photosynthesis does not occur.

Regarding sustained photosynthetic activity, a further advantage of elevated CO_2 combined with high temperature is the improved WUE found due to the decrease in stomatal conductivity (Martínez-Lüscher et al. 2015). According to Robredo et al. (2007), depletion of soil water content may therefore be

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postponed under these conditions, enabling the plant to sustain a high level of photosynthetic activity at high rates for longer. Martínez-Lüscher et al. (2015) also found that photosynthesis is enhanced and photorespiration reduced in high CO_2, high temperature and well-watered environments. However, under extreme water stress conditions, photorespiration was the highest in high CO_2 and temperature, which translates into decreased carboxylation and thus photosynthesis (Salazar-Parra et al. 2012; Leibar et al. 2015).

In the latter environments a marked increase in the electron transport rate (Leibar et al. 2015; Salazar-Parra et al. 2015) was also found. Excess electrons may react with O_2, generating reactive oxygen species (ROS) that could cause photo-oxidative damage to cell constituents, such as chlorophyll and carotenoids. The enhanced photorespiration (due to restricted carboxylation) might be a protective mechanism of the plant to increase the CO_2:O_2 ratio, and limit the accumulation of ROS, thereby mitigating oxidative stress especially under drought conditions (Voss et al. 2013).

Apart from the direct donation of electrons to O_2 in light, ROS may also be formed in plants in response to environmental stresses, such as drought or very high temperatures. This occurs when there is an excess of light excitation energy present as a result of decreased photosynthesis (Aranjuelo et al. 2008). Pan et al. (2018) found excessive production of ROS under heat stress conditions that caused damage to the photosynthetic apparatus, such as inside the chloroplasts where the redox state of Photosystems I and II was altered, as well as to cell structure, membranes and proteins (Zinta et al. 2018). Under dryland cultivation in warm to hot areas with high irradiance, canopy efficiency is drastically reduced by this photo-inhibition (Palliotti et al. 2008).

Numerous studies have found that high ambient CO_2 concentrations may protect the plant against photodamage caused by the higher levels of ROS that were formed by heat and drought stress (Zinta et al. 2014, 2018). Under elevated CO_2, the heat-induced damage to chloroplasts was reduced, while electron transport in Photosystems I and II was promoted by maintaining the redox balance (Pan et al. 2018). Salazar-Parra et al. (2015) found no evidence of either photo-inhibition or damage to leaf protein, chloroplasts or carotenoids under high CO_2 levels, and therefore concluded that CO_2 plays a protective role at current or elevated temperatures against the adverse effects of water stress. Antioxidants, such as phenolic compounds, protect cells against photo-oxidative damage caused by ROS (Król et al. 2014) and, according to Zinta et al. (2014), high CO_2 levels cause the upregulation of antioxidant metabolism which improve cell and tissue protection against ROS. In contrast, Aranjuelo et al. (2008) found that the protective effect of CO_2 was not due to improvements in the antioxidant system, but rather to the enhanced production of pigments *via* the xanthophyll cycle that increased the capacity for the dissipation of excess energy as heat under elevated CO_2.

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In view of the above, the earlier statement should be amended to "elevated CO_2 could alleviate the heat stress-induced limitations to photosynthesis under expected future climatic conditions, provided that sink limitation of assimilate export and thus acclimation of photosynthesis does not occur *and severe water stress is not experienced*."

2.4.5 Water use efficiency and the interaction between scion and rootstock cultivars

The ratio between carbon assimilation and either stomatal conductance or transpiration rate (they are generally closely correlated) are often used to express the efficiency of water use in the plant (WUE) at any given time. Instantaneous WUE (WUE_{inst}) is the ratio when transpiration rate is used, whereas when stomatal conductance is used the ratio is known as intrinsic WUE (WUE_i). With the calculation of WUE_{inst}, night time transpiration is often ignored, although it may be as high as 10-15 % of daytime transpiration, especially in warm and low air RH conditions when plants are well-watered (Schultz & Stoll, 2010; Medrano et al. 2015b). When large changes in air humidity are expected, it would be better to use the WUE_i to avoid the fluctuations in transpiration rate (Salazar-Parra et al. 2012; Leibar et al. 2015).

Any leaf has the capacity to regulate its photosynthetic and transpiration rates according to its environment and thereby constantly changing its WUE. Measurements taken to calculate WUE are done on leaf level and this is effectively used for comparative studies, such as comparing cultivars or study the efficacy of a specific cultivation practice (Medrano et al. 2015b).

However, the extrapolation of WUE from single-leaf measurements to whole plant level is not simple, because of the large effect that the position of a leaf on a shoot or in the canopy has on its WUE (Medrano et al. 2015b). Inside any canopy there is a large variation in terms of leaf exposure, physiological activity (also related to leaf age) and level of light interception during the day, which explains why the correlation between leaf measurements and whole plant/canopy values is weak (Douthe et al. 2018). Another reason is the largely unknown factor of dark respiration on whole plant level. Leaf respiration rates are measured, but the root system is responsible for the largest respiratory loss in carbon – about 70-80 % of total plant respiration (Serra et al. 2014; Medrano et al. 2015b) and is normally not measured. Douthe et al. (2018) also stated the possibility that grape berry and bunch respiration rates during ripening might have an important impact on the net carbon exchange of the plant and thus the WUE.

The upscaling of leaf WUE to whole plant or vineyard level is often done in literature where it is assumed that the WUE calculated for sample leaves is equal to the vine canopy as a whole. Both Salazar-Parra et al. (2012) and Leibar et al. (2015) found improved WUE_{inst} ratios under climate change (high temperatures and high CO_2) conditions where cuttings were water-stressed. They concluded that grapevine water use efficiency would improve under future climatic conditions. Stevens et al. (2008)

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made the suggestion to restrict irrigation application in order to improve WUE in the short term when water supply is limited. Douthe et al. (2018) also reported higher WUE_{inst} in water stressed leaves, but in contrast found that the estimated WUE_{inst} in well-watered canopies was much higher than in canopies with limited water available. According to Medrano et al. (2015b), plant/canopy WUE_i of water stressed vines were either higher, the same or lower, compared to well-watered vines, depending on the cultivar and the year (reigning growing conditions). Thus, they recommended that WUE_i (although an indicator of drought resistance) should not be used as the only parameter when screening cultivars for this characteristic.

Bota et al. (2016) confirmed that substantial genotypic variation in WUE_i occurs among cultivars. These differences were mainly ascribed to differences in stomatal behaviour (and thus conductance), rather than photosynthesis. Various attempts have been made to group cultivars according to the sensitivity of stomatal response to environmental stress, particularly water stress. However, too many discrepancies were found in research results due to the climatic conditions and environment, the intensity and duration of the stress, as well as the specific rootstock used with the scion cultivar (Chaves et al. 2010). A strict classification of cultivars as either isohydric or anisohydric is therefore not considered feasible (Chaves et al. 2010; Bota et al. 2016).

The environment and the effect of various kinds of stress on plant physiological behaviour have already been discussed, but not the important effect that the rootstock genotype and root system in general have on plant stress response. The ideal rootstock within a specific location is one that is able to sustain growth and grape ripening under adverse biotic and abiotic soil and environmental conditions (Hunter et al. 2016) and should therefore be specifically selected based on its natural properties to buffer the vine against unavoidable environmental stress. Root systems should be large and dense with deep penetration into the subsoil layers to optimally utilise the available soil volume – a large number of medium and fine roots (< 5 mm) will increase the water and nutrient absorption capacity of the root system (Hunter et al. 1995; Hunter, 1998).

When grafting a scion cultivar onto a rootstock, an interaction/integration between the two different genotypes is enforced. This interaction significantly affects all vegetative growth parameters as well as the ratio of biomass allocation to shoots and roots, respectively (Tandonnet et al. 2010). The rootstock affects the vigour of the scion by its ability to take up water and nutrients (Serra et al. 2014) and can modify the scion's response to edaphic stresses (Stevens et al. 2008), while the scion cultivar strongly affects the degree of root system development and growth (Tandonnet et al. 2010). The latter authors found that any difference in root:shoot biomass partitioning is primarily determined by the two genotypes involved, while inadequate supply of water or nutrients in the soil would increase the allocation/balance between the roots and the shoots (Hare et al. 1997; Salazar-Parra et al. 2015;

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Simonneau et al. 2017). This is probably due to the decrease in root cytokinin production under the adverse conditions (Hare et al. 1997), which would result in decreased shoot growth and increased root growth (Field et al. 2009). Cytokinins inhibit lateral root initiation and growth (Laplaze et al. 2007). This might be overturned should cytokinin levels decrease under stress conditions, with the result that not only the growth of existing roots, but the development of a finely branched, dense root system is obtained.

Another important aspect of the rootstock-scion interaction is the efficacy of root-to-shoot signalling that affects stomatal conductance (Serra et al. 2014) and thus leaf gas exchange in response to decrease in soil water status (Hare et al. 1997). Stomatal conductance in a grafted grapevine depends on both hydraulic and chemical signals (Peccoux et al. 2018).

High hydraulic conductivity within the root (Lovisolo et al. 2008) as well as between the rhizosphere and soil-root interface (Peccoux et al. 2018) is an important characteristic of drought-tolerant rootstocks. Higher hydraulic conductivity is partly due to higher aquaporin expression and activity (Gambetta et al. 2012). Holbrook et al. (2002) argued that a precursor or signal (other than ABA) is produced in roots that could trigger aquaporin operation and thus improve hydraulic conductivity. Whether ABA is directly involved in aquaporin expression and activity or not, they provide important pathways for water between plant cells and tissues, and between root tips, where the strongest aquaporin expression signal was found (Vandeleur et al. 2009), and leaves (Maurel et al. 2015). High hydraulic conductivity is associated with large xylem vessels, but also with an increased vulnerability to cavitation (Lovisolo & Schubert, 1998). Aquaporins are also involved in embolism repair, which occurs overnight in the relative absence of transpiration due to stomatal closure (Lovisolo et al. 2008).

ABA is well-known for limiting transpiration in plants by inducing stomatal closure through its effect on K^+ and Cl^- ion fluxes (Leung & Giraudat, 1998) and thus guard cell turgor. Due to the sometimes weak correlation between leaf water potential and stomatal conductance in plants that are experiencing a drought, the conclusion was made that, since stomata respond to the soil water deficit and not to the leaf water potential (Hare et al. 1997), a long distance signal from the roots must cause the closing of the stomata.

Since ABA was the only compound found to increase in the roots during soil drying, Davies & Zhang (1991) concluded that ABA is synthesised in roots during conditions of soil water deficit and then mobilised to the leaves in order to induce stomatal closure. Zhang & Outlaw (2001) also mentioned the strong correlation between accumulation of root-source ABA in the apoplast of the guard cells and the decline in stomatal conductance. More recent studies, however, have shown that stomatal closure is possible without root-derived ABA (Holbrook et al. 2002) and that it is more closely related to ABA production in the leaves (Holbrook et al. 2002; Soar et al. 2006) and subsequent translocation to the

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xylem sap. Cramer (2010) found that the ABA concentration in particularly the xylem sap was positively correlated to stomatal closure in response to water deficit, and neither root nor leaf ABA was directly responsible for a decrease in stomatal conductance and transpiration (Soar et al. 2006). The latter study also found an increase in the expression of important genes involved in ABA biosynthesis in the leaves in response to high vapour pressure deficit (VPD), but not in the roots. It therefore seems as if production of ABA is stimulated in the specific tissues directly exposed to the stress, since Zhang & Outlaw (2001) found that direct water-stressing of leaves increased the biosynthesis of ABA therein.

The response to any growth regulator (including ABA) depends on a change in the concentration of the phyto-hormone as well as an increasing sensitivity of the target tissue (Hare et al. 1997). Abscisic acid concentration in a plant cell at any given time is dynamic and depends on a complex of processes (*in situ* biosynthesis, catabolism, export to other cells, import from adjacent cells) occurring in response to environmental signals (Cutler & Krocho, 1999; Zhang & Outlaw, 2001). Furthermore, the sensitivity of guard cells to endogenous ABA might be affected by stress-related alterations in cytokinin levels (Hare et al. 1997) as well as the decrease in leaf water potential that normally results from water deficit conditions (Chaves et al. 2010).

Several conjugates of ABA and its metabolites have been reported (Cutler & Krochko, 1999), with ABA glucose ester (ABA-GE) being the main catabolite found in high water deficit treatments. Previously it was stated that these glucose conjugates have no biological activity, are not to be considered as a form of reserve for ABA, and appear to be a major pathway for the inactivation of ABA (Cutler & Krochko, 1999). In contrast, Jiang & Hartung (2008) postulated the existence of an ABA-GE transporter to release substantial amounts of this conjugate into the xylem under stress conditions, since it is not bio-membrane permeable. The ABA-GE is translocated through the stem xylem without any loss to the surrounding tissues, because it is extremely hydrophilic (Sauter et al. 2002). β-D-glucosidase is the enzyme that releases free ABA from its conjugates (Hartung et al. 2002) and its activity was found to increase substantially in conditions of salt stress (Sauter et al. 2002). It could very well be possible that the activity of these enzymes would increase under any abiotic stress condition, including water stress. ABA-GE could therefore be considered a pool of reserve ABA to be quickly released to control stomatal opening (Balint & Reynolds, 2013).

Whether ABA reaches the guard cells *via* the leaves or the roots, in its pure form or released from the ester conjugate, it remains an effective and specific stress tolerance mechanism for plants growing in drying soils and environments.

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2.5 CURRENT RESEARCH AND SHORTCOMINGS OF STUDIES ON THE EFFECT OF CLIMATE CHANGE ON THE GRAPEVINE

2.5.1 Climate change research on the grapevine

A lot of research has been (and is still currently being) done on the relationship between environmental factors and the physiological and growth response (vegetative and reproductive) of the grapevine. There are significant differences between these experiments, especially regarding the test environment and/or infrastructure; the type and age of the plant material used; the size of the samples and the number of repetitions performed as well as the treatment levels of the potential climatic stress factors (refer to Table 2.4 for a non-extensive list as illustration). The treatments applied also differ significantly regarding the extent/degree of the stress applied, the phenological stage(s) of application and its duration. The grapevine is able to recuperate after stress conditions, which depends on the genotype but also on the climatic environment of the vineyard location (Herrera et al. 2019). Reversible strains (elastic) might become plastic (irreversible) should stress conditions continue for too long (Hunter & Myburgh, 2001).

Results of these experiments are often contradictory, which may partly be due to a difference in experiment design and/or methodology used, rather than the treatment effect. Interpretation is thus often difficult, but the knowledge acquired is of extreme importance for wine grape cultivation in future.

Climate models may be used to determine the suitability of a region for a specific purpose and are often used in combination with crop models to generate future projections of yield, phenology and possible stress indicators for grapevines (as already discussed). Even sophisticated methods have their limitations, since certain assumptions and generalisations are always required in the programming (Fraga et al. 2016), while other factors (such as air relative humidity and wind speed that affect evapotranspiration) are often omitted (Bois, 2019), decreasing the accuracy of predictions.

Meta-analysis is also a tool used in research to combine the findings of various research projects. However, according to Curtis & Wang (1998), it is limited in establishing causal relations where categorical groups created by the meta-analyst were not randomly assigned treatments within the primary studies. Meta-analysis is therefore not a substitute for a well-designed, multi-factorial experiment.

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Table 2.4 Comparison between methodologies and treatment levels of research done on the effect of climatic stress factors on the grapevine.

Test environment and/or infrastructure	Examples of publications
Commercial or experimental vineyards	Patakas et al. (1997); Flexas et al. (2002); Stevens et al. (2008); Martínez & Chacón (2010); De Bei et al. (2011); Dayer et al. (2013); Galat Giorgi et al. (2013); Zufferey (2013a); Bota et al. (2016)
Free-Air CO_2 enrichment (FACE)	Bindi et al. (2001); Long et al. (2004); Reineke & Selim (2019)
Open Top Chambers (OTC)	Moutinho-Pereira et al. (2009); Sadras & Moran (2013); Edwards et al. (2017)
Outdoors, in pots	Flexas et al. (2002); Palliotti et al. (2008)
Growth chambers; green/glasshouses; temperature gradient greenhouses	Düring (1998); Lovisolo et al. (2008); Field et al. (2009); Salazar-Parra et al. (2012; 2015); Król et al. (2014); Hochberg et al. (2015); Kizildeniz et al. (2015); Leibar et al. (2015); Martínez-Lüscher et al. (2015); Morales et al. (2016); Hernández-Montes et al. (2019)
Type of plant material	**Examples of publications**
Microvine	Torregrosa et al. (2017)
Fruit-bearing cuttings; own roots	Salazar-Parra et al. (2012; 2015); Kizildeniz et al. (2015); Leibar et al. (2015); Martínez-Lüscher et al. (2015); Torregrosa et al. (2017)
Vines on own roots	Galat Giorgi et al. (2013)
Grafted vines	Patakas et al. (1997); Flexas et al. (2002); Lovisolo et al. (2008); Palliotti et al. (2008); Stevens et al. (2008); Moutinho-Pereira et al. (2009); Martínez & Chacón (2010);De Bei et al. (2011); Dayer et al. (2013); Zufferey (2013a); Hochberg et al. (2015); Bota et al. (2016); Reineke & Selim (2019)
Age of plant material	**Examples of publications**
Seedlings, 8 weeks	Król et al. (2014)
Mature vines, age unknown	Zufferey (2013a); Edwards et al. (2017)
1 -2 years	Flexas et al. (2002); Lovisolo et al. (2008); Hochberg et al. (2015)
3 – 5 years	Düring (1998); Field et al. (2009); Palliotti et al. (2008); De Bei et al. (2011)

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7 – 10 years	Patakas et al. (1997); Stevens et al. (2008); Moutinho-Pereira et al. (2009); Martínez & Chacón (2010); Galat Giorgi et al. (2013); Bota et al. (2016); Reineke & Selim (2019)
12 years	Dayer et al. (2013)
20 years	Bindi et al. (2001); Flexas et al. (2002)
Sample size and number of repetitions	**Examples of publications**
14 vines per treatment; 2 repetitions	Bindi et al. (2001)
18 vines per treatment	Lovisolo et al. (2008)
20 vines (10 control; 10 treatment)	Palliotti et al. (2008)
4 repetitions/years	Stevens et al. (2008)
30 vines (15 control; 15 treatment)	Field et al. (2009)
2 repetitions/seasons	Sadras & Moran (2013)
8 vines per treatment; 2 repetitions	Salazar-Parra et al. (2012)
40 plants per treatment; 2 repetitions	Zufferey (2013a)
4 repetitions	Król et al. (2014)
8 vines per chamber; two repetitions	Hochberg et al. (2015)
10 plants per treatment; done once	Kizildeniz et al. (2015)
8-10 vines per treatment	Leibar et al. (2015)
12 vines per treatment; done once	Martínez-Lüscher et al. (2015)
5-6 vines per treatment; 2 measures per vine	Salazar-Parra et al. (2015)
10 plants per cultivar (23 cultivars); 3 repetitions	Bota et al. (2016)
12 vines per treatment; 3 repetitions/years	Edwards et al. (2017)
18 vines (9 control; 9 treatment) per measurement time; 2 measurement times	Reineke & Selim (2019)
Various treatment levels of potential climatic stress factors	**Examples of publications**
Water	
100 % water capacity *vs* 12-14 days no irrigation	Düring (1998)
Daily irrigated *vs* irrigation stopped for 6 days	Flexas et al. (2002)
90 % of max available *vs* 40 % of max available	Palliotti et al. (2008)
8 $ML.ha^{-1}.yr^{-1}$ *vs* 5 $ML.ha^{-1}.yr^{-1}$	Stevens et al. (2008)

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100 % (5 ML.ha^{-1}.yr^{-1}); then 50 %; 30 % and 10 %	De Bei et al. (2011)
100 % ET *vs* 25 % ET	Dayer et al. (2013)
70 % soil moisture *vs* 30 % soil moisture; 14 days	Król et al. (2014)
Field Water Capacity (FWC) *vs* 60 % of FWC	Leibar et al. (2015)
100 % of max available *vs* 40 % of max available	Salazar-Parra et al. (2015)
Well-watered at flowering, then no irrigation	Bota et al. (2016)
<u>Temperature</u>	
Soil temperature 13 °C and 23 °C	Field et al. (2009)
Ambient *vs* Ambient + 2 °C	Sadras & Moran (2013)
24/14 °C *vs* 28/18 °C	Salazar-Parra et al. (2012)
Heat waves (ambient + 6-10 °C)	Galat Giorgi et al. (2013)
25/20 °C *vs* 35/30 °C	Hochberg et al. (2015)
Ambient *vs* Ambient + 4 °C	Kizildeniz et al. (2015)
24/14 °C *vs* 28/18 °C	Leibar et al. (2015)
24/14 °C *vs* 28/18 °C	Martínez-Lüscher et al. (2015)
Ambient *vs* Ambient + 4 °C	Salazar-Parra et al. (2015)
Ambient *vs* Ambient + 2 °C	Edwards et al. (2017)
Control *vs* Control + 10 °C	Hernández-Montes et al. (2019)
<u>CO_2</u>	
Ambient; 550 ppm; 700 ppm	Bindi et al. (2001)
375 ppm *vs* 700 ppm	Salazar-Parra et al. (2012)
400 ppm *vs* 700 ppm	Kizildeniz et al. (2015)
375 ppm *vs* 700 ppm	Leibar et al. (2015)
Ambient *vs* 700 ppm	Martínez-Lüscher et al. (2015)
400 ppm *vs* 700 ppm	Salazar-Parra et al. (2015)
Ambient *vs* 650 ppm	Edwards et al. (2017)
Ambient (395±0.4 ppm) *vs* 460±12 ppm	Reineke & Selim (2019)
Phenological stages of application and/or duration	**Examples of publications**
3 and 15 consecutive days before budbreak	Galat Giorgi et al. (2013)

Dormancy to flowering	Field et al. (2009)
From budbreak for 5 months	Bindi et al. (2001)
At 5-6 months after budbreak; for 10 days	Lovisolo et al. (2008)
For 2 weeks	Król et al. (2014)
Whole season	De Bei et al. (2011); Reineke & Selim (2019)
From prior to budburst for 3 years	Edwards et al. (2017)
After budding; for 4 seasons	Stevens et al. (2008)
Shoot length 70 cm; for 7 days	Hochberg et al. (2015)
Flowering, pre-véraison, véraison; stress 7 days, recovery 7 days	Hernández-Montes et al. (2019)
Flowering to fruit ripeness	Zufferey (2013a)
Fruit set to véraison	Palliotti et al. (2008)
From berry set to ripeness	Dayer et al. (2013); Kizildeniz et al. (2015); Leibar et al. (2015); Martínez-Lüscher et al. (2015)
Véraison to ripeness	Salazar-Parra et al. (2012; 2015)
From after véraison; for 2 growing seasons	Sadras & Moran (2013)

Factor or combination of factors	Examples of publications
Water	Patakas et al. (1997); Düring (1998); Flexas et al. (2002); Lovisolo et al. (2008); Palliotti et al. (2008); Stevens et al. (2008); Martínez & Chacón (2010); De Bei et al. (2011); Dayer et al. (2013); Zufferey (2013a); Król et al. (2014); Bota et al. (2016)
Temperature	Field et al. (2009); Galat Giorgi et al. (2013); Hochberg et al. (2015); Torregrosa et al. (2017)
CO_2	Bindi et al. (2001); Moutinho-Pereira et al. (2009); Reineke & Selim (2019)
Water and Temperature	Sadras & Moran (2013); Hernández-Montes et al. (2019)
Temperature and CO_2	Martínez-Lüscher et al. (2015); Edwards et al. (2017)
Water, Temperature and CO_2	Salazar-Parra et al. (2012; 2015); Kizildeniz et al. (2015); Leibar et al. (2015)

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2.5.1.1 Effect of test environment and/or infrastructure

A lot of the research on the effect of changing climate factors was done in greenhouses, glasshouses or in growth chambers (Table 2.4). Even with a highly transparent cover and tightly controlled environment (e.g. Perez Peña & Tarara, 2004), the air temperature inside increased by about 2.5 °C and the irradiation decreased by 10 %. According to Long et al. (2004), OTC's block UV-B radiation, affecting the irradiation received by plants, while the chamber effect (space restriction) on results may be large. Poorter et al. (2016) found that the ratio between the daily amount of sunlight and temperature (photothermal ratio) was consistently lower inside the glasshouses or growth chambers, which may affect overall physiology, growth rate and morphology of the plants. They also reported constant air humidity in growth chambers, while it may vary in glasshouses due to the available options of windows in the roof, the installation of fans, amount and frequency of irrigation of plants, etc.

Düring (1998) found that carbon assimilation in leaves that developed and grew in the relative low light intensity of a glasshouse (400 ppm) decreased when exposed to ambient light. This photo-inhibition was further increased under stress conditions (water deficit or waterlogging).

The WUE_i values of fruit-cuttings in glasshouses were more than 100 % higher than those of field grown vines (Morales et al. 2016). When discussing research results of OTC, Long et al. (2004) advised that results should be reported as relative values or percentage variation from the control values. Although the specific values are not expected to be similar compared to field-conditions, the direction of a response should be the same. The OTC studies tend to overestimate biomass, production and yield (Leakey et al. 2009).

The FACE studies use lower CO_2 levels (550-600 ppm) compared to the 700 ppm often used in growth chambers, which could complicate comparison between findings. It has the advantage that all the tissues started to grow under the test conditions (no pre-growth in ambient environments), but the test population is very small with a low number of replications.

2.5.1.2 Type and age of plant material

Fruit-cuttings on own roots are often used in climate change experiments (Table 2.4), since the size and quality of the yield may also be investigated. However, it has the limitation of using own roots and thus excluding the interaction effect between the scion and rootstock. The physiological functioning and growth response of the grafted vine, and therefore a closer simulation of the behaviour in commercial vineyards, cannot be investigated in this way. The age of the vines in Table 2.4 differed between 8 weeks and 20 years old. It is expected that the response rate and magnitude as well as adaptation capabilities of applied treatments will differ largely among them, which will complicate the interpretation and comparison between findings.

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2.5.1.3 Sample size and number of repetitions

The small sample size and low number of repetitions often mentioned in Table 2.4 could be a cause for concern regarding the validity and repeatability of the results. The use of small populations is understandable due to the lack of physical space available in these experimental set-ups, but then the number of repeats should ideally be increased to improve the level of confidence in the results.

2.5.1.4 Various treatment levels, duration and timing of application of potential climatic stress factors

The vine has a high capacity of adapting to changing environmental conditions, which could be plastic or elastic, depending on a large array of internal and external factors. However, there is a very large variation in especially drought and temperature treatments (Table 2.4), regarding the severity of the stress as well as the duration and time of application. Also, the treatment criteria were often too vague when referring to the specific temperatures or irrigation volumes applied. Using "ambient temperature" as control only makes sense when this temperature is specifically monitored and mentioned. The effect of a changed environment on plant growth, functioning and product quality is subject to the reference conditions (as already discussed). It would also differ within the same plant, depending on the tissue type (roots, leaves or bunches); the tissue age (old, thick roots compared to young, fine roots and also young *vs* old leaves) and the phenological stage (and thus the reigning source:sink balance) when these changes are made.

It should also be kept in mind that CO_2 levels are continuously increasing. About a decade ago, the atmospheric concentration was 375 ppm, while currently it is at 410 ppm. The predicted "doubling in current levels by the end of the century" (as often quoted from the IPCC) will then also depend on the year of reference.

An ideal situation would be where treatment criteria of projects are standardised to simplify comparisons and interpretation of the results. However, this would be extremely difficult due to practical factors such as available funds, infrastructure and technical assistance.

2.5.2 Research opportunities/knowledge gaps with application value

It is important to keep in mind that vineyards in future would be planted, and would develop and grow under already-changed climatic scenarios, which is not the same as submitting an unstressed, already established cutting/grapevine to these conditions. Differences in leaf anatomy, morphology and physiology have been found between existing leaves being exposed to stress environments and leaves developing and growing within these environments (as already discussed). The change in climate will be gradual and although currently planted vines would most likely continue to grow in "future

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climates", there should be sufficient time/opportunity for them (as well as the grape producer and industry) to adapt.

Literature available on the effect that future climatic conditions may have on grapevines and winegrape production evoked a few questions:

- How would newly-planted commercial "vineyards of the future" function under projected climate conditions?
- How will such vineyards cope in wine regions that are currently already considered as warm?
- Would current cultivation practices still apply, or would significant adaptations be required in future?

The closer research treatments can simulate the "real" climatic conditions that are expected, the easier the translation between the research results and the producer will be. This would enable the wine industry to be pro-active in assessing important future impacts of climate change so that producers will be well-informed and ready to implement suitable adaptive measures, such as replacing current cultivars with more suitable options or adjusting vineyard management practices to decrease the environmental impact and footprint and ensure economic sustainability.

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CHAPTER 3: INTEGRATIVE EFFECTS OF CLIMATE CHANGE FACTORS (TEMPERATURE, CO_2 AND WATER) ON SOME PHYSIOLOGICAL PROCESSES IN YOUNG, GRAFTED GRAPEVINES

ABSTRACT :

The physiological activity of young, potted grapevines was measured at four week intervals during the first 12 weeks after planting. The effect of different combinations of ambient temperature (maximum ranges of 27-31 °C, compared to 30-34 °C), ambient CO_2 (400 ppm *vs* 800 ppm) and soil water (irrigation to water holding capacity and 50 % thereof), applied immediately after planting, on physiological performance of young vines was investigated under glasshouse conditions. Two scion cultivars (Shiraz and Merlot), both grafted onto rootstock 101-14 Mgt, were used in this study. Stomatal conductance, transpiration and photosynthetic rates decreased during the course of the growth period in all treatment combinations and both cultivars. Both chlorophyll *a* and *b* concentration in primary leaves decreased during this time, while the chlorophyll *a/b* ratio showed a slight increase. At 8 and 12 weeks after planting, photosynthesis and total chlorophyll were negatively correlated. A strong, positive correlation existed between total chlorophyll and leaf nitrogen concentration. Elevated CO_2 levels resulted in better WUE_i in grapevines, due to a relatively stronger stimulating effect on the photosynthetic rate compared to stomatal conductance. Synergy between high CO_2 and high temperature with regards to photosynthesis was observed, especially when water stress was not severe. The CO_2 was able to mitigate the negative effect of water deficit on physiological activity to a certain extent. Shiraz and Merlot had similar reaction patterns to the treatment factors, but Merlot seemed more sensitive to water deficit and at the same time was more responsive to increased CO_2 levels. The study showed that as long as water deficit does not become too severe (at levels where structural damage starts to occur), physiological activity of young grapevines would be enhanced by projected climates in future.

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3.1 INTRODUCTION

Over the last few decades, the average temperature during the grapevine growing season has increased in most of the global wine producing regions. This warming was not uniform, with higher warming rates in the Northern than in the Southern Hemisphere (Jones et al. 2005; Webb et al. 2013) and a higher increase at higher than at lower latitudes. A higher frequency of temperature extremes was measured (Jones, 2007), while higher day and night temperatures (especially during spring) have been reported (Koch & Oehl, 2018). Atmospheric CO_2 is continually on the rise with current levels at 410 ppm (NOAA-ESRL, 2019), compared to about 340 ppm in 1980. This is considered to be the main cause of warming (IPCC, 2014) and higher CO_2 and temperature levels would thus be an inseparable combination in future climates.

Changing snow and rainfall patterns are not as consistent as that of temperature, but climatic models generally indicate a wetter climate for higher latitude regions (such as New Zealand, the Mosel Valley in Germany and the north of Oregon in the USA) and a drier climate for Southern Europe, Australia and South Africa (Webb et al. 2013). In many winegrowing regions of the world, the water requirement of vineyards (300-700 mm) is already higher than the annual mean precipitation (Medrano et al. 2015). Higher ambient temperature would further increase evapotranspiration, negatively affecting the yield due to water stress in the vines (Fraga et al. 2016).

It is very difficult to define clear relationships between climatic conditions and grapevine performance (Schultz, 2011), due to the large natural adaptive physiological capacity (plasticity) of the grapevine (Jones & Alves, 2013; Seguin & Garcia de Cortazar, 2015). Multi-factorial research on the combined effect of increased CO_2, increased temperature, and decreased water availability on plant response is therefore an important objective (Hunter et al. 2010; Salazar-Parra et al. 2012; Zinta et al. 2018) to expand knowledge on the mechanisms that regulate growth and physiological functioning of the grapevine in reaction to the changing environment.

Any response of a plant to its environment is ultimately aimed at sustaining its growth and reproduction (Alsina et al. 2007). Therefore, a balance must be maintained between carbon assimilation and the utilisation and distribution of assimilates in the plant to ensure growth and maintenance, but also to provide for the storage of reserves. Simply stated, carbon assimilation is dependent on the supply of CO_2 to the chloroplasts, the carboxylation activity of RubisCO (ribulose-1,5-bisphosphate carboxylase/oxygenase), availability of RuBP (ribulose-1,5-bisphosphate) as substrate, and the export rate of assimilates from the leaves to prevent feedback inhibition.

There are conflicting reports on the effect of increased temperature and CO_2 on stomatal conductance. Greer & Weston (2010) and Pan et al. (2018) found a decrease in conductance with an increase in

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temperature, Edwards et al. (2017) found no difference, while Sadras and Moran (2013) and Urban et al. (2017) stated that stomatal conductance increases with an increase in temperature. High CO_2 levels induce partial closure of stomata and therefore a decrease in conductance (Long et al. 2004; Martínez-Lüscher et al. 2015). However, in warm, dry environments (or during drought periods) plants can respond to elevated CO_2 by increasing their stomatal conductance (Purcell et al. 2018). This again, seems to contradict the findings of Flexas et al. (2002), Alsina et al. (2007) and Palliotti et al. (2008) who stated that the rate of stomatal conductance decreased under drought conditions. How the stomata would react to the combination of high CO_2, temperature and low water availability needs to be clarified, especially due to the link with transpiration and the degree of water stress induced in the plant.

Elevated CO_2 levels (Long et al. 2004; Aranjuelo et al. 2015) and water stress conditions (Flexas et al. 2002; Salazar-Parra, 2012) decreased mesophyll conductance. Temperature, on the other hand, increased conductance exponentially between 10 ^{o}C and 40 ^{o}C (Bernacchi et al. 2002). The diffusional pathway of CO_2 from the leaf boundary layer into the chloroplast would determine the internal CO_2 concentration (C_i) and thus the CO_2:O_2 ratio. The higher the ratio, the more photosynthesis is enhanced in relation with photorespiration (Long, 1991; Zufferey et al. 2000).

High ambient CO_2 increased the rate of photosynthesis (Long et al., 2004; Pan et al. 2018). A temperature increase enhanced photosynthesis up to 30 ^{o}C (Greer & Weedon, 2012), whereas at higher temperatures it started to decrease again. When these two factors were combined, a synergistic effect was found with regards to the photosynthetic rate (Alonso et al. 2008; Edwards et al. 2017), while the optimal temperature range for photosynthesis increased with simultaneous increase in ambient CO_2 (Kriedemann et al. 1976; Long, 1991; Alonso et al. 2008; Greer & Weedon, 2012). This was ascribed to (amongst others) an increase in carboxylation rate of RuBP by RubisCO (Alonso et al. 2009).

Chlorophyll content in leaves may be used as an indication of malnutrition (Girardin et al. 1985) or leaf senescence during the growth season (Martínez-Lüscher et al. 2015), while a breakdown in chlorophyll could be a sign of photo-oxidative damage by reactive oxygen species (ROS) in response to environmental stresses, such as high temperatures (Pan et al. 2018) or drought (Palliotti et al. 2008). The effect of climate change parameters on chlorophyll is not clear. Ping et al. (2015) and Salazar-Parra et al. (2015) found that water stress had no effect on chlorophyll, while Leibar et al. (2015) found an increase in both chlorophyll *a* and *b* in water-stressed plants. In direct contrast, chlorophyll decreased in other studies as result of water stress (Sanchez et al. 1983; Zhang et al. 2011; Urban et al. 2017). According to Palliotti et al. (2008), new leaves that developed after the onset of drought contained lower chlorophyll *a* and *b* concentrations than existing leaves. Leibar et al. (2015) reported an increase in both chlorophyll *a* and *b* in response to the combination of high temperatures and high CO_2 levels, with a

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decrease in chlorophyll *a/b* ratio. In contrast, no difference in chlorophyll *a* and *b* respectively was found by Martínez-Lüscher et al. (2015), yet there was a significant increase in chlorophyll *a/b* ratio.

A strong sink strength (and thus high export rate from the leaves) is required to sustain high carbon assimilation rates (Salazar-Parra et al. 2015). Sugars tend to increase in leaves where export is limited (Hunter et al. 1994), resulting in down-regulation of photosynthesis (Keller, 2010; Salazar-Parra et al. 2012). Prevention of carbohydrate accumulation in leaves might be achieved by the development of new, strong sinks (e.g. new vegetative or reproductive structures), an increase in the growth rate or storage ability of existing sinks, or a higher respiration rate (Leibar et al. 2015; Morales et al. 2016).

When severe water stress was experienced under high CO_2 and temperature conditions, photorespiration was enhanced to the detriment of photosynthesis (Salazar-Parra et al. 2012; Leibar et al. 2015). It is therefore expected that elevated CO_2 levels may alleviate heat stress-induced limitations to photosynthesis under expected future climatic conditions, provided that sink limitation of assimilate export (and thus acclimation of photosynthesis) does not occur and severe water stress is not experienced.

Loveys et al. (2003) observed that the temperature effect on photosynthesis depends on the temperature at which the specific leaves developed. Purcell et al. (2018) found different stomatal responses to elevated CO_2 conditions, depending on the temperature and water supply conditions already experienced. It is thus expected that the effect of one climatic stress factor on grapevine functioning and growth would depend on the severity of other stress factors and that the environment in which the vine developed would also affect the level of plant response. In future, vineyards would be planted and cultivated under a different climatic environment compared to the current conditions. It stands to reason that their growth and functioning would be different than, for example, a mature, established vineyard growing under current climatic conditions that is suddenly exposed to future projected climates.

In this part of the study the combined effect of projected climate change conditions (high ambient temperature, elevated CO_2 and water deficit) on the physiology of grafted grapevines during the first 12 weeks after planting was measured under controlled conditions in glasshouse compartments. This is a novel approach to gain a better understanding of how young vines would function and grow (at leaf, root and whole-plant level) under future climates during the very important young vineyard establishment stage. Shiraz was chosen based on its proven record in warm wine producing areas with water scarcity, while it was expected of Merlot to provide better insights into the behaviour of more stress-sensitive scion cultivars. The rootstock 101-14 Mgt was selected due to its perceived sensitivity to water stress conditions.

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3.2 MATERIALS AND METHODS

3.2.1 Study location and glasshouse compartments

Four glasshouse rooms situated at ARC Infruitec-Nietvoorbij, Stellenbosch, were used to accommodate the different treatments. The rooms were 2.4 m X 6.0 m each and prepared according to the treatment criteria depicted in Table 3.1 and the schematic layout in Figure 3.1. The experiment comprised of five consecutive growth cycles (planting times during the first week of February and the first week of September), using Shiraz (SH 470) as scion cultivar for the first three, and Merlot noir (MO 348) for the other two. Both scions were grafted onto rootstock 101-14 Mgt. The potted vines were randomly allocated per glasshouse compartment in a randomised complete block design.

Table 3.1 Treatment combinations randomly allocated in four glasshouse compartments for five growth cycles

PARAMETER	TREATMENTS											
	C0T0			C1T0			C0T1			C1T1		
Vine age (weeks)	4	8	12	4	8	12	4	8	12	4	8	12
CO_2 levels (ppm)	400	400	400	800	800	800	400	400	400	800	800	800
Temperature (°C)	15/27	15.5/29	16/31	15/27	15.5/29	16/31	18/30	18.5/32	19/34	18/30	18.5/32	19/34
Water treatments	(wet): WC (dry): $\frac{1}{2}$WC	(wet): WC (dry): $\frac{1}{2}$WC	(wet): WC (dry): $\frac{1}{2}$WC	(wet): WC (dry): $\frac{1}{2}$WC	(wet): WC (dry): $\frac{1}{2}$WC	(wet): WC (dry): $\frac{1}{2}$WC	(wet): WC (dry): $\frac{1}{2}$WC	(wet): WC (dry): $\frac{1}{2}$WC	(wet): WC (dry): $\frac{1}{2}$WC	(wet): WC (dry): $\frac{1}{2}$WC	(wet): WC (dry): $\frac{1}{2}$WC	(wet): WC (dry): $\frac{1}{2}$WC

C0: Lower CO_2 (400 ppm); C1: Higher CO_2 (800 ppm); T0: Lower temperature; T1: Higher temperature (T0 max + 3°C); WC: Water-holding capacity

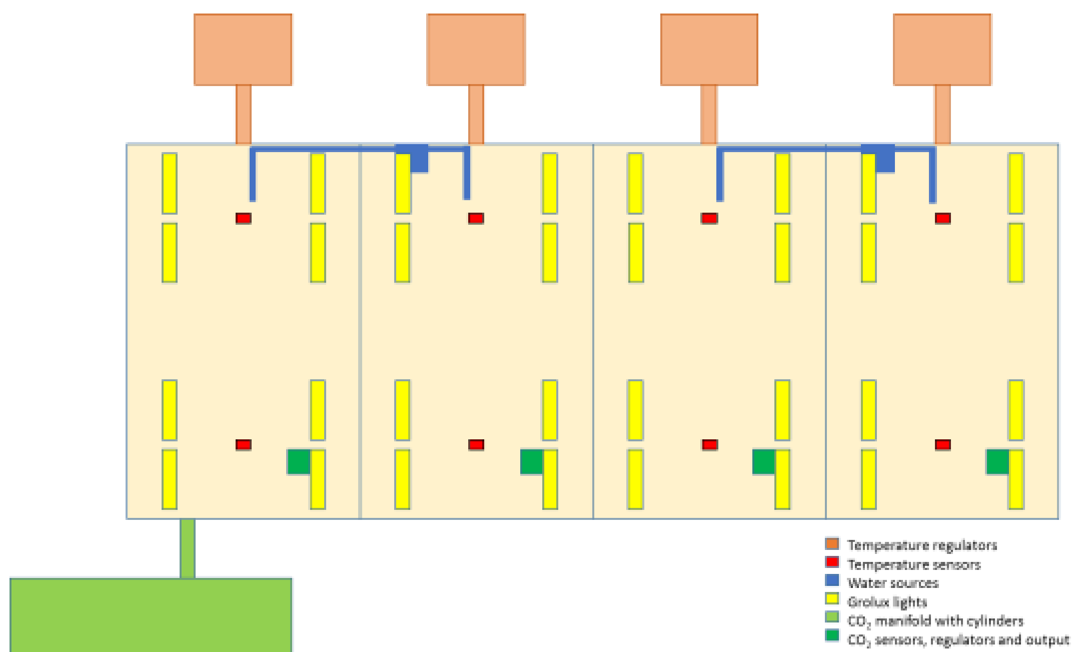

Fig. 3.1 Schematic presentation of the layout of the four glasshouse compartments and sensors used in this trial (not according to scale).

3.2.1.1 Light augmentation and distribution

Eight double IP65 PVC enclosures provided with 1.2 m Grolux tubes (36W) (Sylvaia, Germany) were installed in each room to supplement natural light and extend daylight hours when required. These were linked to timers that switched on between 06:00 and 18:00 every day. However, when the light intensity and distribution in the rooms were monitored, it was found that the lights had no significant effect on the light intensity inside the glasshouse rooms (Fig. 3.2).

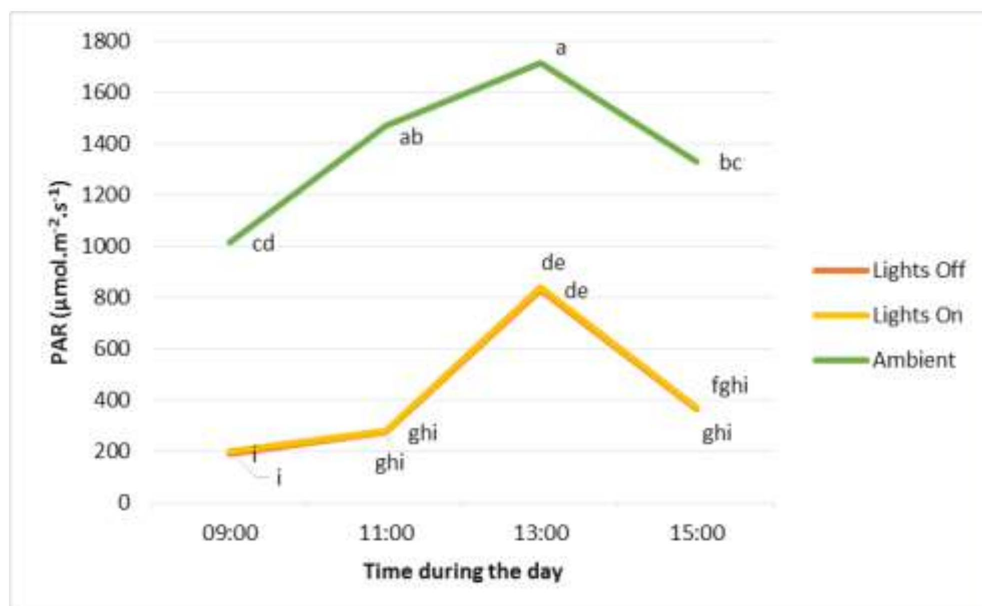

Fig. 3.2 Comparison between ambient PAR, and the PAR in the centre of one of the glasshouse rooms (with and without the lights on) during the course of the day. Data points with the same letters do not differ significantly ($p \leq 0.05$).

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Before and after each growth cycle the Photosynthetic Active Radiation (PAR) at five fixed points per room was measured to determine the degree of variation within and between rooms. Although variation was found between the points within the rooms (Fig. 3.3), especially at 13:00 and 15:00 (data not shown), the average light intensity and distribution patterns among the four rooms did not differ significantly. It was concluded that light exposure in the various rooms was comparable during the course of each growth cycle and that it would not have been an important nuisance variable in this study.

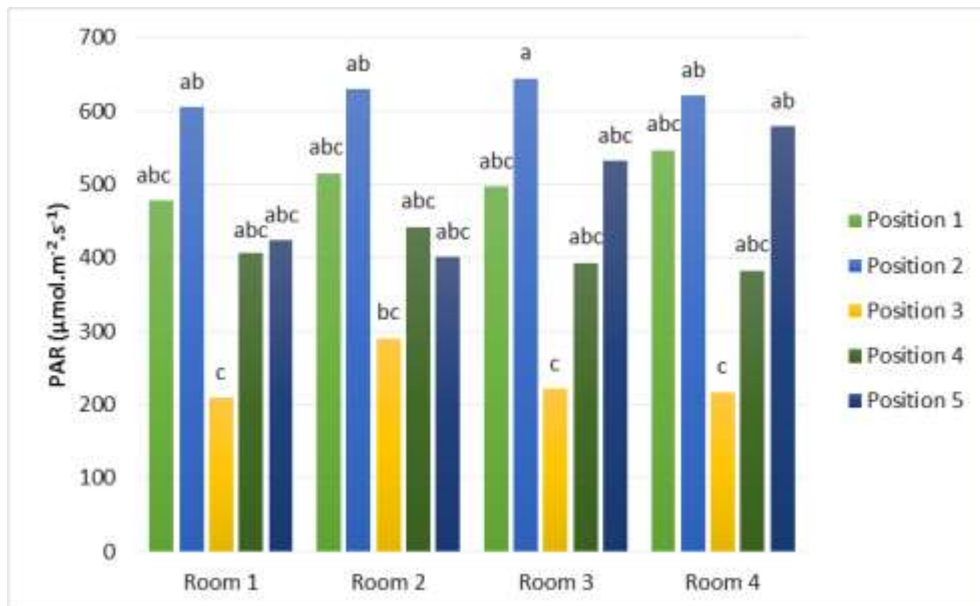

Fig. 3.3 Comparative distribution of average photosynthetic active radiation within each glasshouse room at five fixed measurement points. Bars with the same letters do not differ significantly ($p \leq 0.05$).

3.2.1.2 CO_2 control

An automatic change-over manifold containing two sets of four CO_2 cylinders for continuous CO_2 supply was set up outside the glasshouse. The manifold was connected with CO_2 gas lines to each room for the supply of CO_2 at 1 kPa at the centre of the rooms. Each room was supplied with a NEMA Wall-Mount CO_2 Level Controller specifically designed for greenhouses (model CM-0043-WP-NM from CO2Meter Inc., Florida). The CO_2 levels were continuously monitored and automatically adjusted according to the two treatments (C0 - 400 ppm; C1 - 800 ppm) by means of a solenoid valve being linked to the controller. The required levels were successfully maintained during the growth periods (Fig. 3.4).

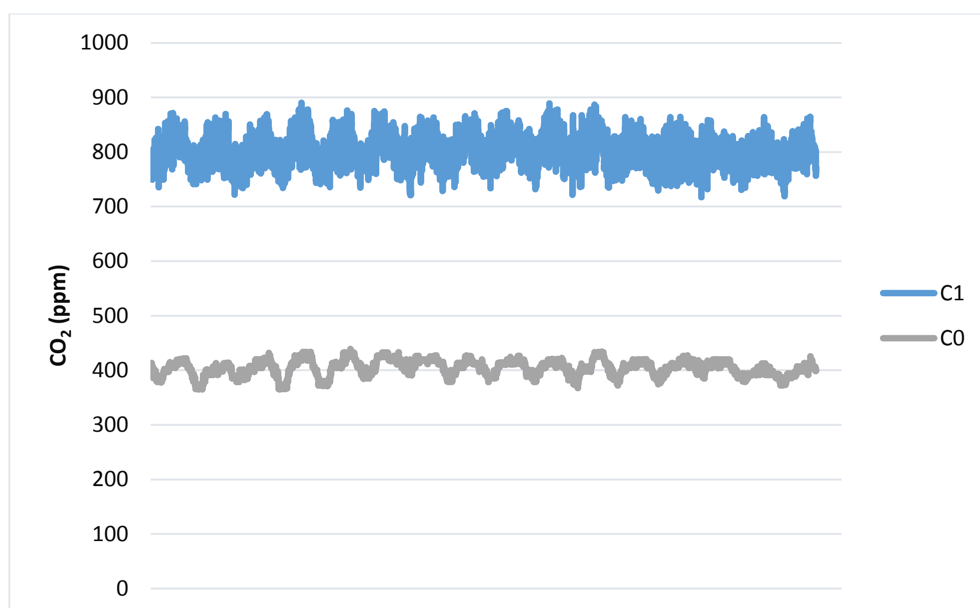

Fig. 3.4 Example of average CO_2 levels obtained over four growth cycles in the C0 and C1 treatment rooms during the second measurement period at 8 weeks after planting.

3.2.1.3 Temperature control

Air temperature (°C) was continuously monitored by two Vaisala HMP60 Temperature/Humidity probes in each room. The probes were positioned on opposite sides of each glasshouse room (Fig. 3.1). The temperature in each room was maintained by a separate air conditioner (DAIKIN UAT09JY1; Daikin Industries Ltd, Japan). The respective long-term average maximum temperatures for the three months October to December at Klawer Wine Cellar, South Africa, were used for T0 (27 °C, 29 °C and 31 °C), with T1 being three degrees warmer. The matching minimum/night temperatures for the region could however not be reached and maintained by the temperature control system. Thus, the minimum temperatures for the T0 treatments were adjusted to 15 °C, 15.5 °C and 16 °C for the three respective months of each growth cycle, with the corresponding T1 temperatures set at 3 °C higher (Table 3.1). The required levels were successfully maintained during the growth periods (Fig. 3.5).

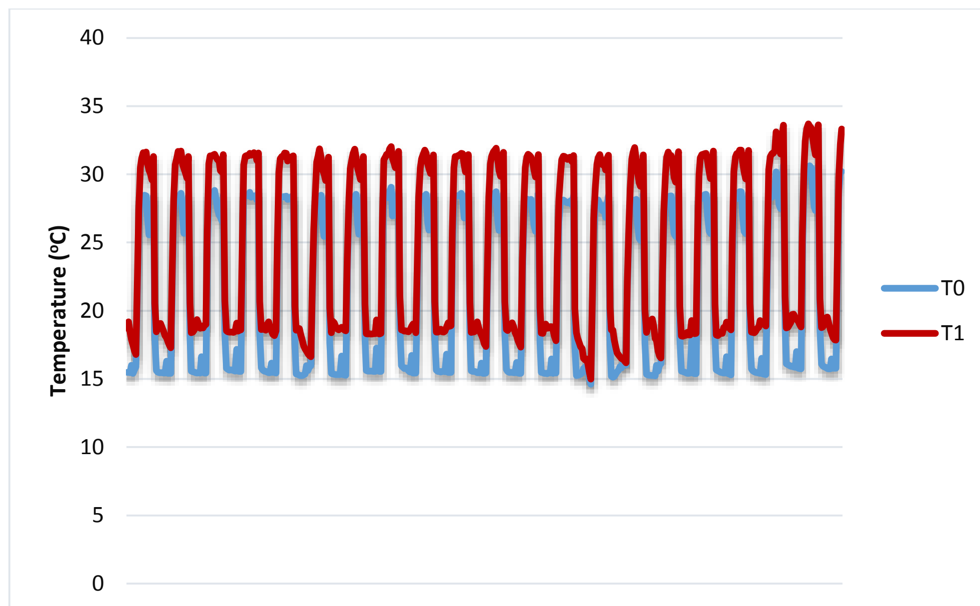

Fig. 3.5 Example of average minimum and maximum temperatures obtained over five growth cycles in the T0 and T1 treatment rooms during the 7 to 9-week period after planting.

3.2.1.4 Irrigation

Three samples, of approximately 7 L each, of the soil obtained from the site at Robertson (with known mass) were oven-dried for 24 h at 105 °C and weighed again to determine the soil water content of the soil upon arrival. After that, the dry soil was put in planting pots (with known mass) and water added until the soil was saturated and water began to seep out from below. The pots were then covered to prevent evaporation from the surface and left for two days to allow for drainage. The pots containing the wet soil were weighed and the pot mass subtracted to obtain the mass of the soil at water-holding capacity. The difference in mass between the soil upon arrival and the soil at water-holding capacity is the amount of water that was added to each pot directly after planting to ensure that all the vines started off with the same amount of available water.

During the growth period, a randomised selection of eight (wet) pots in each compartment was weighed and the average amount of water lost per pot after the previous irrigation determined. The pre-plant mass of the specific vine in each pot was accounted for. This calculated amount of water was then supplied in the (wet) treatments and 50 % of that in the (dry) treatments. Each pot was provided with a full-flow 2 $L.h^{-1}$ dripper in the middle of the pot, adjacent to the vine trunk. Differential irrigation commenced in the second week after planting and was applied twice weekly by controlling the duration of supply by means of manually operated taps.

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The water used for irrigation was analysed after three of the growth cycles by a SANAS Accredited Testing Laboratory (in accordance with ISO 17025:2005). In all growth cycles tested, the water met the criteria of the South African Water Quality Guidelines for irrigation.

3.2.1.5 Relative humidity

The relative humidity (RH) inside the rooms was monitored, but it was not investigated as an experimental factor. Differences were found in the daily maximum and minimum humidity between the various rooms, but they were not large and the fluctuation patterns were very similar (Fig. 3.6). It was therefore not expected that the RH would play a significant differentiating role in the physiological and vegetative growth response of the young grapevines to the various treatment combinations.

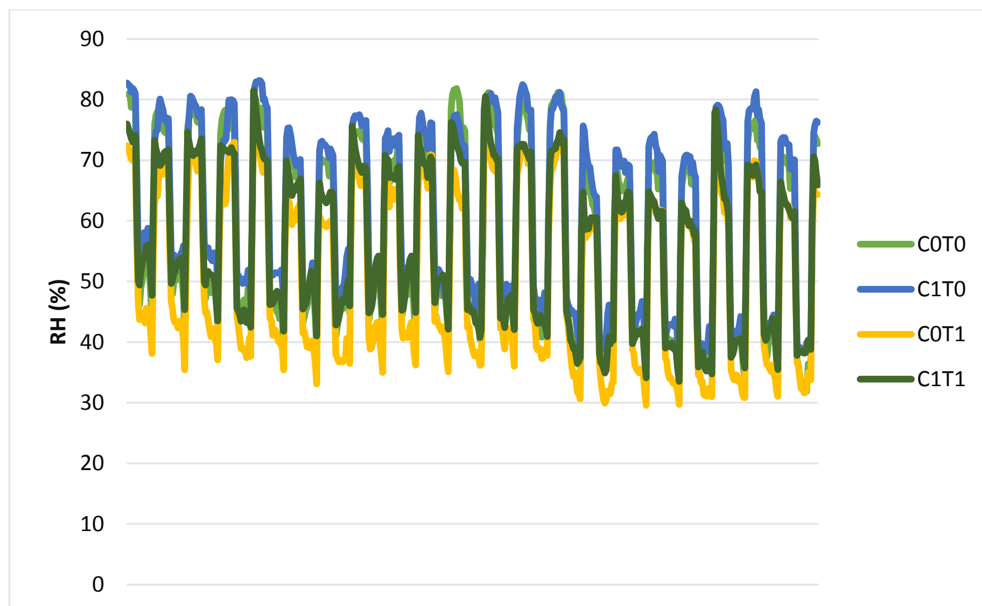

Fig. 3.6 Example of average daily fluctuation in relative humidity obtained over five growth cycles in the respective rooms during the 7 to 9-week period after planting.

3.2.1.6 Potting soil

The soil used for each growth cycle was separately obtained from the same fallow vineyard site in Robertson, South Africa, and transported to the experiment location. Each plastic planting pot (25 cm diameter; roughly 7.2 L) was provided with a Bidim-layer at the bottom before the vine was planted in 6.50 kg of soil. The soil was a sandy clay loam with a high pH (Table 3.2).

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Table 3.2. Texture analysis and pH of the soil used at each planting. Values in rows followed by the same letter do not differ significantly ($p \leq 0.05$)

SOIL	First planting	Second planting	Third planting	Fourth planting	Fifth planting	Average
pH_{KCl}	7.33 ab	7.10 bc	7.57 a	6.90 c	7.53 a	*7.29*
Clay (%)	29.67 a	27.00 a	29.00 a	27.00 a	17.67 a	*26.07*
Silt (%)	12.67 a	17.33 a	12.67 a	18.00 a	17.33 a	*15.60*
Sand (%)	57.67 a	55.67 a	58.33 a	55.00 a	65.00 a	*58.33*

Soils were analysed before each planting date by a SANAS Accredited Testing Laboratory, in accordance with ISO 17025:2005 (further details in Chapter 5). No additional nutrients were provided to the young, growing vines for the duration of the study.

3.2.1.6 Grafted vines

Vines were obtained from a SAPO (South African Plant Improvement Organisation) accredited nursery in the Wellington/Paarl region. Shiraz (SH 470), grafted onto rootstock 101-14 Mgt, was used for the first three growth cycles and Merlot noir (MO 348), grafted onto the same rootstock, for the last two cycles. Before planting, vines were pruned back to two buds and roots (only those originating from the basal node were kept) cut to a length of 10 cm (Fig. 3.7). Shoot removal and weed control were continuously done after planting during growth cycles to ensure optimal growth of the vines under the respective growth conditions. Primary shoot tips were not removed and all developing secondary shoots were allowed to grow.

Fig. 3.7 Example of a grafted vine just before planting.

Figure 3.8 depicts an example of an experimental layout used for a specific growth cycle. Since each block represents a group of 9 potted vines, 108 vines were established (54 per irrigation treatment) per room. Thus 432 vines were used for each growth cycle.

The CO_2 and temperature combination of the respective rooms were randomly re-allocated between consecutive growth cycles. Also, within the rooms, water supply was randomly allocated within the blocks.

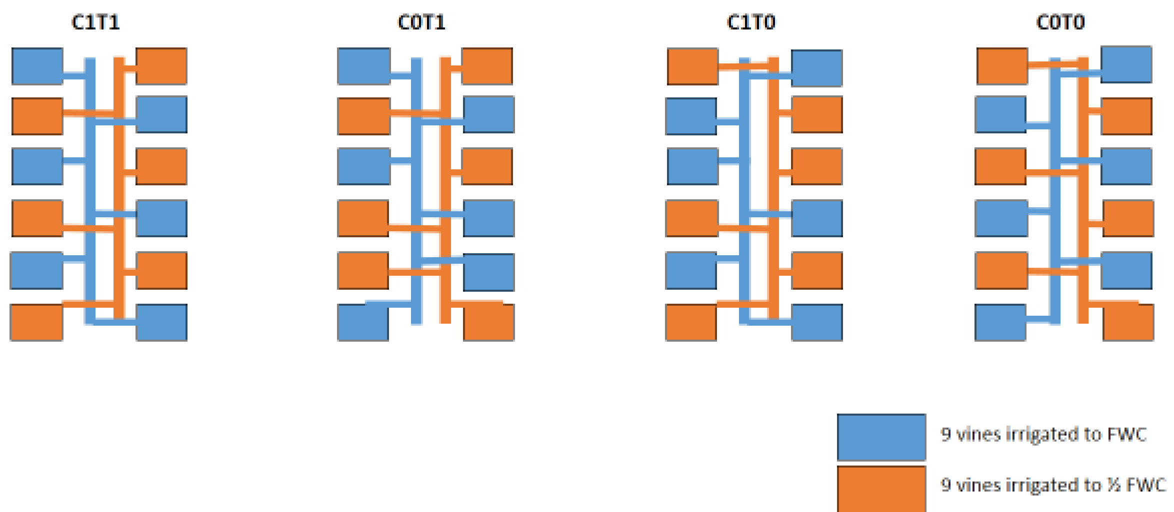

Fig. 3.8 An example of a statistical layout for one planting cycle in this study.

3.2.2 Measurements

3.2.2.1 Physiology

Photosynthesis (A_N) and transpiration (E) rates, stomatal conductance (g_s) and internal CO_2 (C_i) levels of basal leaves on primary shoots (nodes 2-4; one leaf per vine; six replicates per treatment combination) was measured at midday at each of the three sampling dates using a LI-6400 portable photosynthesis system (LI-COR Biosciences, Lincoln, Nebraska, United States). The PAR of the LED light source (6400-02B) was set at 350 µmol.m^{-2}.s^{-1}. During the measurements, CO_2 levels in the sample chamber were controlled by means of a CO_2 injector system (6400-01) according to the treatment criteria and a constant flow rate of 300 µmol.s^{-1} was applied. Midday stem water potential of basal leaves on primary shoots (nodes 2-4; one leaf per vine; six replicates per treatment combination) was determined with a pressure chamber (Scholander et al. 1965) at each of the three sampling dates. Leaves were bagged using plastic bags covered with aluminium foil at least two hours before measurement, using the method described in Begg & Turner (1970).

3.2.2.2 Chlorophyll (total, *a* and *b*)

The method as described in Hunter & Visser (1989) was used. The plant material of six vines was combined for each of the three replications of each treatment combination at the respective times of sampling. Primary leaves (all leaves from basal to apical part of shoot combined) were analysed after 4, 8 and 12 weeks of growth for their chlorophyll concentrations.

A representative leaf sample of 5 g (fresh mass) was cut into pieces of 1 cm^2. The leaf material was added to 100 cm^3 80 % aqueous acetone containing 0.1 g $CaCO_3$ and macerated with a Janke & Kunkel Ultra-Turrax T25 macerator at room temperature for 60 seconds at 20 500 rpm. The homogenate was

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left to settle in the dark at 5 °C for 24 h, after which the sediment was completely discoloured. Absorbancies at 645 nm and 663 nm were determined with a LKB Biochrom Ultrospec spectrophotometer (II E) (Biochrom, Cambridge, England; Model 4057) using 2 mm quartz cells.

The equations used for the determination of chlorophyll concentration are:

Chlorophyll *a* = $(0.0127A_{663} – 0.00269A_{645}) \times 20\,000 = \mu g.g^{-1}$ fresh mass

Chlorophyll *b* = $(0.0229A_{645} – 0.00468A_{663}) \times 20\,000 = \mu g.g^{-1}$ fresh mass

Total chlorophyll = $(0.0202A_{645} + 0.00802A_{663}) \times 20\,000 = \mu g.g^{-1}$ fresh mass

3.2.2.3 Leaf nitrogen (N) content (%)

Each leaf sample (2 g fresh mass) was washed with a Teepol solution, rinsed with de-ionised water and dried overnight at 70 °C in an oven. The dried leaves were then milled and ashed at 480 °C, shaken up in a 50:50 HCl (32 %) solution for extraction through filter paper (Campbell & Plank, 1998; Miller, 1998). Total N content of the ground leaves was determined through total combustion in a Leco Truspec® CN N-analyser (Leco Africa, Kempton Park, South Africa).

3.2.3 Statistical layout of project

The data was subjected to analysis of variance (ANOVA) using the General Linear Models Procedure (PROC GLM) of SAS software (Version 9.2; SAS Institute Inc., Cary, USA). The ANOVA was performed in order to evaluate the main influences/effects of cultivar, CO_2, temperature and H_2O, as well as to detect interaction effects among these factors. Measurements over sampling times were included in a split-plot analysis of variance with sampling times as sub-plot factor (Little & Hills, 1978) where applicable. The Shapiro-Wilk test was performed on the standardised residuals from the model to verify normality (Shapiro & Wilk, 1965). Levene's test showed dissimilarity of cultivar variances (Levene, 1960). To correct for variance differences between cultivars, a weight was included in the ANOVA. The weight was the inverse of the experimental error of each cultivar (John & Quenouille, 1977). Fisher's least significant difference was calculated at the 5 % level to compare means of the factors (main effects) and factor interaction means (Ott & Longnecker, 2001). A probability level of 5 % was considered significant for all significance tests. The Pearson product moment (Pearson) correlation tests were performed using XLSTAT (Version 2015.1.03.15485, Addinsoft, Paris).

3.3 RESULTS AND DISCUSSION

3.3.1 Midday physiological activity

At 4 weeks after planting, stomatal conductance (g_s) (Fig. 3.9) and transpiration (E) (Fig. 3.10) of both Merlot and Shiraz significantly increased with water supply in the T0 chambers. Merlot showed this level of response to water supply also in the T1 rooms, but the g_s and E of Shiraz remained relatively low in T1, even with water provision. The stem water potential (ψ_s) was less negative under well-watered conditions, as expected (Fig. 3.11), while the T1 temperatures resulted in more negative ψ_s than T0. Except for C0(dry), the ψ_s in Merlot was consistently higher than that of Shiraz, despite the higher E of the cultivar. This is interesting, since g_s (and thus E) of Shiraz is generally found to be poorly linked to a decrease in ψ_s (Bota et al. 2016) due to its so-called anisohydric behaviour (Soar et al. 2006). In contrast, linear correlations between ψ_s and g_s were found for both cultivars (Fig. 3.12).

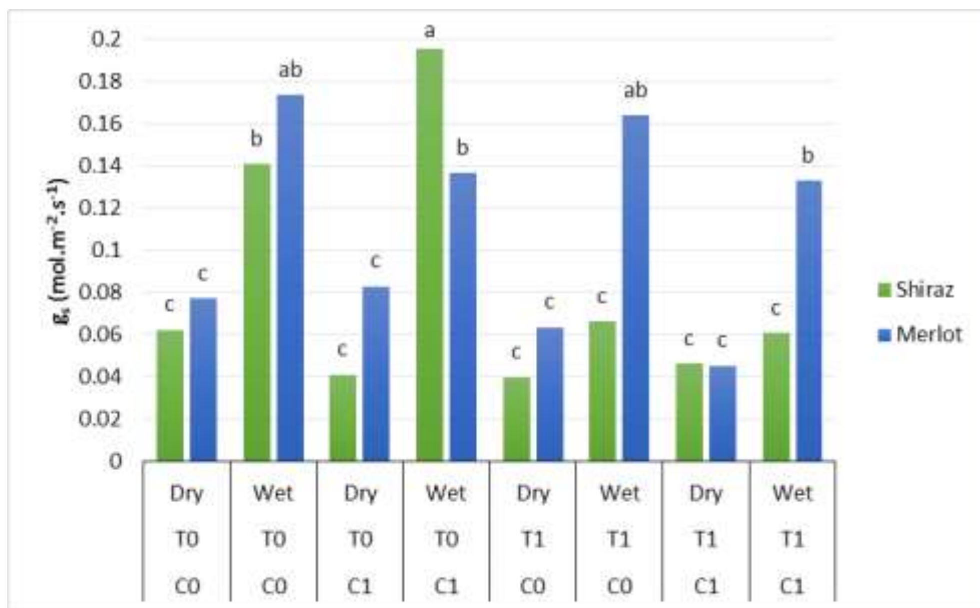

Fig. 3.9 Stomatal conductance of Shiraz and Merlot in the various treatment combinations at 4 weeks after planting. Bars with the same letters do not differ significantly ($p \leq 0.05$).

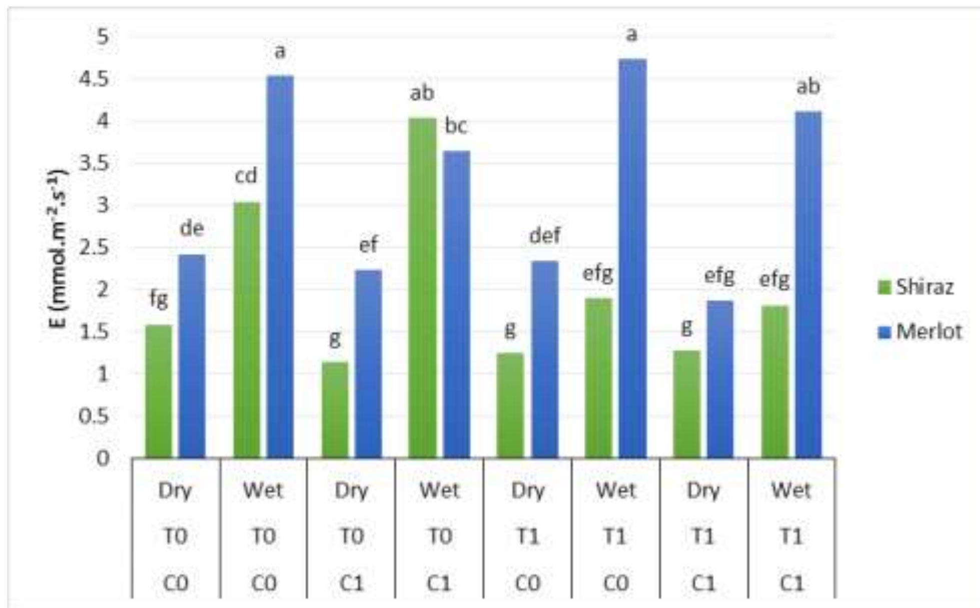

Fig. 3.10 Transpiration rate of Shiraz and Merlot in the various treatment combinations at 4 weeks after planting. Bars with the same letters do not differ significantly ($p \leq 0.05$).

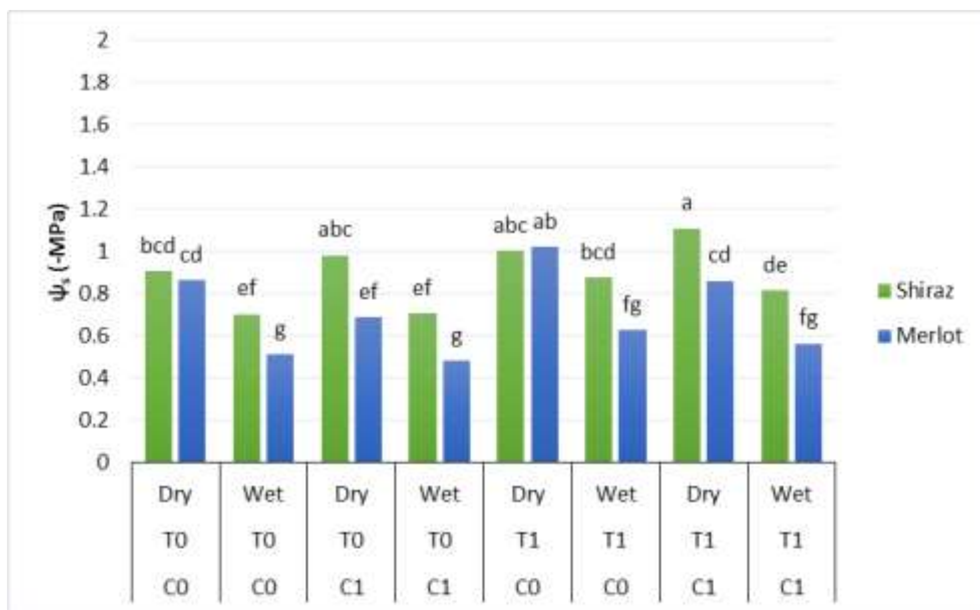

Fig. 3.11 Stem water potential of Shiraz and Merlot in the various treatment combinations at 4 weeks after planting. Bars with the same letters do not differ significantly ($p \leq 0.05$).

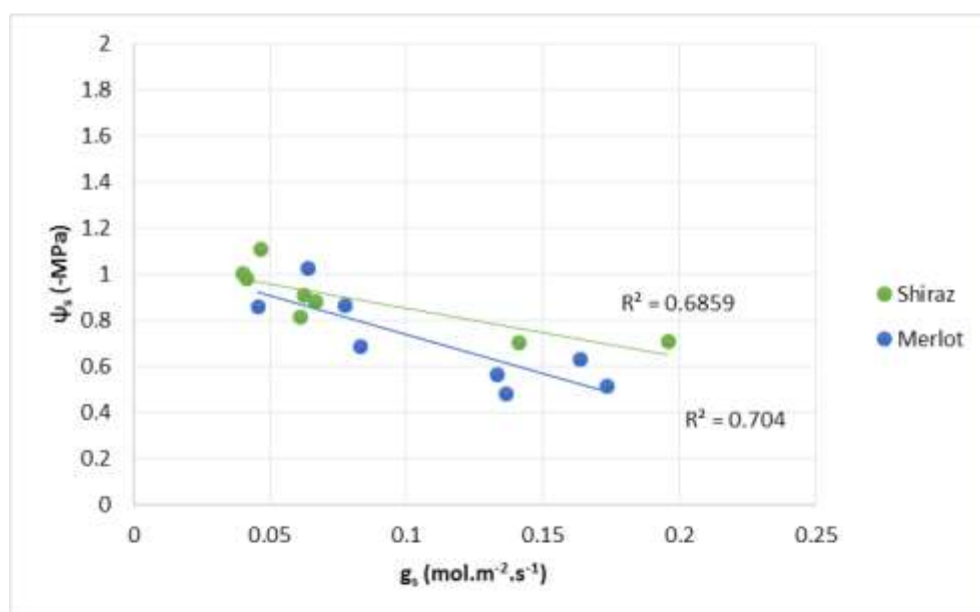

Fig. 3.12 Relationship between stem water potential and stomatal conductance of Shiraz and Merlot in the various treatment combinations at 4 weeks after planting.

At the most, vines (both cultivars) at 4 weeks after planting were moderately water-stressed, with the lowest ψ_s in the region of -1 MPa (Myburgh, 2018). Stomatal conductance was however lower than expected. Lovisolo et al. (2010) linked mild water stress with conductance rates of 0.15 mol.m^{-2}.s^{-1}, while g_s of lower than 0.05 mol.m^{-2}.s^{-1} (which is normally associated with severe water stress) was measured in C1(dry) and/or T1(dry) conditions. It is therefore concluded that another factor, most probably light intensity, caused the low levels of g_s.

Elevated CO_2 seemed to decrease both g_s and E, which is in accordance with the findings of Kriedemann et al. (1976), Long et al. (2004) and Edwards et al. (2017), but it significantly increased the C_i in the leaves (Fig. 3.13). It is therefore expected that A_N under C1 conditions will be higher than in C0, due to increased carboxylation rate by RubisCO (Long et al. 2004) and decreased photorespiration rate (Kriedemann et al. 1976; Flexas et al. 2002; Zinta et al. 2014; 2018). It was found that increased CO_2 concentrations increased the A_N in all combinations (Fig. 3.14). The synergistic effect of combined high temperature and high CO_2 on A_N (Alonso et al. 2008; Edwards et al. 2017) was confirmed in both (wet) and (dry) treatments, likely because the vines at 4 weeks after planting were only moderately water-stressed.

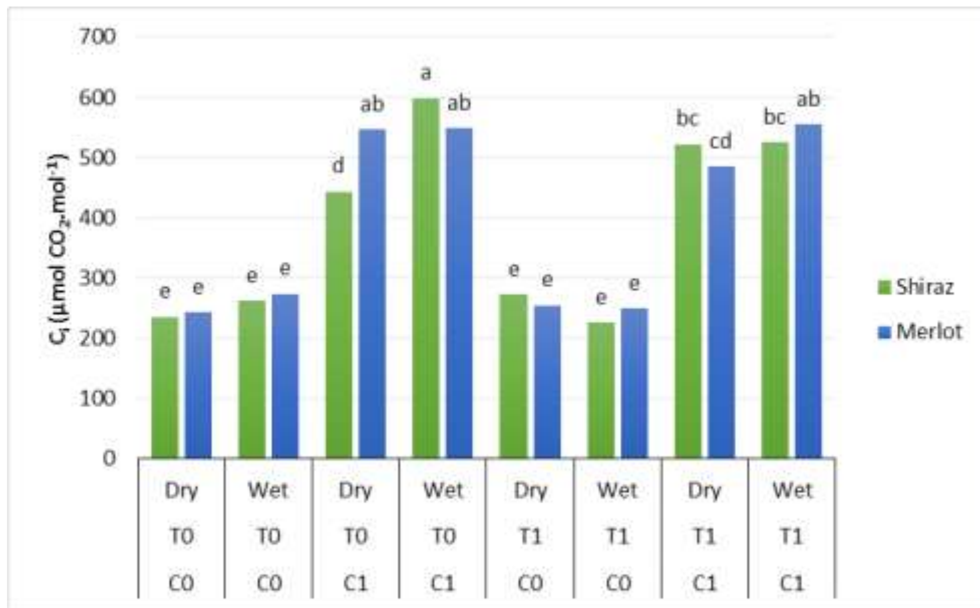

Fig. 3.13 Leaf internal CO_2 of Shiraz and Merlot in the various treatment combinations at 4 weeks after planting. Bars with the same letters do not differ significantly ($p \leq 0.05$).

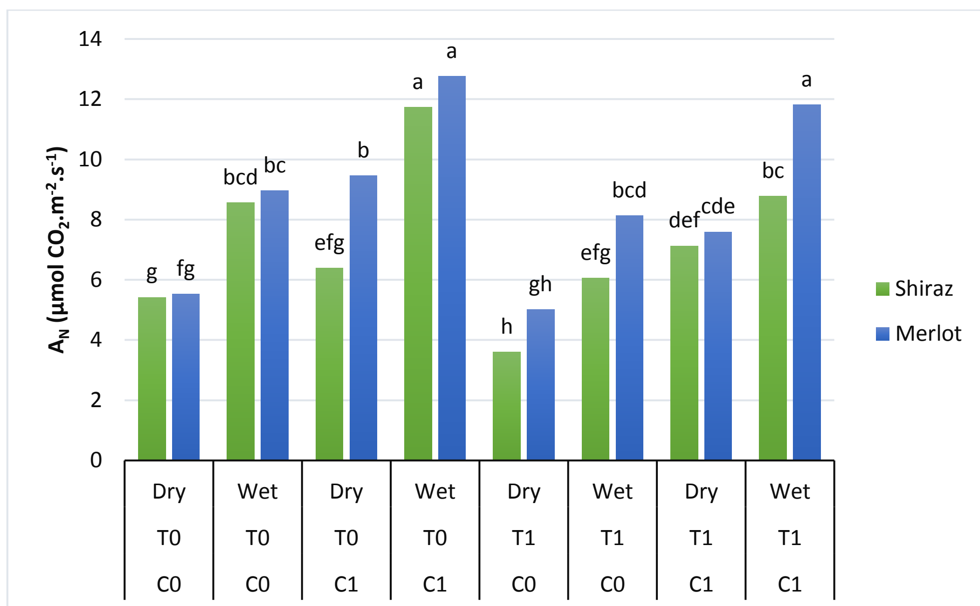

Fig. 3.14 Photosynthetic rate of Shiraz and Merlot in the various treatment combinations at 4 weeks after planting. Bars with the same letters do not differ significantly ($p \leq 0.05$).

The efficiency of water use by a plant may be expressed as either the ratio between A_N and E (instantaneous water use efficiency - WUE_{inst}) or between A_N and g_s (intrinsic water use efficiency - WUE_i). Both were calculated and showed very similar tendencies, but since the WUE_i is more often used in related literature, this measure is reported for comparative purposes.

Bota et al. (2016) compared the g_s and WUE_i of 23 cultivars (10-year old and grafted onto Richter 99 rootstock) under field conditions in response to progressive water stress. All vines were irrigated at

flowering and after that, no more water was supplied. Measurements (one leaf per plant; four replicates) were taken at véraison, during berry ripening and at grape ripeness. Shiraz had lower WUE_i than Merlot when the vines were moderately water-stressed ($\psi_s \geq -0.8$ MPa). They ascribed it to higher g_s in Shiraz than Merlot relative to A_N. The only treatment that seems to fit this description was C1T0(wet), where the conductance of Shiraz was significantly higher and A_N lower than in Merlot resulting in a lower WUE_i (Fig. 3.15). In all the other treatments, g_s (and A_N) of Merlot was higher than that of Shiraz, causing Shiraz to generally have higher WUE_i ratios than Merlot. These measurements were taken relatively late in the season (véraison and later). This may contribute to the differences in results, since this study primarily focused on the vegetative growth phase of the vines.

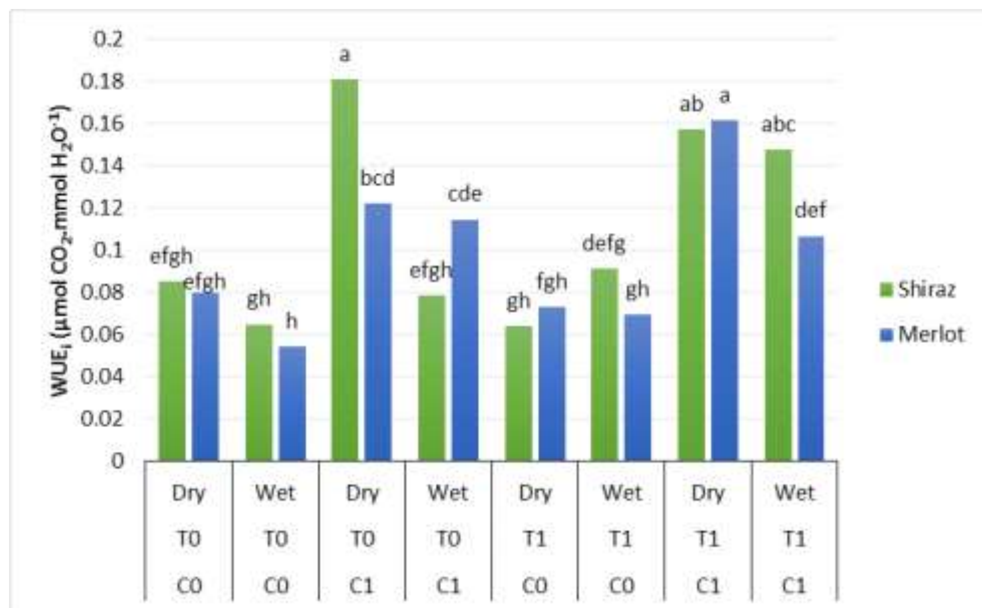

Fig. 3.15 WUE_i of Shiraz and Merlot in the various treatment combinations at 4 weeks after planting. Bars with the same letters do not differ significantly ($p \leq 0.05$).

The first signs of restricted A_N in response to water supply are due to restriction of stomatal and mesophyll conductance (Lovisolo et al. 2010; Schultz & Stoll, 2010) which are reflected in the ψ_s and C_i. When A_N rate was plotted against C_i (Figs 3.16-3.17) and ψ_s (Figs 3.18-3.19), three groups could be distinguished in both cultivars at 4 weeks after planting. The C1(wet) group had the highest A_N rate, followed by the C0(wet) and C1(dry) treatments with similar rates of A_N, and then the C0(dry) treatments with the lowest A_N activity.

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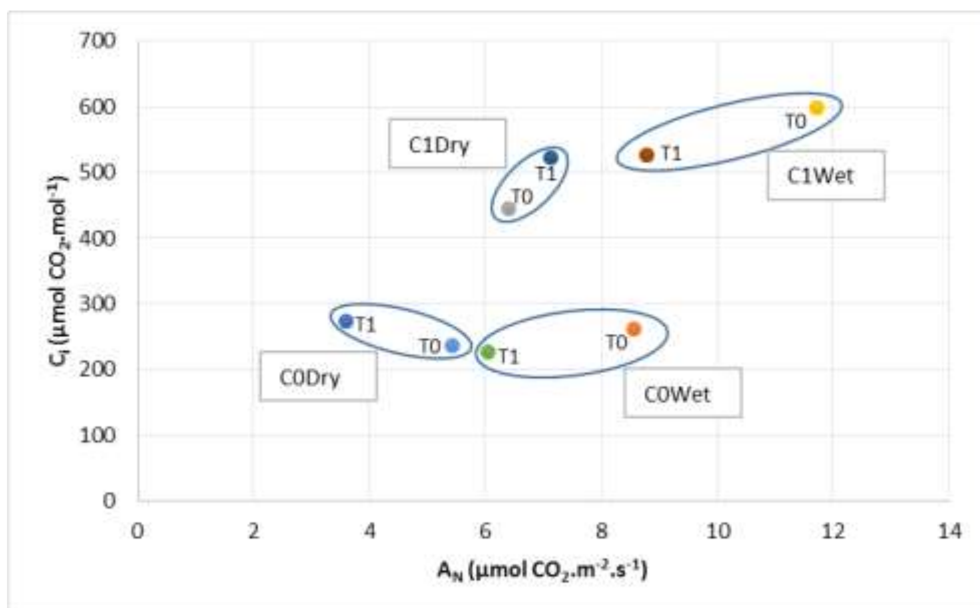

Fig. 3.16 Relationship between leaf internal CO_2 concentration and photosynthesis of Shiraz in the various treatment combinations at 4 weeks after planting.

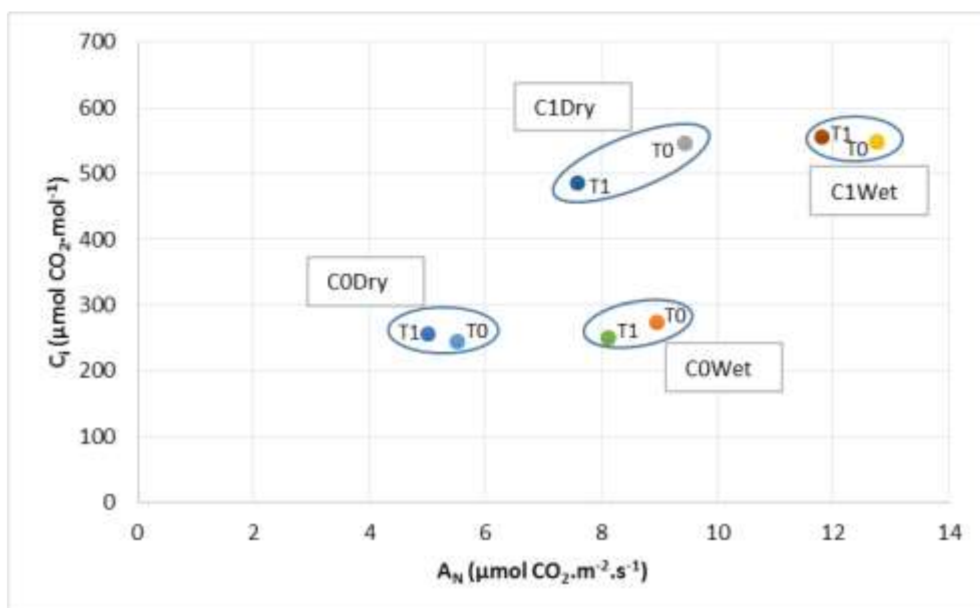

Fig. 3.17 Relationship between leaf internal CO_2 concentration and photosynthesis of Merlot in the various treatment combinations at 4 weeks after planting.

The positive relationship between C1 levels and C_i is clear (Figs 3.16-3.17), while temperature and water supply did not affect C_i significantly (Table 3.3) [The higher C_i in the C1T0(wet) than C1T0(dry) treatment in Shiraz is linked with the higher g_s]. Under comparable C_i and temperature conditions, water supply positively affected A_N.

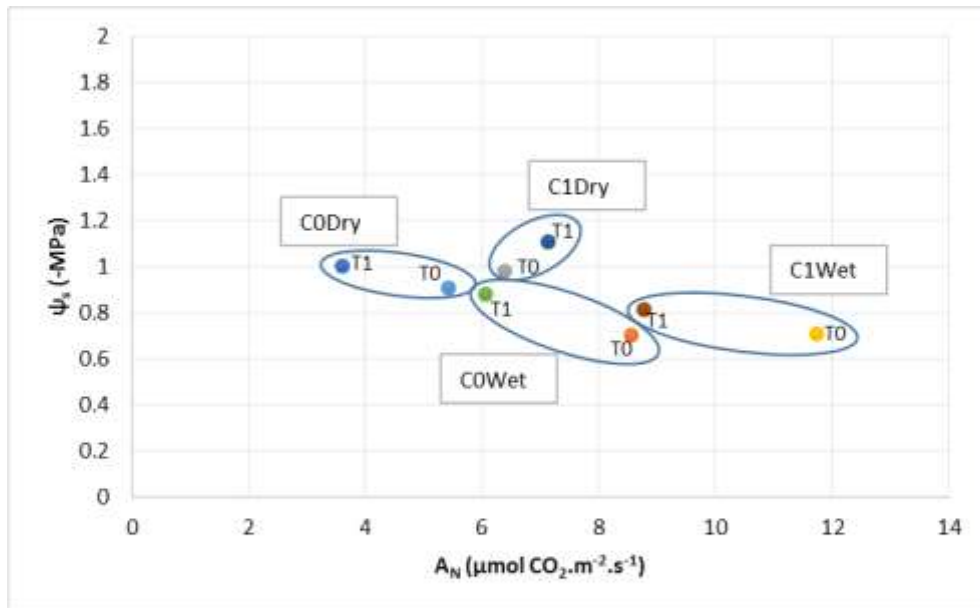

Fig. 3.18 Relationship between stem water potential and photosynthesis of Shiraz in the various treatment combinations at 4 weeks after planting.

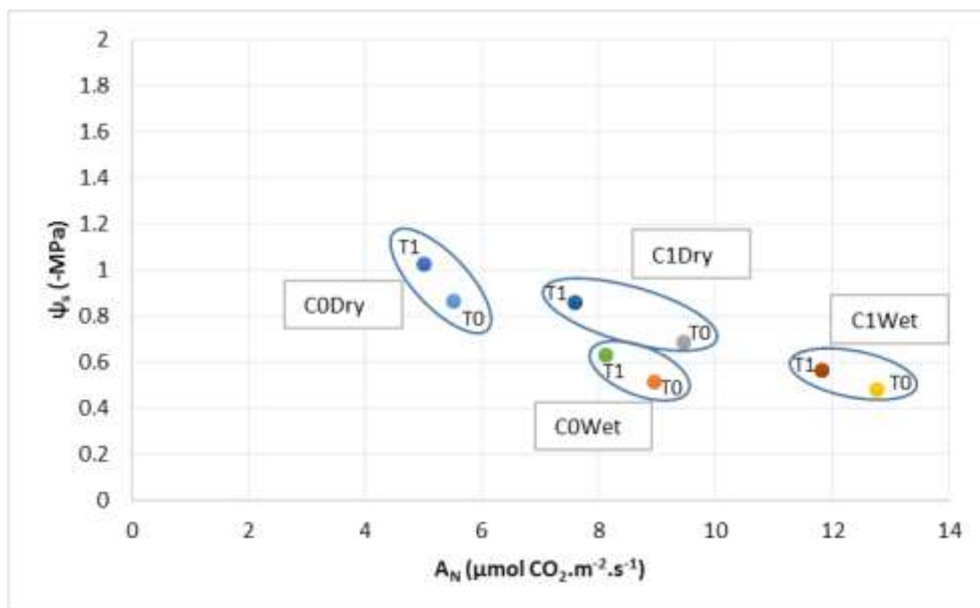

Fig. 3.19 Relationship between stem water potential and photosynthesis of Merlot in the various treatment combinations at 4 weeks after planting.

Water supply increased the ψ_s in both cultivars (Figs 3.18-3.19), while it seemed as if C1 improved ψ_s under similar temperature and water levels, especially in Merlot. Under comparable xylem water potential and temperature, A_N was increased in the higher CO_2 conditions. Higher CO_2 levels increased the rate of A_N where the stem water potentials were similar. This enhancing effect of CO_2 cancelled out the negative effect of T1 compared to T0 on A_N, since the C1T1 combinations had higher A_N activity than C0T0, in both well-watered and water deficit vines.

Table 3.3. Indication of significance level of main treatment factor and interaction effects

	Cv	Weeks	CO_2	Temp	H_2O	Cv x Weeks	Cv x CO_2	Cv x Temp	Cv x H_2O	Weeks x CO_2	Weeks x Temp	Weeks x H_2O	CO_2 x Temp	CO_2 x H_2O	Temp x H_2O
g_s	*	*** 24 %	*	*** 3 %	*** 12 %	*** 1 %	*	NS	NS	NS	*** 3 %	*** 6 %	NS	*	*** 1 %
E	*	*** 17 %	**	** 1 %	*** 16 %	*** 7 %	NS	*	NS	***	*** 1 %	*** 3 %	NS	**	*
C_i	*	*** 2 %	*** 67 %	NS	NS	*	NS	NS	NS	*** 2 %	NS	*** 1 %	NS	*** 1 %	NS
A_N	NS	*** 33 %	*** 10 %	*** 1 %	*** 6 %	*** 2 %	*	NS	*	*	**	*** 2 %	NS	*	**
WUE_i	NS	*** 4 %	*** 39 %	NS	*	** 1 %	NS	NS	NS	*** 2 %	NS	*** 3 %	NS	*** 3 %	NS
ψ_s	***	*** 16 %	*** 3 %	*** 5 %	*** 16 %	*** 10 %	*	***	*	*** 2 %	NS	*** 1 %	NS	*	NS

	Cv x Weeks x CO_2	Cv x Weeks x Temp	Cv x Weeks x H_2O	Cv x CO_2 x Temp	Cv x CO_2 x H_2O	Cv x Temp x H_2O	Weeks x CO_2 x Temp	Weeks x CO_2 x H_2O	Weeks x Temp x H_2O	CO_2 x Temp x H_2O	Cv x CO_2 x Temp x H_2O	Cv x Weeks x Temp x H_2O	Cv x Weeks x CO_2 x H_2O	Cv x Weeks x CO_2 x Temp	Weeks x CO_2 x Temp x H_2O	Cv x Weeks x CO_2 x Temp x H_2O
g_s	NS	** 1 %	NS	NS	NS	*	NS	NS	*** 1 %	NS	NS	***	NS	NS	NS	NS
E	NS	**	*	NS	NS	NS	NS	*	**	NS	NS	*** 1 %	NS	NS	NS	NS
C_i	NS	NS	***	NS	NS	NS	NS	NS	*	NS	NS	***	NS	*	NS	NS
A_N	NS	NS	NS	NS	NS	NS	NS	NS	NS	**	*	NS	NS	NS	NS	NS
WUE_i	NS	NS	*** 1 %	NS	NS	NS	NS	NS	*	NS	NS	*** 1 %	NS	*	NS	NS
ψ_s	NS	**	*	NS	NS	NS	NS	NS	**	NS	NS	NS	***	**	NS	NS

(*, ** and *** indicate significance at $p \leq 0.05$, 0.01 and 0.001, respectively. NS indicates no significant difference ($p > 0.05$). The percentage values included in the table indicate the contribution by the specific factor or interaction to the total variance of each parameter measured (values only included if contribution is > 1 %).
Cv: cultivar; Temp: temperature; Weeks: number of weeks after plant (measurements taken at 4, 8 and 12 weeks)

Both g_s and E decreased significantly between 4 and 8 weeks after planting (Figs 3.20-3.21). Once again, stomatal conductance was very low with rates (< 50 $mmol.m^{-2}.s^{-1}$) comparable to severe water stress (Lovisolo et al. 2010), which was not reflected in the ψ_s measurements. Interestingly, g_s of Shiraz at 8 weeks after planting had a very strong linear correlation with ψ_s ($r = 0.889$; $p = 0.011$), which was not the case for Merlot ($r = 0.616$; $p = 0.104$) (data not shown). The rates of g_s in response to the treatment combinations were very similar between the cultivars, with the exception of C0T0(wet) where conductance in Merlot was higher than in Shiraz. C1(wet) conditions clearly enhanced g_s in both cultivars.

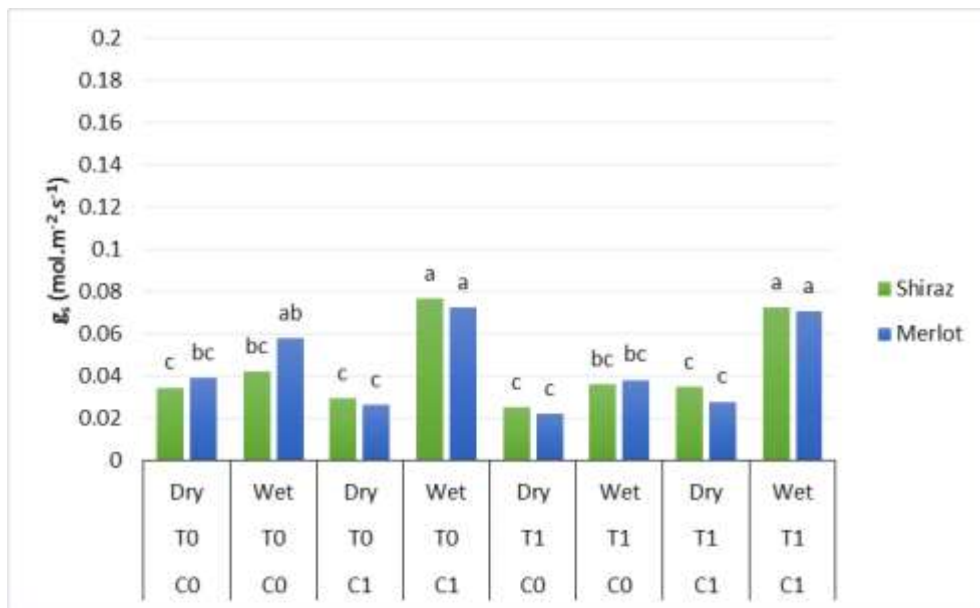

Fig. 3.20 Stomatal conductance of Shiraz and Merlot in the various treatment combinations at 8 weeks after planting. Bars with the same letters do not differ significantly ($p \leq 0.05$).

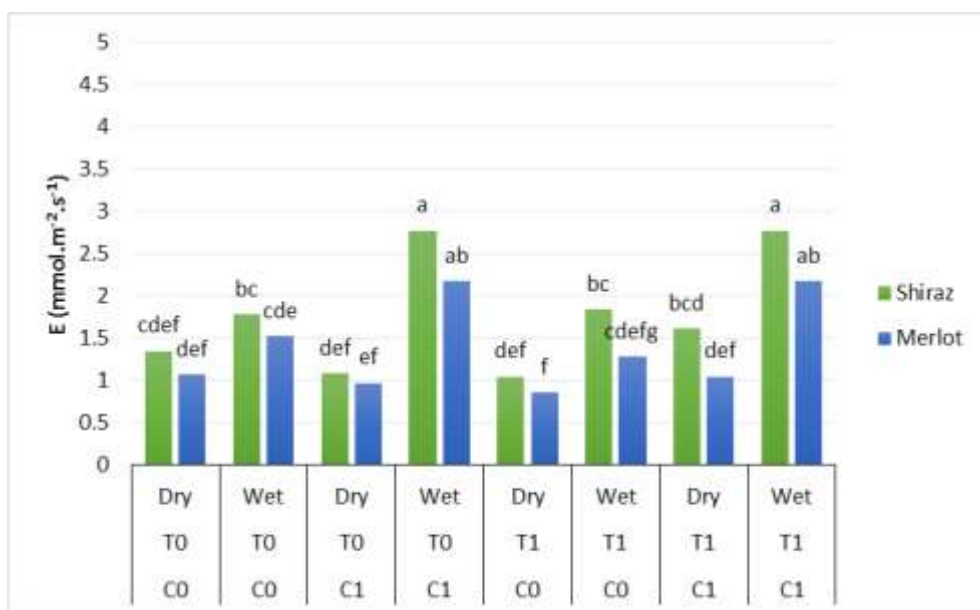

Fig. 3.21 Transpiration rate of Shiraz and Merlot in the various treatment combinations at 8 weeks after planting. Bars with the same letters do not differ significantly ($p \leq 0.05$).

Transpiration rates followed the same trends as g_s, but with Shiraz consistently transpiring at higher rates than Merlot. The xylem water potential measurements support this by generally indicating lower water stress for Shiraz than Merlot (the only two exceptions being the C1T0 treatments) (Fig. 3.22). Stem water potential in Shiraz was never more negative than -1.4 MPa, which is considered the boundary between strong and severe water stress (Myburgh, 2018). In both T1(dry) treatments Merlot experienced severe water stress. Down-regulation of A_N may have occurred due to lower PSII efficiency and RubisCO activity (Flexas & Medrano, 2002; Ping et al. 2015). This seems to explain the very low A_N rate for Merlot in the C0T1(dry) treatment, but not the relatively high A_N under C1T1(dry) conditions (Fig. 3.23).

Photosynthetic activity decreased significantly from 4 to 8 weeks after planting in both cultivars. The relative effect of the respective treatment combinations stayed the same over time, with higher A_N rates measured in elevated CO_2 conditions and adequate water supply. The temperature regime in T0 was also more favourable to A_N than T1.

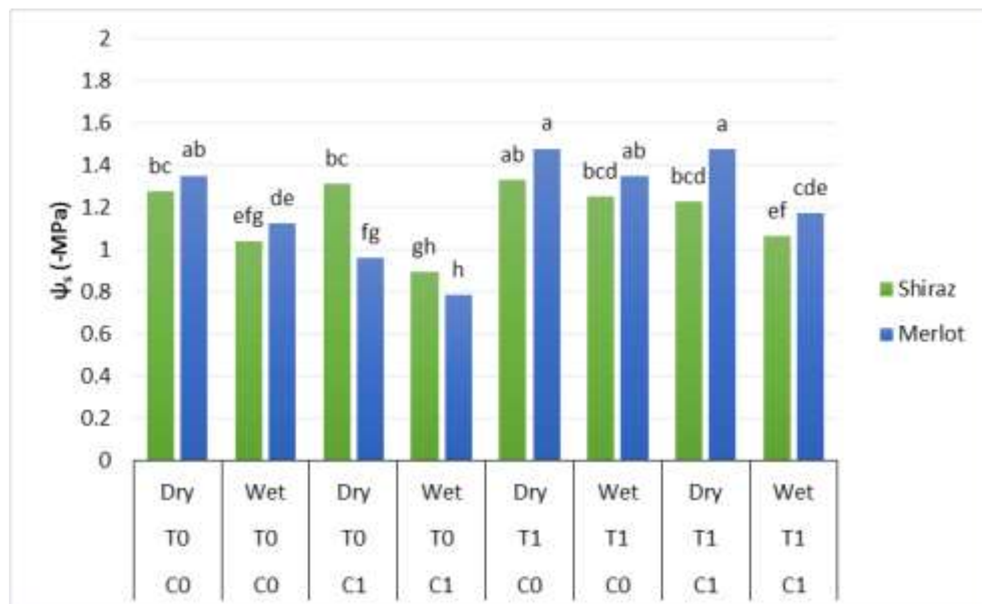

Fig. 3.22 Stem water potential of Shiraz and Merlot in the various treatment combinations at 8 weeks after planting. Bars with the same letters do not differ significantly ($p \leq 0.05$).

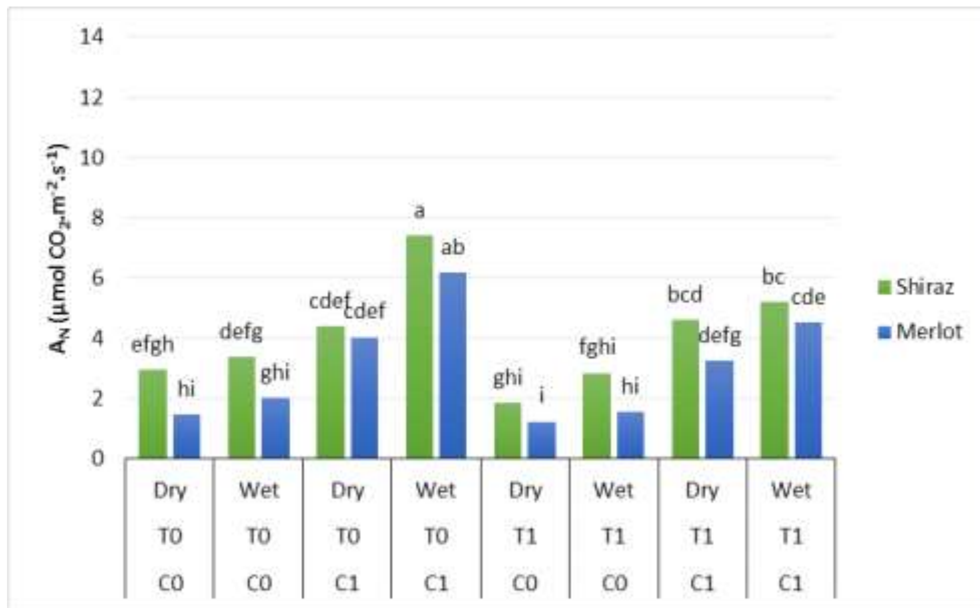

Fig. 3.23 Photosynthetic rate of Shiraz and Merlot in the various treatment combinations at 8 weeks after planting. Bars with the same letters do not differ significantly ($p \leq 0.05$).

There are many reports on the positive effect of high ambient CO_2 on the WUE of plants by increasing A_N relative to g_s and E (Long et al. 2004; Robredo et al. 2007; Edwards et al. 2017). It was also found that g_s in high temperature-high CO_2 environments is similar to that in ambient temperature-ambient CO_2, but that E was higher in the former (Leibar et al. 2015; Edwards et al. 2017; Douthe et al. 2018).

These effects are visible in Figures 3.20 to 3.25 should any of the C0/C1 combinations be compared. For example, if C1T1(dry) is compared to C0T1(dry), both have very low g_s rates of less than 40 mmol.m^{-2}.s^{-1} (Fig. 3.20). The C1 treatment, however, transpired at a higher rate than the C0 (Fig. 3.21), while the ψ_s (and thus water stress experienced) are similar (Fig. 3.22). Photosynthesis was higher in the C1 environment (Fig. 3.23), resulting in a significantly higher WUE (Figs 3.24-3.25). These trends were found in both temperature regimes, since the T0 treatment in this study was in the range of 27-31 °C and the T1 in the 30-34 °C range. Both are comparable (or higher) than high temperature treatments in literature (Salazar-Parra et al. 2012; Leibar et al. 2015; Martínez-Lüscher et al. 2015).

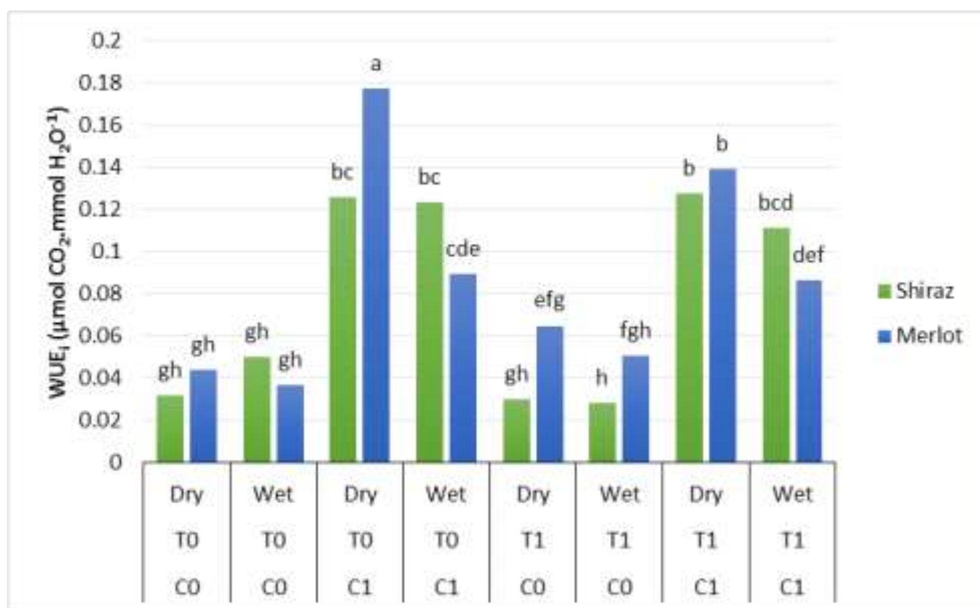

Fig. 3.24 WUE_i of Shiraz and Merlot in the various treatment combinations at 8 weeks after planting. Bars with the same letters do not differ significantly ($p \leq 0.05$).

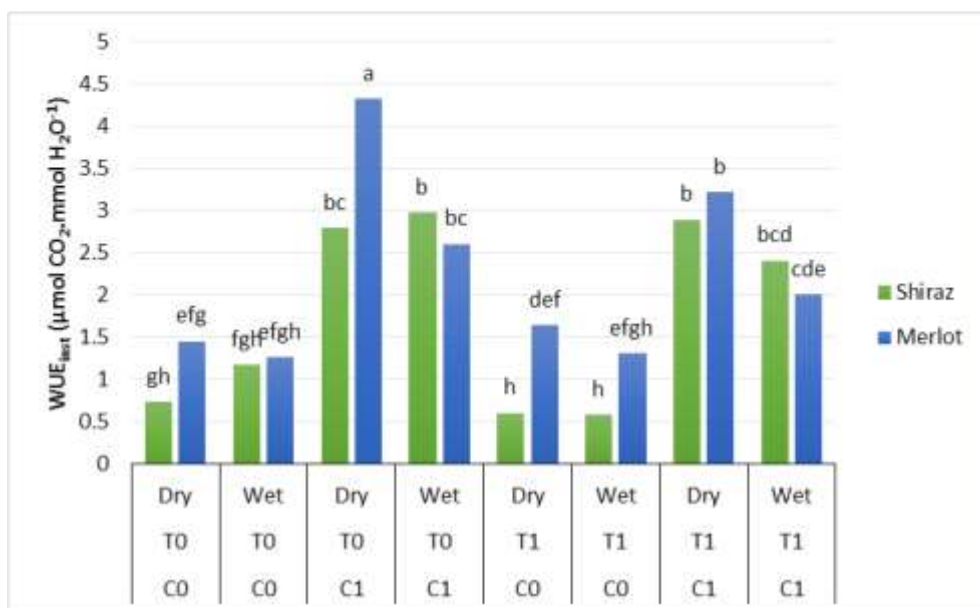

Fig. 3.25 WUE_{inst} of Shiraz and Merlot in the various treatment combinations at 8 weeks after planting. Bars with the same letters do not differ significantly ($p \leq 0.05$).

At 8 weeks, the effect of the increased CO_2 levels on A_N became more apparent, and two groups were formed based on that parameter (Figs 3.26-3.29). Differences between cultivars became more visible, with Shiraz showing higher rates of A_N than Merlot at comparable C_i and Merlot displaying higher levels of water stress at the same temperature and CO_2, with the possible exception of C1T0(wet).

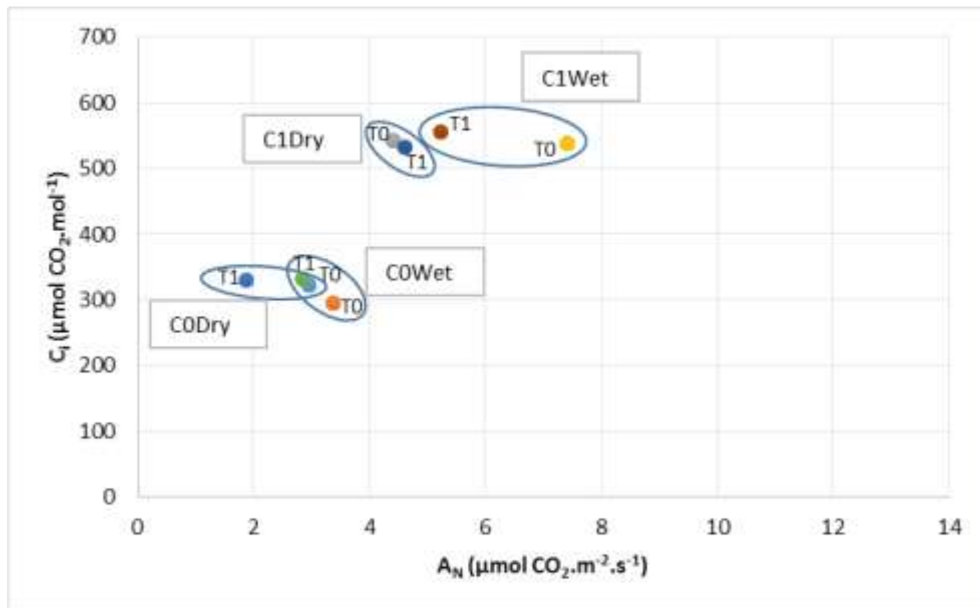

Fig. 3.26 Relationship between leaf internal CO_2 concentration and photosynthesis of Shiraz in the various treatment combinations at 8 weeks after planting.

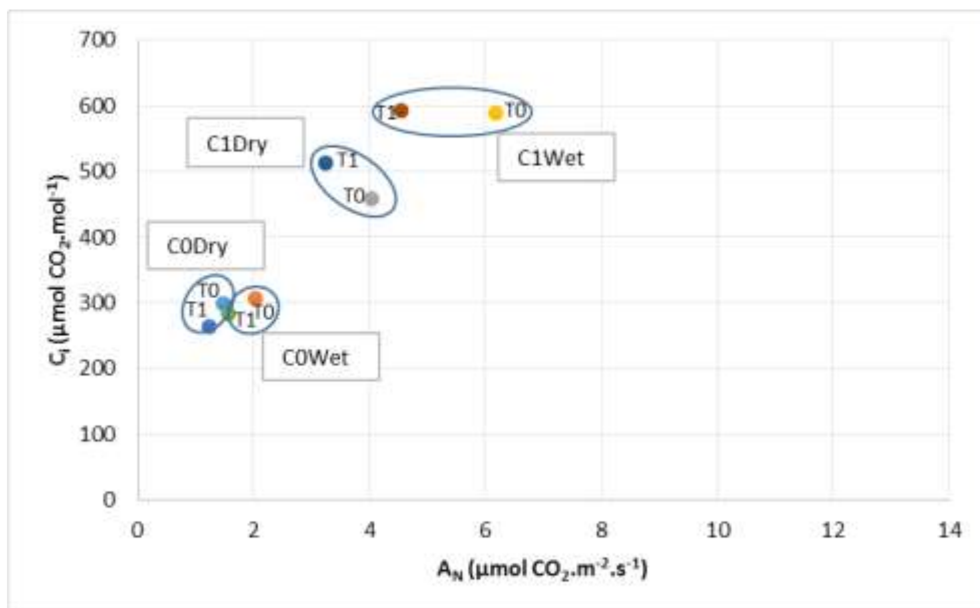

Fig. 3.27 Relationship between leaf internal CO_2 concentration and photosynthesis of Merlot in the various treatment combinations at 8 weeks after planting.

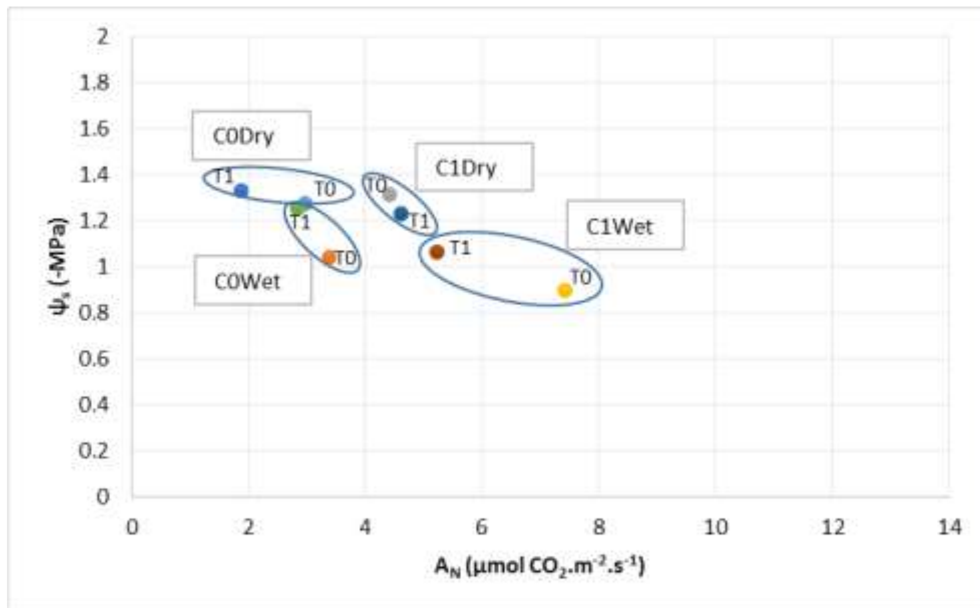

Fig. 3.28 Relationship between stem water potential and photosynthesis of Shiraz in the various treatment combinations at 8 weeks after planting.

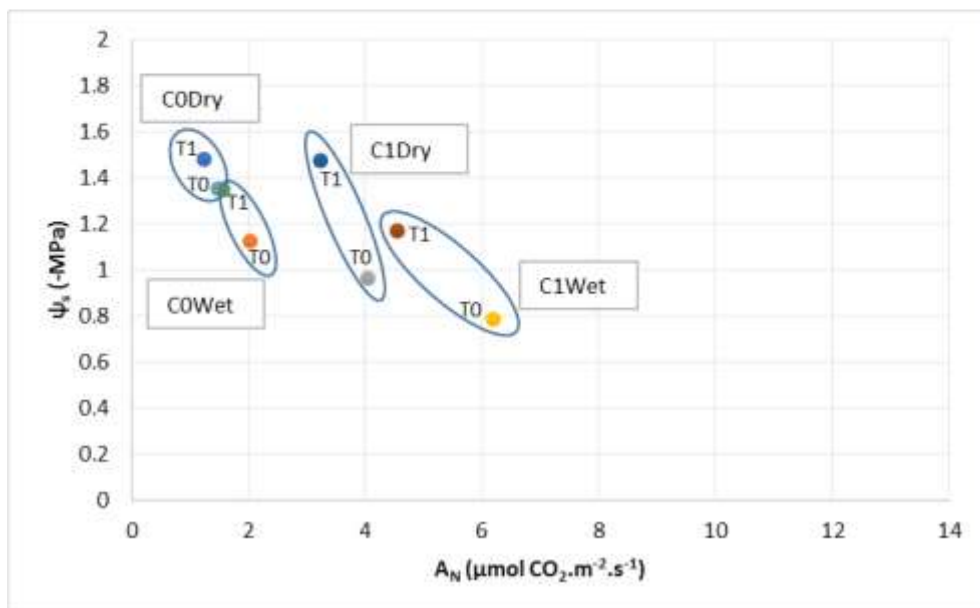

Fig. 3.29 Relationship between stem water potential and photosynthesis of Merlot in the various treatment combinations at 8 weeks after planting.

At 12 weeks after planting, physiological activity of basal leaves in both cultivars was very low. This is in accordance with literature where A_N, g_s as well as E decreased with leaf age as the growth season progresses (Hunter et al. 1994; Salazar-Parra et al. 2012; Medrano et al. 2015; Douthe et al. 2018). Despite the low specific rates, the vines still reacted to the environmental treatment combinations. The g_s (Fig. 3.30) and E (Fig. 3.31) of Shiraz and especially Merlot were enhanced by water supply and also increased CO_2 levels. Transpiration rates of Shiraz were consistently higher than those of Merlot, and

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this cultivar did not experience severe water stress in any of the treatments (Fig. 3.32). In contrast, Merlot was severely water-stressed in T0(dry) and the C0T1(wet) treatments (ψ_s < -1.4 MPa) and extremely stressed in the T1(dry) combinations with $\psi_s \leq$ -1.8 MPa. This might be explained by the stronger linear relationship between ψ_s and stomatal opening of Shiraz compared to Merlot (Fig. 3.33). This is in contrast with the findings of Bota et al. (2016) and supports the statement that the response of a cultivar to water stress is not genetically determined (Simonneau et al. 2017), but is more dependent on the intensity and duration of the water deficit (Chaves et al. 2010) and the phenological stage at which this stress occurs (Poni et al. 1993).

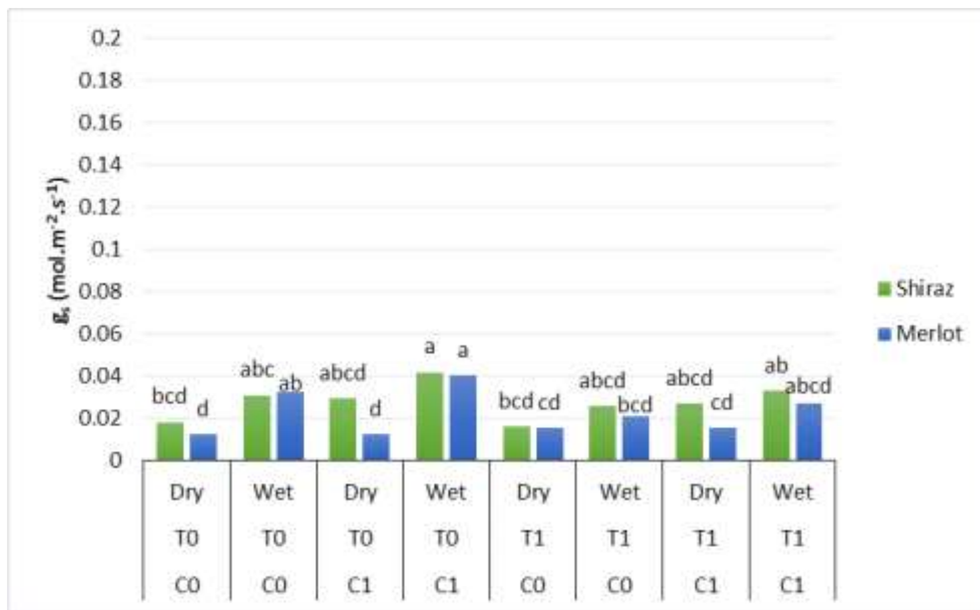

Fig. 3.30 Stomatal conductance of Shiraz and Merlot in the various treatment combinations at 12 weeks after planting. Bars with the same letters do not differ significantly ($p \leq 0.05$).

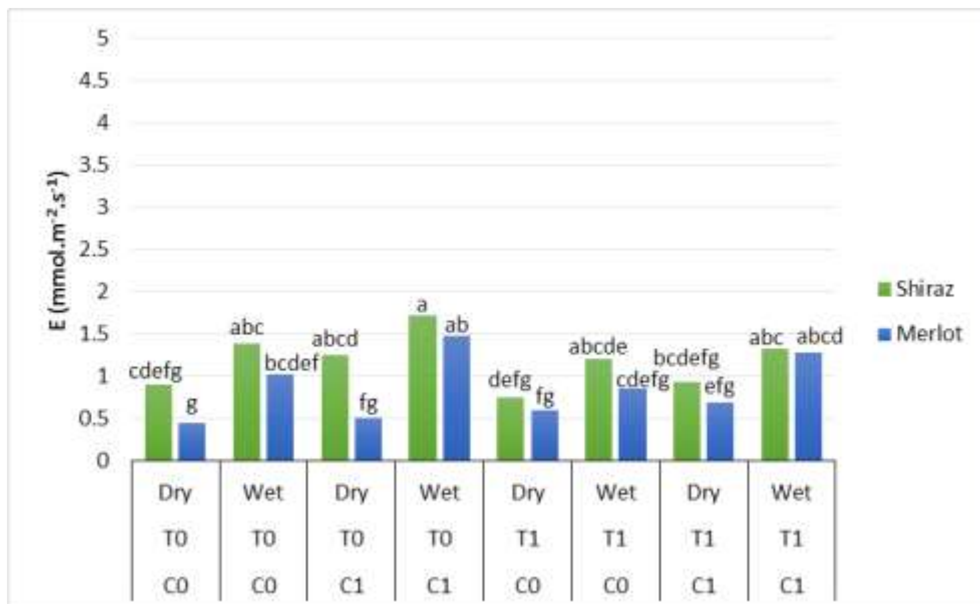

Fig. 3.31 Transpiration rate of Shiraz and Merlot in the various treatment combinations at 12 weeks after planting. Bars with the same letters do not differ significantly ($p \leq 0.05$).

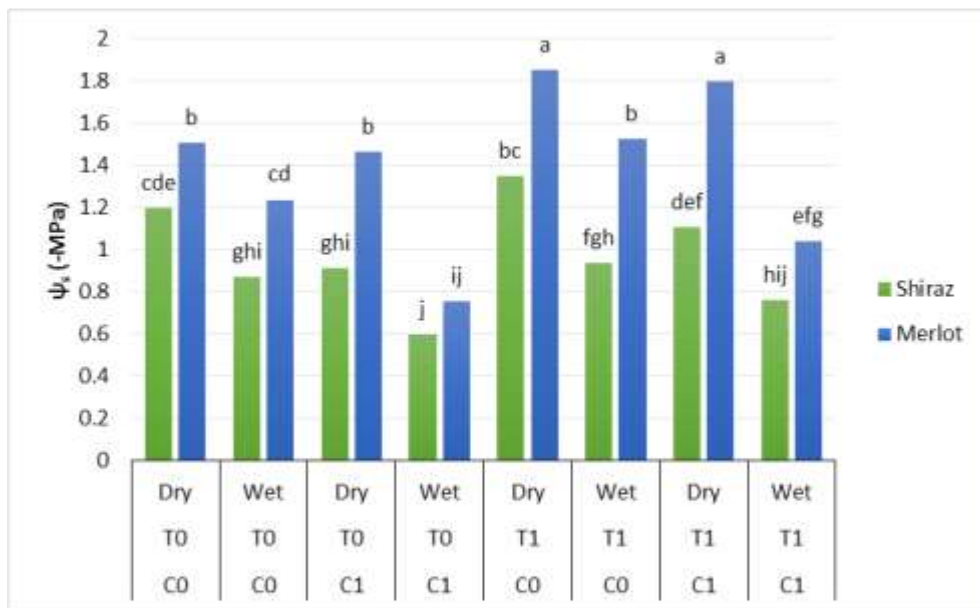

Fig. 3.32 Stem water potential of Shiraz and Merlot in the various treatment combinations at 12 weeks after planting. Bars with the same letters do not differ significantly ($p \leq 0.05$).

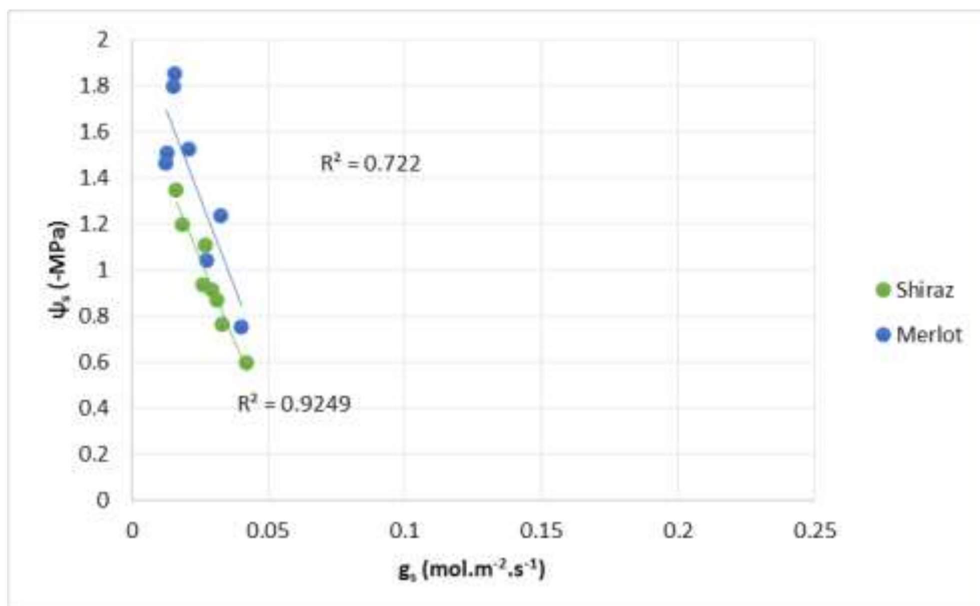

Fig. 3.33 Relationship between stem water potential and stomatal conductance of Shiraz and Merlot in the various treatment combinations at 12 weeks after planting.

At 12 weeks after planting, A_N was better sustained in Shiraz than in Merlot. Only the C1(wet) treatments in the latter cultivar displayed mentionable levels of activity (Fig. 3.34), with basically no A_N occurring under C0(dry) conditions. Shiraz was less sensitive to water deficit than Merlot, with only the C0(dry) treatments with an A_N rate of lower than 2 µmol $CO_2.m^{-2}.s^{-1}$, compared to Merlot where A_N in most of the treatment combinations was lower than 2 µmol $CO_2.m^{-2}.s^{-1}$.

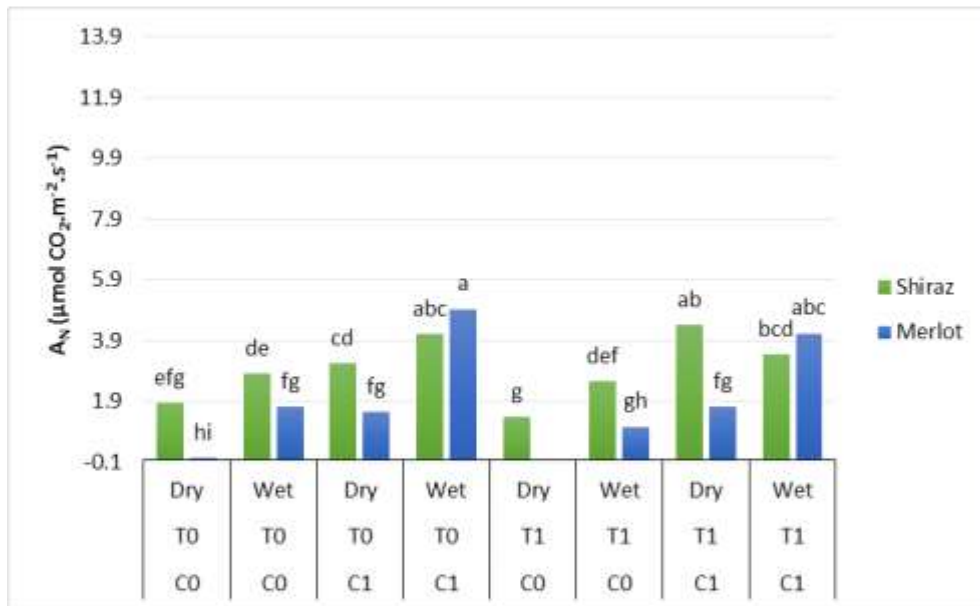

Fig. 3.34 Photosynthetic rate of Shiraz and Merlot in the various treatment combinations at 12 weeks after planting. Bars with the same letters do not differ significantly ($p \leq 0.05$).

The WUE_i of both cultivars was improved under elevated CO_2 conditions (in both T0 and T1 regimes as previously discussed) (Fig. 3.35), irrespective of the level of water supply. A high WUE_i value generally indicates drought resistance in a cultivar (Medrano et al. 2015), but these ratios may be very misleading, as illustrated by Merlot in the C1(dry) treatments. High WUE_i values that are comparable to those at 4 and 8 weeks after planting were found, which might be interpreted as sustained physiological activity and efficient use of available water. However, this parameter only expresses the ratio between A_N and g_s and gives no indication of the specific rates at which these two processes occur.

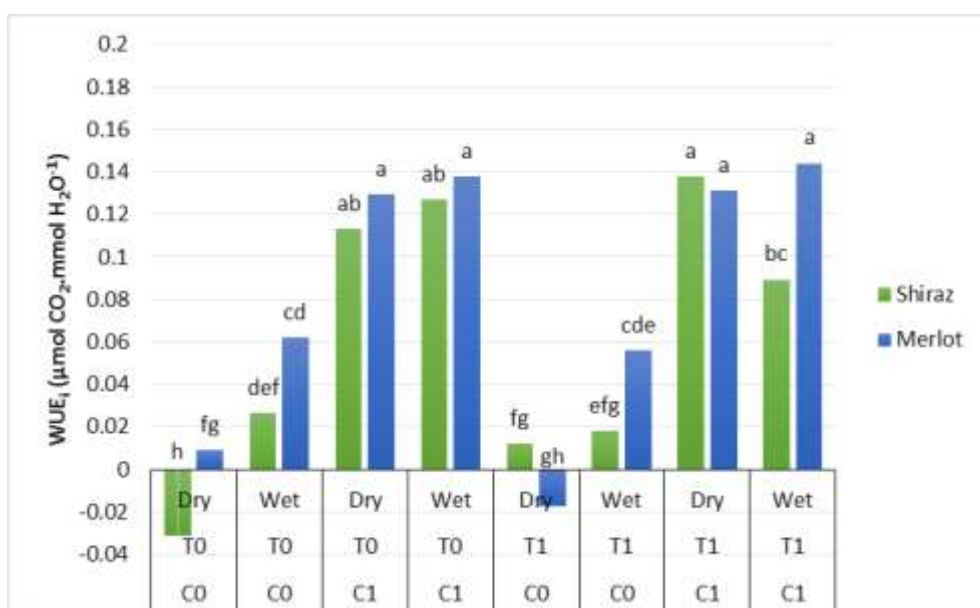

Fig. 3.35 WUE_{inst} of Shiraz and Merlot in the various treatment combinations at 12 weeks after planting. Bars with the same letters do not differ significantly ($p \leq 0.05$).

Shiraz was able to maintain A_N and prevent excessive water stress in the vine under high temperature and water deficit conditions in the presence of high CO_2 levels (Fig. 3.36). In this study, Merlot appeared to be less drought resistant than Shiraz, but should be able to function well under high temperature conditions, provided that CO_2 levels are high and sufficient water is available (Fig. 3.37).

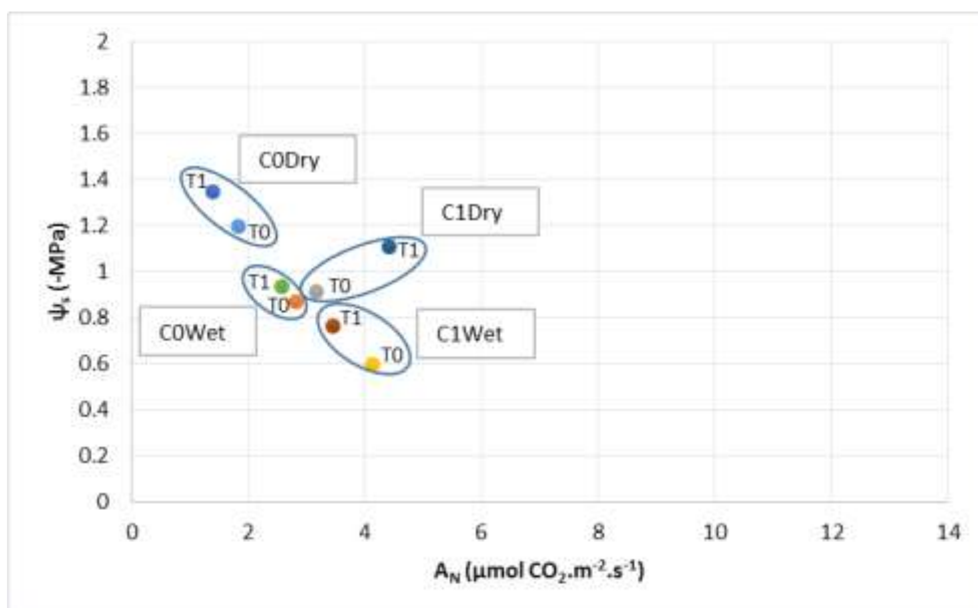

Fig. 3.36 Relationship between stem water potential and photosynthesis of Shiraz in the various treatment combinations at 12 weeks after planting.

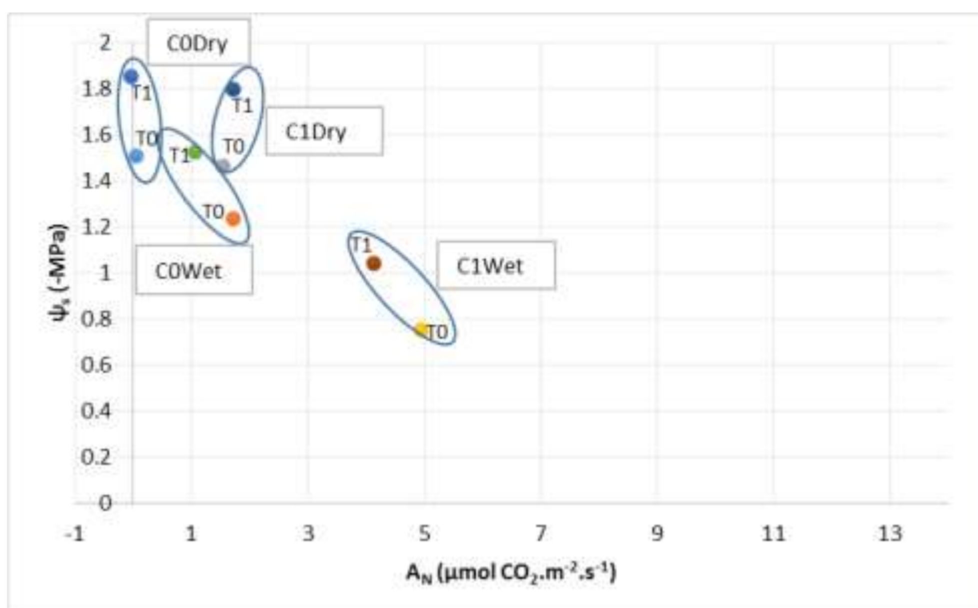

Fig. 3.37 Relationship between stem water potential and photosynthesis of Merlot in the various treatment combinations at 12 weeks after planting.

3.3.2 Chlorophyll concentration

At 4 weeks, chlorophyll (chl) *a* levels seemed to be higher in C0 than C1 treatments for both cultivars, while water supply and temperature had no significant effect (Fig. 3.38). This higher chl *a* in C0 environments was more evident in the leaves of Merlot than in those of Shiraz. Chlorophyll *b* levels were higher in Merlot than in Shiraz leaves, especially at the higher temperatures (Fig. 3.39). For Shiraz, C0 treatments seemed to result in higher chl *b* concentrations than C1 under higher temperatures. The chl *b* in Merlot leaves was more responsive to the environmental conditions, with higher levels under C0 than under C1 conditions and under T1 than under T0 temperatures. When high CO_2 levels and temperatures were combined, both chl *a* and *b* were lower compared to C0T0 treatments, under water-stressed and well-watered conditions. This is in contrast with Martínez-Lüscher et al. (2015) who found no effect on chl *a* and *b* under elevated CO_2, as well as Leibar et al. (2015) who found an increase in both. The highest leaf chl *a* and *b* concentrations ($\mu g.g^{-1}$ fresh mass) were already found at 4 weeks after planting in both cultivars, after which they decreased during the following weeks (Figs 3.38-3.39). These findings are contrary to those of Filimon et al. (2016) who reported an increase in leaf chl *a* and *b* concentrations until véraison before they started to decrease.

Fig. 3.38 Chlorophyll *a* of Shiraz and Merlot in the various treatment combinations at 4, 8 and 12 weeks after planting. Bars with the same letters within the same week do not differ significantly ($p \leq 0.05$).

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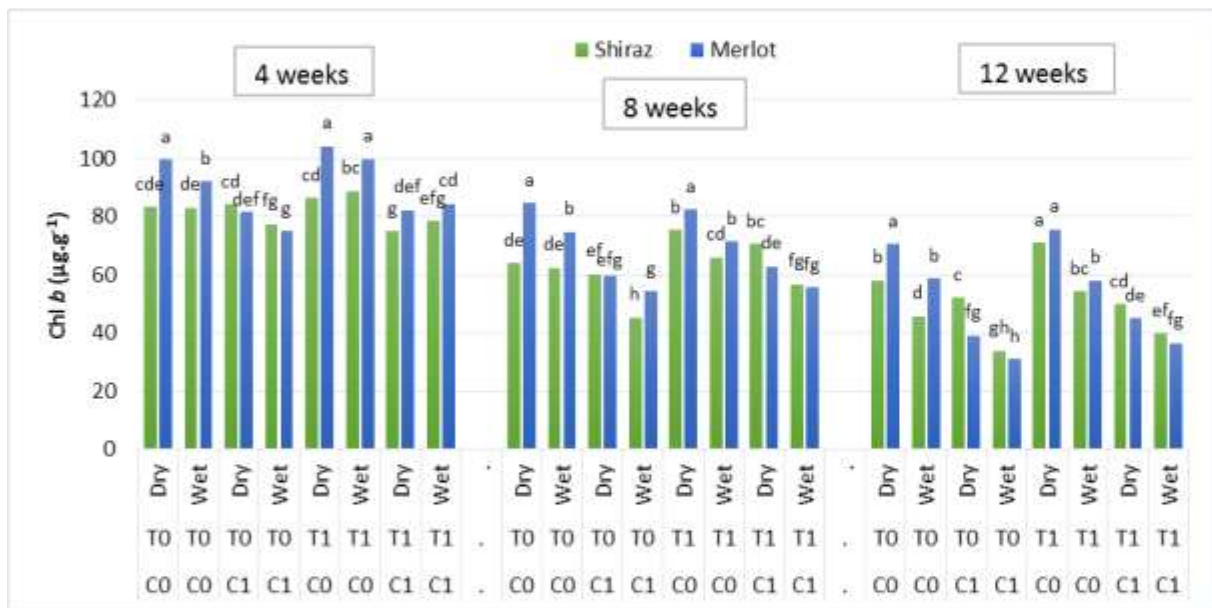

Fig. 3.39 Chlorophyll *b* of Shiraz and Merlot in the various treatment combinations at 4, 8 and 12 weeks after planting. Bars with the same letters within the same week do not differ significantly ($p \leq 0.05$).

Chlorophyll *a* in Shiraz decreased faster and to lower levels than those of Merlot during the following 8 weeks. The effect of the various treatment combinations was similar for both cultivars, but more clearly discernible for Merlot. Chlorophyll *a* breakdown was enhanced by high CO_2 levels and water supply, while T0 temperatures also resulted in lower chl *a* compared to T1. Chlorophyll *b* also decreased between 4 and 12 weeks after planting in both cultivars. This pattern of degradation was similar to that of chl *a*, with a faster and higher decrease in elevated CO_2, T0 and well-watered conditions. This resulted in higher chlorophyll concentrations under ambient CO_2, higher temperatures and water-stress conditions at the end of the 12 weeks. Haque et al. (2006) also found a faster rate of chlorophyll decrease under elevated CO_2 conditions. Although Leibar et al. (2015) found an increase in both chl *a* and *b* in water-stressed plants, this is in contrast with most studies where limited water resulted in lower chlorophyll levels (e.g. Sanchez et al. 1983; Zhang et al. 2011; Urban et al. 2017).

The relative breakdown rates over time of chl *a* compared to chl *b* differed between the cultivars and treatment combinations (Figs 3.38-3.39), but the chl *a/b* ratio remained relatively constant in both cultivars, with a slight increase towards the end of the growth period (Fig. 3.40). The effect of the treatments was the same between cultivars, with higher chl *a/b* ratios in (wet) than (dry), T0 than T1 and C1 than C0 conditions.

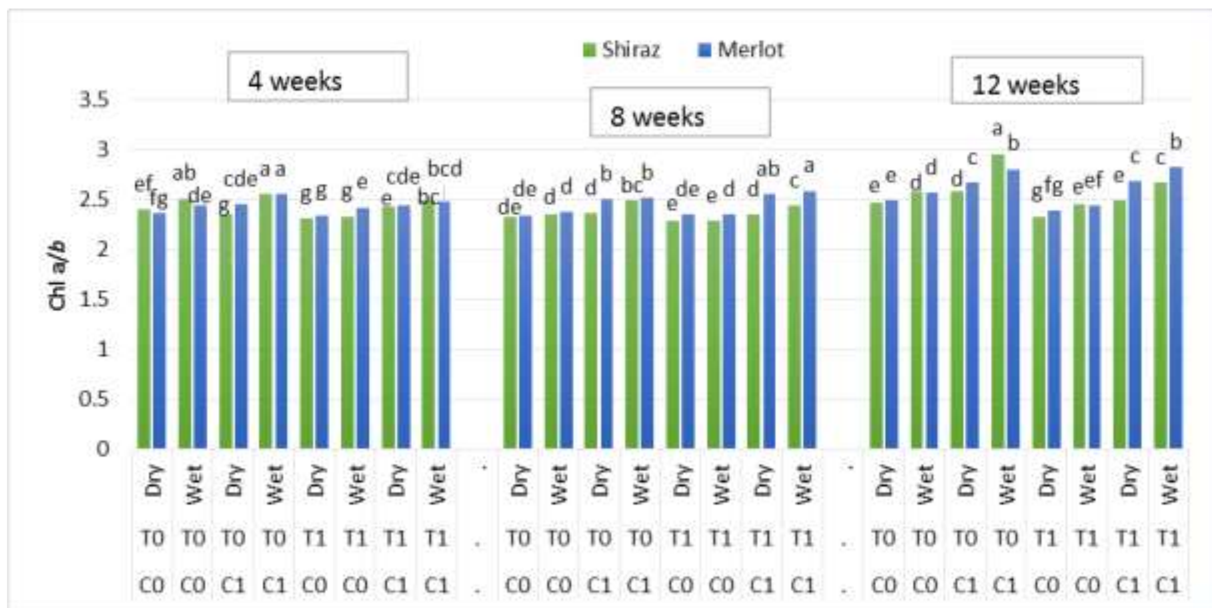

Fig. 3.40 Chlorophyll a/*b* of Shiraz and Merlot in the various treatment combinations at 4, 8 and 12 weeks after planting. Bars with the same letters within the same week do not differ significantly ($p \leq 0.05$).

3.3.3 Trends over the growth period

Stomatal conductance (Fig. 3.41) and E (Fig. 3.42) were closely linked during the weeks monitored and both parameters tended to decrease, which is in accordance with Salazar-Parra et al. (2012) who also found a decrease in g_s and E during the course of their study on the effect of climate factors (CO_2, temperature and water supply) on fruit cuttings. At 4 weeks after planting, g_s of both cultivars was enhanced by the combination of lower temperature and water supply, while Shiraz seemed more inhibited by the higher temperatures than Merlot, with the latter cultivar displaying high g_s and E in all the (wet) treatments. The combination of water supply and elevated CO_2 seemed of more importance during the following weeks with higher g_s and E at 8 weeks after planting in both cultivars, irrespective of the temperature. Of all the environmental factors (CO_2, water and temperature), the level of water supply had the strongest effect on g_s and E (Table 3.3). Since only basal leaves were measured during the course of this trial, leaf age could also have contributed to the decreases found. Patakas et al. (1997) found lower E in older leaves due to a decrease in g_s. This is supported by the data in Table 3.3 where the time (in weeks after planting) difference between measurements contributed more to the total variance for both g_s and E than any of the climatic parameters.

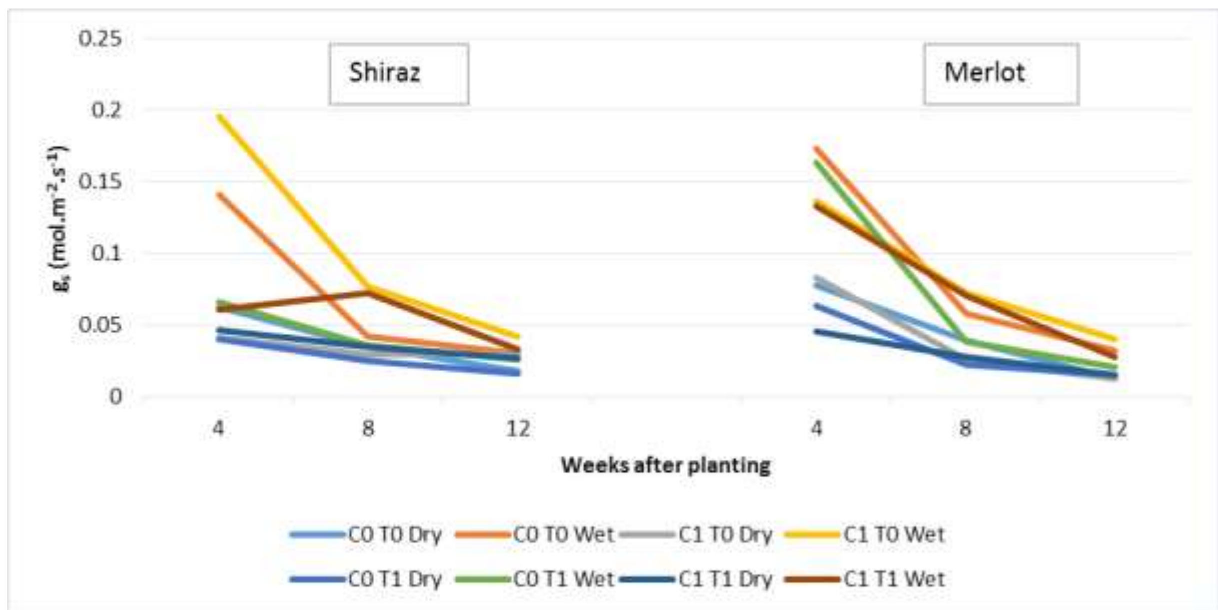

Fig. 3.41 Stomatal conductance of Shiraz and Merlot in the various treatment combinations at 4, 8 and 12 weeks after planting.

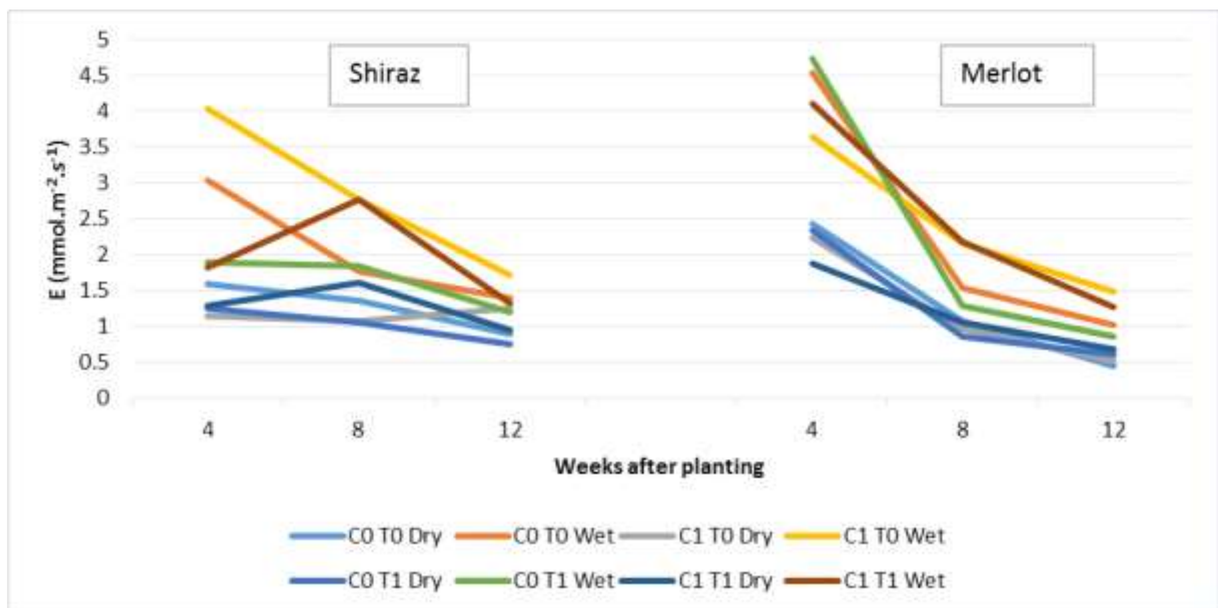

Fig. 3.42 Transpiration rate of Shiraz and Merlot in the various treatment combinations at 4, 8 and 12 weeks after planting.

The patterns of g_s and E were reflected in the ψ_s (Fig. 3.43). At 4 weeks after planting, it seemed as if water supply was the main determinant for stem water potential, especially for Merlot. The level of water stress increased between 4 and 8 weeks after planting in al treatment combinations for both cultivars. Between 8 and 12 weeks, ψ_s in the Shiraz seemed to stabilise, with no level of ψ_s indicating severe water stress (< -1.4 MPa; Myburgh, 2018). In contrast, Merlot was severely water stressed in the T1(dry) treatments. In both cultivars, the stem water potential increased in the C1(wet) treatments

between 8 and 12 weeks, which corresponds to the sharp decrease in both g_s and E during the same period. Under the temperature conditions of this study and providing that water is available, higher CO_2 levels resulted in higher xylem water potentials compared to current ambient levels.

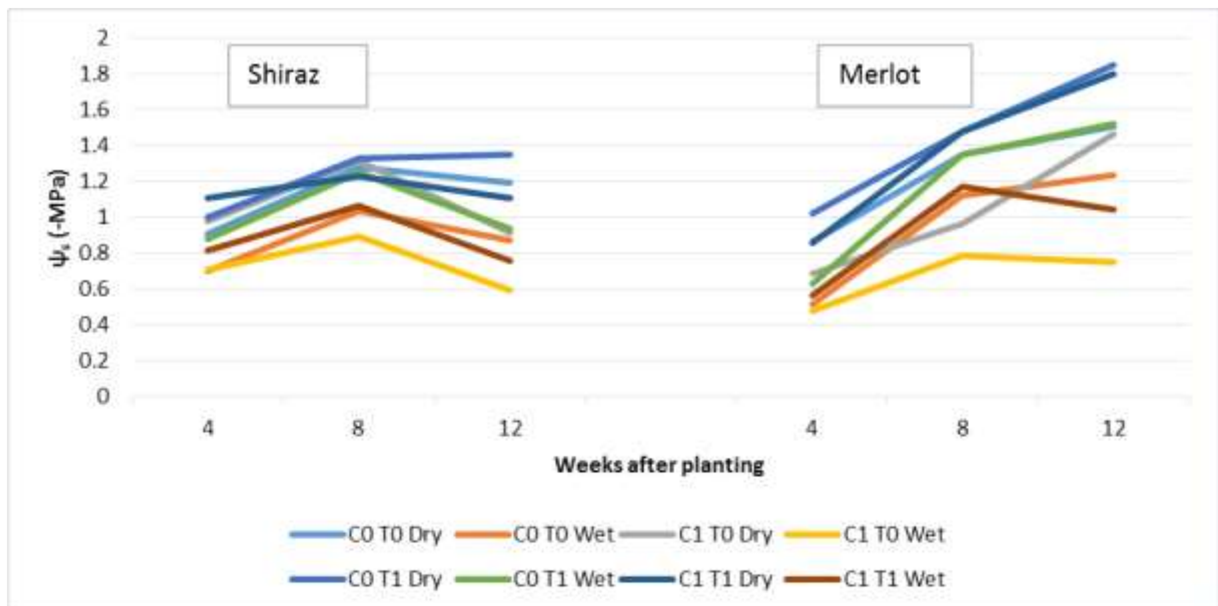

Fig. 3.43 Stem water potential of Shiraz and Merlot in the various treatment combinations at 4, 8 and 12 weeks after planting.

The mesophyll CO_2 concentration was considerably enhanced by elevated CO_2 and remained relatively constant at high levels during the whole growth period in those treatments for both cultivars (Fig.3.44). Even in ambient CO_2 levels of 800 ppm, C_i seemed to be limited to a maximum of 600 µmol CO_2.mol^{-1}, which could be an indication of the leaf CO_2 saturation point. Photosynthesis is therefore not limited by available CO_2, but the rate would (at least) depend on RUBP availability and RubisCO activity. It is expected that A_N would be higher in the C1 treatments, due to the enhanced carboxylation of RuBP relative to its oxygenation in the presence of leaf saturated CO_2 and thus lower photorespiration (Kriedemann et al. 1976; Flexas et al. 2002; Zinta et al. 2014, 2018).

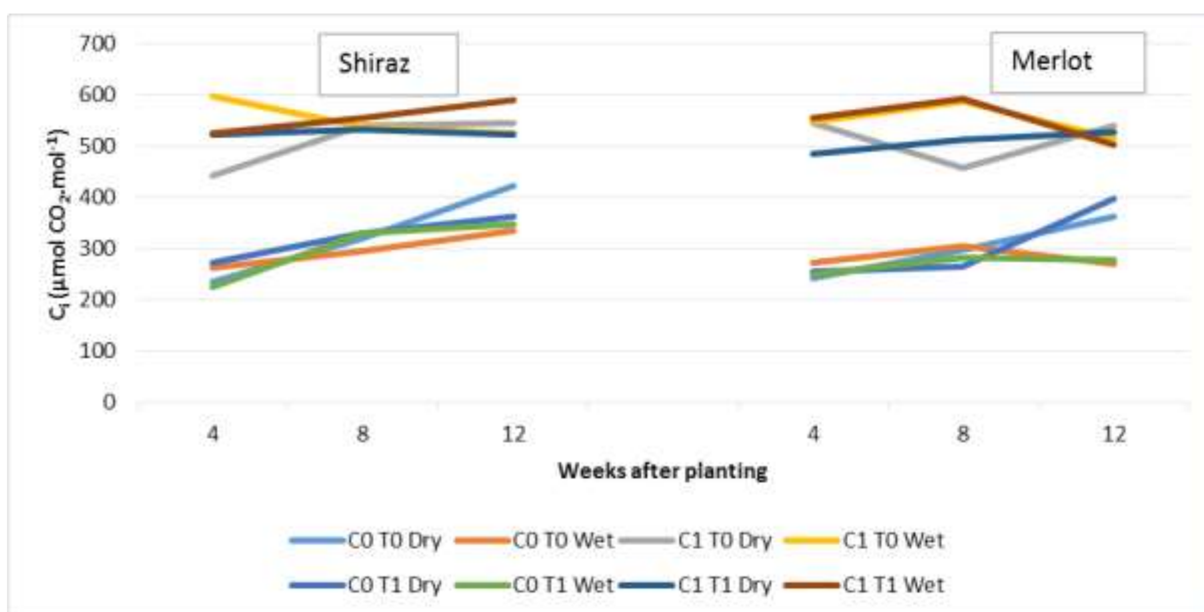

Fig. 3.44 Leaf internal CO_2 of Shiraz and Merlot in the various treatment combinations at 4, 8 and 12 weeks after planting.

Internal CO_2 was significantly lower in the C0 treatments, but increased between 4 and 12 weeks after planting in especially the C0(dry) treatments to reach an equilibrium with the treatment level of 400 ppm. The C_i in the C0(wet) vines remained lower than that at 300 µmol CO_2.mol^{-1}. Ambient CO_2 clearly had a significant effect on mesophyll CO_2, and as single factor contributed 67 % of the total variance found (Table 3.3), while both water supply and temperature did not affect C_i.

Photosynthesis decreased during the growth period for both cultivars in all treatments (Fig. 3.45) with the fastest decrease between 4 and 8 weeks after planting. Between 8 and 12 weeks after planting, the rate of photosynthesis seemed to become more constant, except for the (wet) treatments in Merlot that continued to decrease sharply and indicate the sensitivity of this cultivar to water deficit. This general decrease in photosynthetic rates is in accordance with those found by Hunter & Visser (1988), Archer (1990) and Hunter et al. (1994), in which cases A_N activity decreased (regardless of the leaf position on the shoot) as the season progressed due to leaf senescence. The A_N decreased also in chamber studies to low rates at grape ripeness, irrespective of the growth environment (Salazar-Parra et al. 2012). This decrease was attributed to a decrease in physiological activity due to leaf senescence.

During the 12 weeks after planting, A_N rates were the highest in elevated CO_2 levels and with adequate water supply, while it seemed as if the T0 temperature regime was slightly more favourable than T1. Higher CO_2 levels combined with high temperatures did enhance A_N compared to ambient CO_2 and T0 in both cultivars, which is in accordance with literature (Alonso et al. 2008; Edwards et al. 2017).

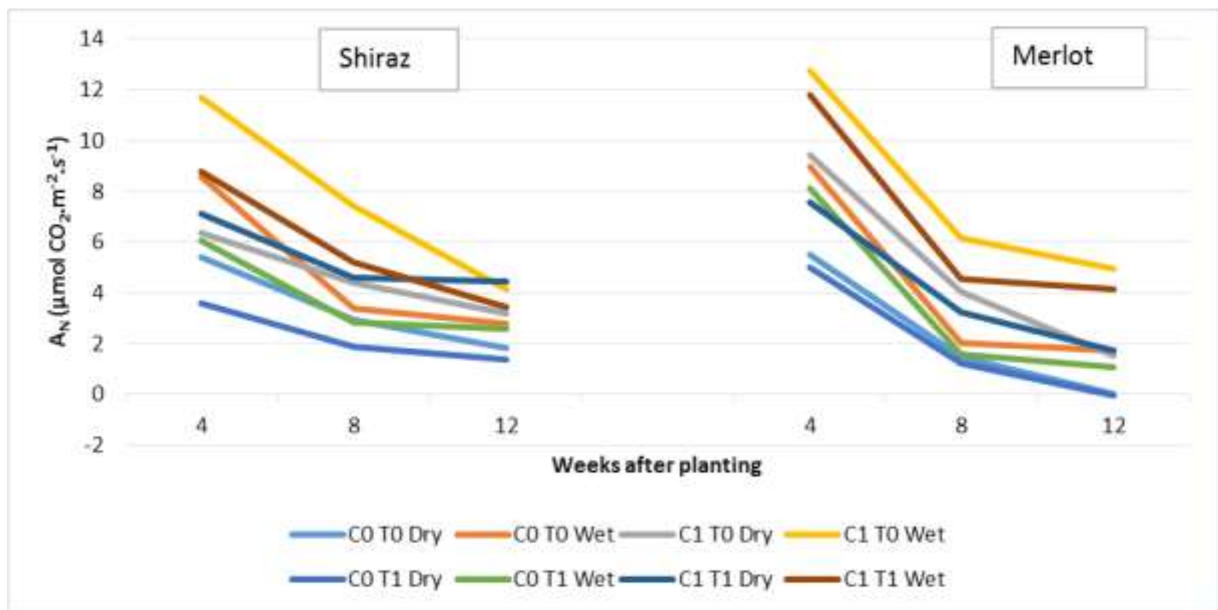

Fig. 3.45 Photosynthetic rate of Shiraz and Merlot in the various treatment combinations at 4, 8 and 12 weeks after planting.

The levels of ambient CO_2, temperature, water supply as well as the age of the basal, mature leaves measured significantly affected A_N ($p \leq 0.001$) (Table 3.3). The leaf age contributed 33 % to the total variance, followed by CO_2 (10 %) and water (6 %). With regards to the environmental conditions enforced, A_N was more strongly affected by CO_2 and water availability than by temperature, although during the growth period maximum temperatures of between 27 °C and 31 °C were consistently more conducive to A_N than 30-34 °C.

WUE_i was significantly higher in elevated CO_2 environments (Fig. 3.46). It was however not due to the lower g_s in the C1 compared to the C0 treatments as was found by Martínez-Lüscher et al. (2015), but rather to the relative higher A_N rates under the conditions of this trial.

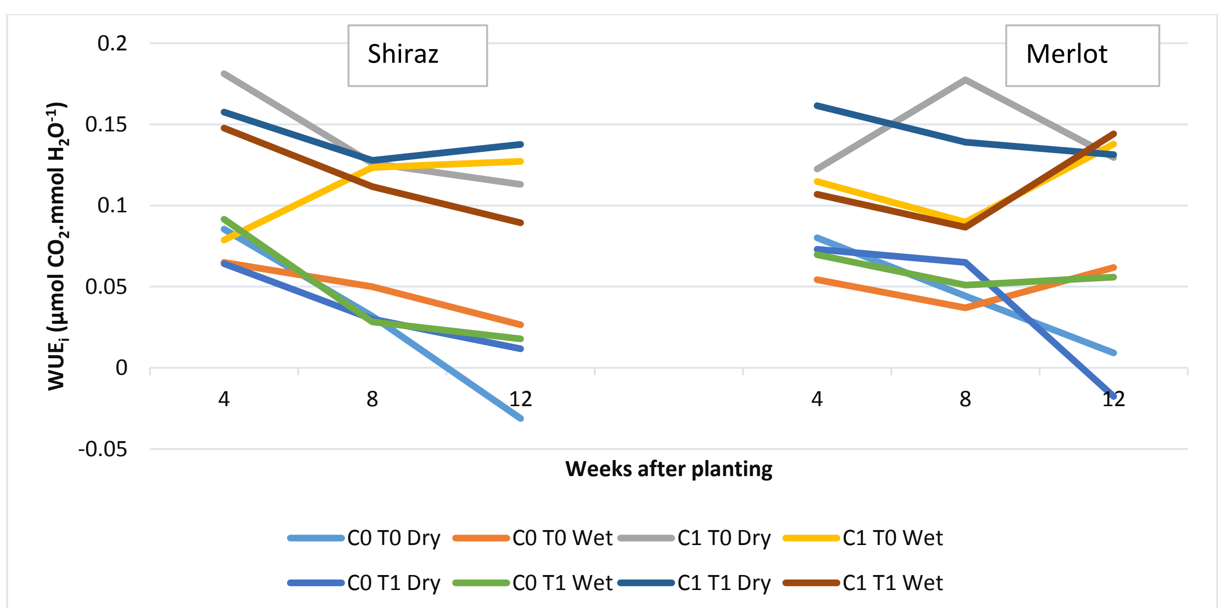

Fig. 3.46 WUE_i of Shiraz and Merlot in the various treatment combinations at 4, 8 and 12 weeks after planting.

The WUE_i of C0(dry) treatments decreased during the growth period, irrespective of the temperature or cultivar. At the end of the growth period the A_N rate in these treatments was so low that negative (or close to zero) ratios were found. Between 8 and 12 weeks after planting the WUE_i of (wet) vines remained relatively constant in Shiraz and increased for Merlot (especially in the C1 treatments) , which highlights the importance of adequate water supply to sustain physiological activity in grapevines throughout the growth period. As already discussed, the advantages of a high WUE_i should always be put in context to the actual A_N activity of the vines and not be evaluated on its own.

Chlorophyll concentrations decreased in all treatment combinations over the 12 weeks after planting (Fig. 3.47). This is in accordance with the natural progress of leaf senescence, but under field conditions breakdown of chlorophyll commenced later in the season at véraison (Filimon et al. 2015). At 12 weeks after planting, the lowest total chlorophyll concentrations were found in the C1T0(wet) treatments and the highest in the C0T1(dry). In contrast, photosynthesis was consistently higher in C1 than C0, T0 than T1 and (wet) than (dry) treatments (Fig. 3.45).

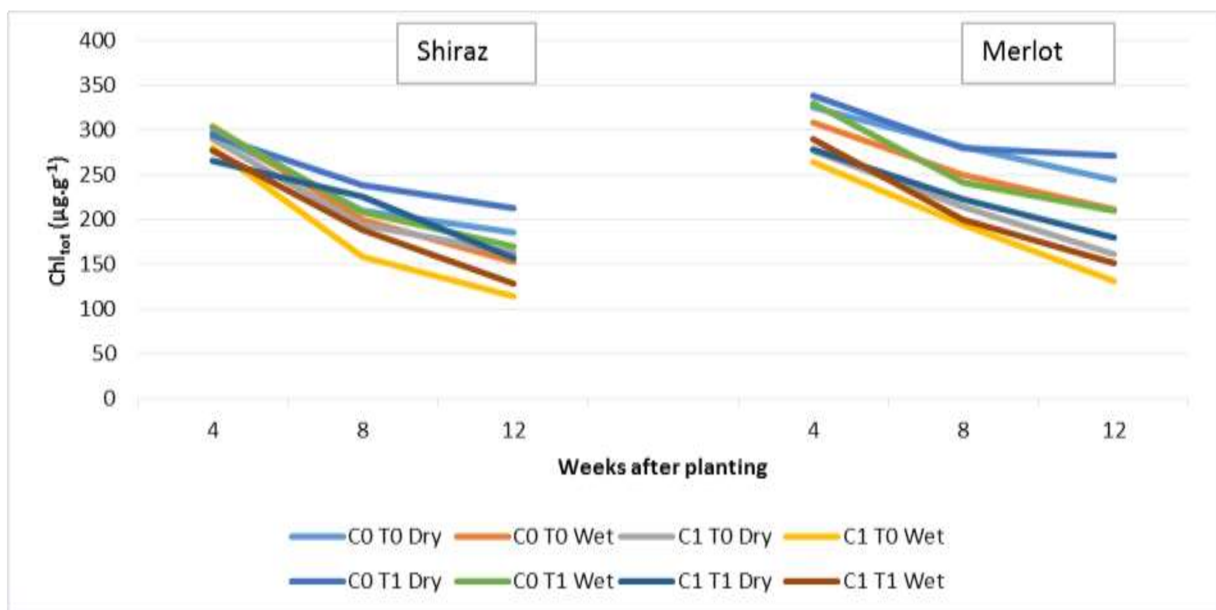

Fig. 3.47 Total chlorophyll concentration of Shiraz and Merlot in the various treatment combinations at 4, 8 and 12 weeks after planting.

Although the process of A_N is directly dependent on the presence of chlorophyll, there is no consistent relationship between chlorophyll concentration (chl_{tot}) and A_N activity in well-exposed leaves in vineyard canopies (Hunter & Visser, 1989). Despite a possible relationship found between the chl_{tot} and the A_N activity for mature, interior canopy leaves that were exposed to lower light conditions (Hunter & Visser, 1989), chl_{tot} is not a reliable index of A_N. Plants seem able to increase chlorophyll efficiency, rather than its synthesis, when conditions that were detrimental to A_N turn favourable again. Photosynthetic rate

increased when N was supplied to N deprived plants (Girardin et al. 1985), while Sanchez et al. (1983) found that plants are able to compensate for up to 40 % lower chlorophyll levels with regards to A_N.

Interestingly, the C1T0(wet) environments were found to be the most conducive to both A_N and the breakdown of chlorophyll, which seems to indicate a poor relationship between chl_{tot} and the rate of A_N. Although A_N of only the basal leaves was measured and the chl_{tot} of a combined sample of all the main leaves on the shoot was determined, it was interesting to find that very strong negative linear correlations exist between chl_{tot} and A_N, especially during the second half of the growth period. At 8 weeks the correlation coefficient (r) for Shiraz was -0.840 (p = 0.009) and for Merlot -0.917 (p = 0.001) (data not shown), while the correlations during week 12 were still very strong in both cultivars (Fig. 3.48). At that same time, very strong positive relationships were found between the leaf nitrogen concentration (%N) and chl_{tot} (Fig. 3.49). Due to the strong link between leaf N level, chlorophyll and RubisCO content (Evans, 1989) it may be suggested that the RubisCO content in C1 leaves decreased as well and that carboxylation efficiency in these treatments was increased to sustain the higher A_N measured. However, since RubisCO analysis was not done in this study, it was found that the highest A_N was found in leaves under elevated CO_2 levels that contained the lowest chl_{tot} and N concentrations and *vice versa*.

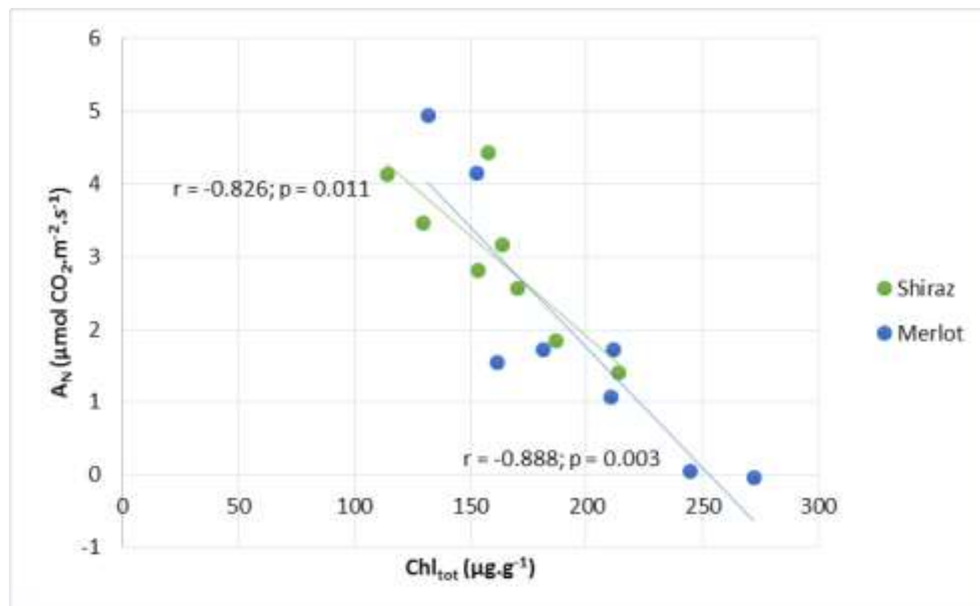

Fig. 3.48 Relationship between photosynthetic rate and total chlorophyll of Shiraz and Merlot leaves in the various treatment combinations at 12 weeks after planting.

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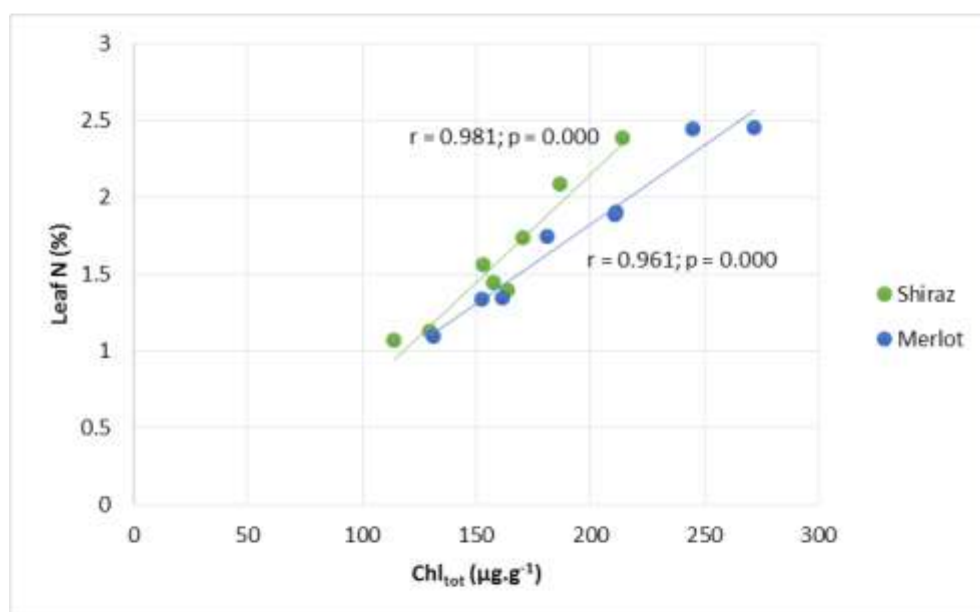

Fig. 3.49 Relationship between nitrogen concentration and total chlorophyll of Shiraz and Merlot leaves in the various treatment combinations at 12 weeks after planting.

High A_N rates are maintained under high ambient CO_2 (due to the increased C_i) even with low leaf chlorophyll and RubisCO content (Drake et al. 1997; Leakey et al. 2009). This would optimise the N use by plants under these conditions, since nitrogen may then be partitioned to other tissues and organs where required (Evans, 1989; Drake et al. 1997; Leakey et al. 2009). This will be discussed in more detail in Chapters 5 and 6.

3.4 CONCLUSIONS

Future climatic conditions (high temperature, increased atmospheric CO_2 and less water available for agriculture) would significantly affect physiological activity in newly planted, grafted grapevines. The CO_2 concentrations and level of water supply had the strongest effect, while the slightly lower temperature conditions of this study seemed to be more conducive to grapevine functioning. However, the synergistic effect between elevated CO_2 and high temperature was observed, especially when water stress was not very severe.

Higher CO_2 levels resulted in increased stomatal conductance and transpiration rate, but also better WUE_i due to the stimulating effect on photosynthesis. It seemed as if the higher CO_2 was able to mitigate the negative effect of water deficit to a certain extent, since higher photosynthetic rates were measured in water-stressed vines in elevated CO_2 conditions than in well-watered vines in the lower CO_2 treatments. Water supply will however remain pivotal for sustained and optimal physiological activity under future climate conditions, especially for more drought sensitive cultivars.

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At the end of the growth period, the highest photosynthetic activity was linked with the lowest chlorophyll and leaf nitrogen concentrations in the well-watered, elevated CO_2 treatments. It seemed as if the efficiency of the chlorophyll was increased under these conditions, while more nitrogen possibly became available to the rest of the plant body to contribute to a larger vegetative growth potential.

It is clear that the effects of a changing climate on grapevine functioning (and growth) will also depend on the cultivar. Shiraz and Merlot had similar reaction patterns to the treatment factors, but the degree of the reactions seemed to differ with Merlot being more sensitive to water deficit and at the same time more responsive to increased CO_2 levels.

The grapevine is very resilient and is able to adapt physiologically to changes in growth conditions in its environment. A sound understanding of the terroir and well-founded choices in cultivars (scion and rootstock combination) and cultivation practices (optimising conditions for vegetative growth and quality grape ripening) should ensure continuing wine grape cultivation in most current viticulture regions.

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CHAPTER 4: INTEGRATIVE EFFECTS OF CLIMATE CHANGE FACTORS (TEMPERATURE, CO_2 AND WATER) ON VEGETATIVE GROWTH OF YOUNG, GRAFTED GRAPEVINES

ABSTRACT:

The vegetative growth of young, potted grapevines was measured at four week intervals during the first 12 weeks after planting. The effect of different combinations of ambient temperature (maximum ranges of 27-31 °C, compared to 30-34 °C), ambient CO_2 (400 ppm vs 800 ppm) and soil water (irrigation to water holding capacity and 50 % thereof), applied immediately after planting, on young vine growth was investigated under glasshouse conditions. Two scion cultivars (Shiraz and Merlot), both grafted onto 101-14 Mgt, were used in this study. Primary shoot and leaf growth increased in both cultivars under high CO_2 conditions. Cultivars reacted differently to environmental variables, with aerial growth of Merlot more strongly enhanced by CO_2 levels than Shiraz. Higher CO_2 resulted in thicker leaves. Biomass accumulation in shoots and leaves continued throughout the growth period. Stronger root growth occurred in elevated CO_2 and in well-watered treatments. Treatment combinations did not affect the inherent growth patterns of the vine and balances between new aerial and root growth were maintained. With proper soil preparation practices and judicious water management, overall vegetative growth of newly planted grapevines should be stronger in future, with a strong buffer capacity against adverse environmental conditions.

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4.1 INTRODUCTION

Any response of a plant to its environment is ultimately aimed at sustaining its growth and reproduction (Alsina et al. 2007). From a purely viticultural point of view, sustainable viticulture (while acknowledging that economic viability is an integral part of it) may be defined as the method of cultivation that achieves the highest yield of ripe grapes of acceptable quality over years, with no reduction in vine vegetative growth (Howell, 2001). Therefore, a balance must be maintained between carbon assimilation and the distribution and utilisation of assimilates throughout the plant to ensure growth and maintenance, but also provide for the storage of reserves. This biochemical exchange between a producing organ and a consuming organ is expressed as a source:sink relationship (Carbonneau, 1996).

A strong sink strength (and thus high export rate from the leaves to the receiving sink) is required to sustain high carbon assimilation rates (Salazar-Parra et al. 2015). Sugars tend to build up in leaves where export is limited (Hunter et al. 1994), resulting in down-regulation of photosynthesis (Keller, 2010; Salazar-Parra et al. 2012). Prevention of carbohydrate accumulation in leaves might be achieved by the development of new, strong sinks (e.g. new vegetative or reproductive structures), an increase in the growth rate or storage ability of existing sinks, or a higher respiration rate (Leibar et al. 2015; Morales et al. 2016).

The sink strength is an expression of the ability to import assimilates, which could be measured as the absolute growth rate or net accumulation rate of dry matter (Ho, 1988). The import rate is regulated by the metabolic activity of the sink and could be altered by either changing the strength of the specific sink or the relative strength of competing sinks. Sink organs are divided into utilisation sinks (where most of the imported assimilates are used for growth) and storage sinks (where substantial amounts of imported assimilates are stored) (Ho, 1988). According to Hunter et al. (1994), the partitioning of assimilates between sites of production, utilisation and accumulation primarily determine the yield and longevity of grapevines.

Grapevine shoots show slow initial growth directly after bud burst, followed by a sharp increase in growth rate (Coombe, 1992), provided that water supply is not limited (Van Zyl, 1981), which could be described as exponential (Mullins et al. 1992). This period of rapid growth does not persist in grapevines and the growth rate decreases after flowering due to the competitive increase in reproductive sink strength (Mullins et al. 1992), causing the growth curve to become sigmoidal. Shoot growth is accompanied by the lengthening of internodes and the expansion of existing leaves (Pratt, 1988). In late summer the growth rate of the primary shoots decreases, while internode elongation ceases progressively from the basal to the apical part of the shoot. Where water is readily available, shoot growth will continue (albeit at low rate) throughout the season (Van Zyl, 1981). Shoot growth should ideally stop at véraison under field conditions (Archer, 1988) to limit sink competition between growing

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shoots and ripening bunches for the production of high quality wines. After full ripeness is achieved, the translocation of assimilates towards vegetative organs is resumed, possibly to supplement the accumulation of reserves (Hunter & Visser 1988; Bates et al. 2002).

The number of primary leaves on a shoot is determined by its length and vigour (Dokoozlian & Kliewer, 1995). Leaf development of the grapevine follows a well-defined sequence of emergence, unfolding and rapid laminar expansion, followed eventually by senescence and abscission (Kriedemann et al., 1970). A grapevine leaf has a typical sigmoidal growth curve (De la Harpe, 1983), with the rapid growth phase occurring in the second and third weeks after unfolding. The leaf attains full size about four weeks after unfolding (Hale & Weaver, 1962). After that, the specific leaf mass (mass per unit area) continues to increase (Wermelinger & Koblet, 1990), indicating continued translocation to the leaves. This increase in specific leaf mass is caused by an increase in structural components, probably cell walls (Mullins et al. 1992).

There is a strong inter-relationship between shoot and leaf growth (Iland et al. 2011). Wermelinger & Koblet (1990) found a very strong linear relationship between total shoot dry mass and total leaf dry mass per vine in an 18-year-old vineyard, while Costanza et al. (2004) reported linear relationships between both dry and fresh shoot mass and leaf area per shoot, as well as between shoot length and leaf area per shoot for both primary and secondary shoots in a 5-year-old vineyard that experienced no water stress. Therefore, it could be expected that any factor affecting grapevine vigour would have similar, correlative effects on shoot and leaf growth (Iland et al. 2011).

Root growth and distribution are largely affected by physical (Van Huyssteen, 1988) and chemical (Conradie, 1988) soil properties, while the rootstock genotype and cultivation practices (such as planting density and trellis system used) determine the root density (Southey & Archer, 1988; Archer et al. 1988). Callejas et al. (2009) proposed that soil temperature would be the main factor modifying root growth, should all factors affecting root development be optimal. According to Alvarez-Uria and Körner (2007) the critical minimum temperature for significant root growth is 6 ^{o}C, while Woodham & Alexander (1966) found that vines grown in a solution culture and roots kept at a temperature of 30 ^{o}C resulted in stronger root and shoot growth compared to vines where the roots were kept at 11 ^{o}C and 20 ^{o}C. The authors mentioned that these results might have been due to the small contrast between the root and shoot temperature (the latter was kept below 35 ^{o}C), rather than the temperature *per se*. Field et al. (2009) studied the effect of soil temperature between dormancy and flowering and found that the higher soil temperature (23 ^{o}C) resulted in higher shoot biomass and larger leaf area with relatively lower root growth compared to the lower soil temperature of 13 ^{o}C. In mature vines, there are two main peaks of fine root growth during the growth season - around flowering and harvesting (Van Zyl, 1984). In accordance, Callejas et al. (2009) found continuous root growth during the season, with peaks at

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flowering and harvest. Bates et al. (2002) reported a third peak during mid-season, while Eissenstat et al. (2006) found little evidence of a bimodal root growth pattern or a growth peak after harvest, with large variation in root growth patterns between seasons. According to Araujo and Williams (1988), root growth only commences in young vines when the canopy is already well-developed and also stated that root growth occurs when excess photosynthetates are available in the leaves. They further observed a large increase in root biomass in two-year-old vines during the last part of the growth season, which they ascribed to the absence of a reproductive sink.

In future, vineyards will be established and cultivated under different climatic conditions compared to current conditions, with higher expected atmospheric CO_2 concentrations, increased temperatures and limited water availability (Morales et al. 2016). When high CO_2 levels were combined with high temperatures (30-35 ^oC; Alonso et al. 2009) and water supply was not limited, there was a synergistic effect on the rate of photosynthesis (Alonso et al. 2008; Edwards et al. 2017). It is thus expected that grapevine vigour (Kriedemann et al. 1976; Galat Giorgi et al. 2013) as well as total growth (vegetative and reproductive) may increase (Bindi et al. 2001; Long et al. 2004; Sadras & Moran, 2013; Torregrosa et al. 2017) under future environmental conditions.

According to Martínez & Chacon (2010), drought may be the main environmental factor limiting the photosynthetic activity of plants. Limited water availability is associated with decreased vigour and growth, with strong reductions in shoot length, leaf number and leaf size (Palliotti et al. 2008). Inadequate supply of water (or nutrients) in the soil would increase allocation to the roots in relation to the shoots (Hare et al. 1997; Salazar-Parra et al. 2015; Simonneau et al. 2017), which is why root growth is less limited by drought conditions than shoot growth (Sharp & Davies, 1989). A decrease in the shoot:root ratio may therefore be an indication of limited water or nutrient availability.

When grafting a scion cultivar onto a rootstock, an interaction between the two different genotypes is enforced and a balance between canopy and root growth is established (Hunter & Volschenk, 2001; Archer & Hunter, 2005; 2010). This interaction significantly affects all vegetative growth parameters as well as the ratio of biomass allocation to shoots and roots, respectively (Tandonnet et al. 2010). The rootstock affects the vigour of the scion by its ability for water and nutrient uptake (Serra et al. 2014) and can modify the scion's response to edaphic stresses (Stevens et al. 2008), while the scion cultivar strongly affects the degree of root system development and growth (Tandonnet et al. 2010).

During the first season after planting, optimal care of the young vine is advised regarding strict weed and pest control and avoidance of any water stress (Creasy & Creasy, 2009; Jackson, 2014) to maximise vegetative growth (both shoot and root growth). Given continued judicious management, proper root system development during the first few years would buffer the vine against adverse climate conditions or environmental stress for the rest of its productive life (Archer & Hunter, 2010).

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In this part of the study the combined effect of projected climate change conditions (high ambient temperature, elevated CO_2 and water deficit) on the vegetative growth of grafted grapevines during the first 12 weeks after planting was measured under controlled conditions in glasshouse compartments. This is a novel approach to gain a better understanding of how young vines would function and grow (at leaf, root and whole-plant level) under future climates during the very important young vineyard establishment stage.

4.2 MATERIALS AND METHODS

4.2.1 Study location and glasshouse compartments

Four glasshouse rooms situated at ARC Infruitec-Nietvoorbij, Stellenbosch, were used to accommodate the different treatments. The rooms were 2.4 m X 6.0 m each and prepared according to the treatment criteria explained in detail in Chapter 3 and summarised in Table 4.1. The experiment comprised of five consecutive growth cycles (planting times during the first week of February and the first week of September), using Shiraz (SH 470) as scion cultivar for the first three, and Merlot noir (MO 348) for the other two. Both scions were grafted onto rootstock 101-14 Mgt. The potted vines were randomly allocated per glasshouse compartment in a randomised complete block design. There were 108 vines per room (54 per irrigation treatment) and thus 432 vines were used for each growth cycle.

Table 4.1 Treatment combinations randomly allocated in four glasshouse compartments for five growth cycles.

PARAMETER	TREATMENTS											
	C0T0			C1T0			C0T1			C1T1		
Vine age (weeks)	4	8	12	4	8	12	4	8	12	4	8	12
CO_2 levels (ppm)	400	400	400	800	800	800	400	400	400	800	800	800
Temperature (°C)	15/27	15.5/29	16/31	15/27	15.5/29	16/31	18/30	18.5/32	19/34	18/30	18.5/32	19/34
Water treatments	Wet: WC Dry: $\frac{1}{2}$WC	Wet: WC Dry: $\frac{1}{2}$WC	Wet: WC Dry: $\frac{1}{2}$WC	Wet: WC Dry: $\frac{1}{2}$WC	Wet: WC Dry: $\frac{1}{2}$WC	Wet: WC Dry: $\frac{1}{2}$WC	Wet: WC Dry: $\frac{1}{2}$WC	Wet: WC Dry: $\frac{1}{2}$WC	Wet: WC Dry: $\frac{1}{2}$WC	Wet: WC Dry: $\frac{1}{2}$WC	Wet: WC Dry: $\frac{1}{2}$WC	Wet: WC Dry: $\frac{1}{2}$WC

C0: Lower CO_2 (400 ppm); C1: Higher CO_2 (800 ppm); T0: Lower temperature; T1: Higher temperature (T0 max + 3°C); WC: Water-holding capacity

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4.2.1.1 Potting soil

The soil used for each growth cycle was obtained from the same fallow vineyard site in Robertson, South Africa, and transported to the experiment location. Each plastic planting pot (25 cm diameter; roughly 7.2 L) was provided with a Bidim-layer at the bottom before the vine was planted in 6.50 kg of soil. The soil was a sandy clay loam with a high pH (Chapter 3). Soils were analysed for their macro and micro nutrient content before each planting date by a SANAS Accredited Testing Laboratory, in accordance with ISO 17025:2005 (further details in Chapter 5). No additional nutrients were provided to the young, growing vines for the duration of the study.

4.2.1.2 Grafted vines

Vines were obtained from a SAPO (South African Plant Improvement Organisation) accredited nursery in the Wellington/Paarl region. Shiraz (SH 470), grafted onto rootstock 101-14 Mgt, was used for the first three growth cycles and Merlot noir (MO 348), grafted onto the same rootstock, for the last two cycles.

Before planting, vines were pruned back to two buds and roots (only those originating from the basal node were kept) cut to a length of 10 cm. A sample of 18 prepared vines was taken and the average fresh mass of the various vine tissues (explained in 4.2.2.1) was separately determined before planting. Nursery vines were similar in size with Merlot vines having denser root systems with especially more medium-size roots than Shiraz (Table 4.2). Shoot removal and weed control were continuously done during the growth cycles to ensure optimal growth of the vines under the respective growth conditions. Primary shoot tips were not removed and all developing secondary shoots were allowed to grow.

Table 4.2 Average mass distribution (expressed as fresh mass per vine) of Shiraz and Merlot before planting.

Parameter	Mass (g) of Shiraz	Mass (g) of Merlot
RS	24.01 a	24.58 a
OT (> 2.0 mm)	4.14 a	4.29 a
OM (0.5 - 2.0 mm)	3.05 b	7.33 a
OF (< 0.5 mm)	0.86 a	0.65 b

Values in rows followed by the same letter do not differ significantly ($p \leq 0.05$). RS: Rootstock trunk; OT: Old thick roots; OM: Old medium roots; OF: Old fine roots

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4.2.2 Measurements

4.2.2.1 Vegetative growth parameters

Primary and secondary shoot length and mass; number and length of internodes on primary shoot; primary and secondary leaf number; leaf area (individual and total per shoot) [by means of a LICOR LI-3100 area meter (Lincoln, Nebraska, USA)] and leaf mass (individual and total per shoot) were determined every 4 weeks at 4, 8 and 12 weeks after planting. Since effort was made to investigate the respective growth responses of the vine shoots and leaves, they will also be discussed separately in the text. The term “shoot” therefore does not include the leaves, while “aerial growth” refers to the shoots and leaves combined. Tendrils and leaf petioles did not form part of any samples.

Roots were separated into old (dark in colour and suberized) and new (soft and light brown to white in colour) roots (Table 4.2). Each group was further separated into thick (> 2.0 mm in diameter), medium (0.5 - 2.0 mm) and fine (< 0.5 mm) roots before analysis. The plant material of six vines was combined for each of the three replications of each treatment combination at the respective times of sampling.

4.2.3 Statistical layout of project

The data was subjected to analysis of variance (ANOVA) using the General Linear Models Procedure (PROC GLM) of SAS software (Version 9.2; SAS Institute Inc., Cary, USA). The ANOVA was performed in order to evaluate the main influences/effects of cultivar, CO_2, temperature and H_2O, as well as to detect interaction effects among these factors. Measurements over sampling times were included in a split-plot analysis of variance with sampling times as sub-plot factor (Little & Hills, 1978) where applicable. The Shapiro-Wilk test was performed on the standardised residuals from the model to verify normality (Shapiro & Wilk, 1965). Levene’s test showed dissimilarity of cultivar variances (Levene, 1960). To correct for variance differences between cultivars, a weight was included in the ANOVA. The weight was the inverse of the experimental error of each cultivar (John & Quenouille, 1977). Fisher’s least significant difference was calculated at the 5 % level to compare means of the factors (main effects) and factor interaction means (Ott & Longnecker, 2001). A probability level of 5 % was considered significant for all significance tests. The Pearson product moment (Pearson) correlation tests were performed using XLSTAT (Version 2015.1.03.15485, Addinsoft, Paris).

4.3 RESULTS AND DISCUSSION

4.3.1 Secondary shoot and leaf growth

Secondary shoot and leaf growth: Since shoot tip removal was not done in this study, the development and growth of secondary shoots and leaves were extremely limited with a total secondary shoot mass

of between 0.12 g and 0.97 g and secondary leaf mass between 0.48 g and 1.99 g per primary shoot at 12 weeks after planting (data not shown). Secondary growth will therefore only be briefly discussed here.

For the most part, secondary growth in Shiraz did not seem to be affected by the treatment combinations (Figs. 4.1-4.2). The most noticeable was the decrease in secondary shoot and leaf growth in the C0T1(dry) treatment. Secondary growth in Merlot was more responsive to the environmental conditions. Higher CO_2 levels enhanced shoot and leaf growth, while the level of water supply affected secondary shoot growth the strongest (Table 4.3). Generally, Merlot displayed stronger secondary growth than Shiraz.

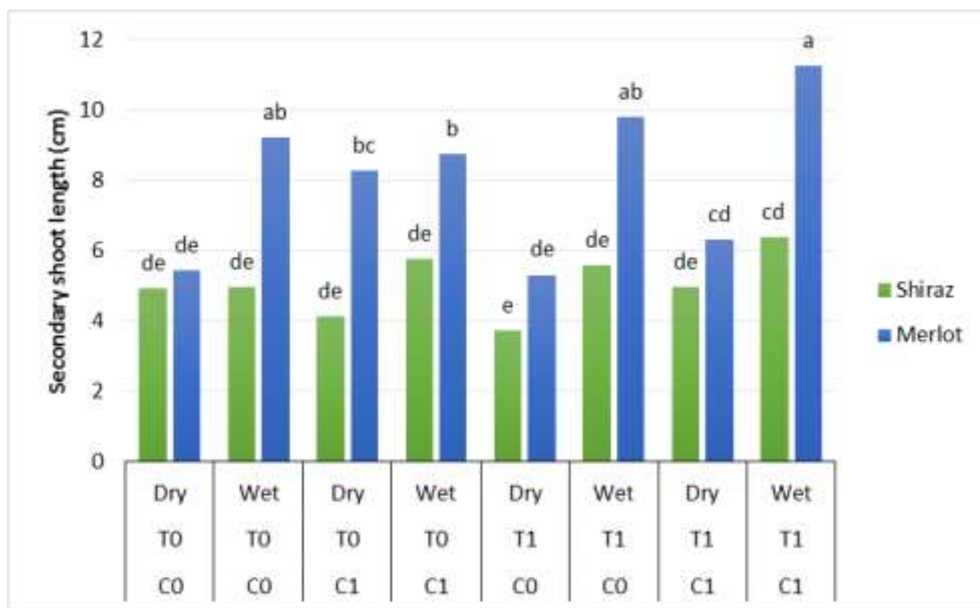

Fig. 4.1 Total secondary shoot length per primary shoot of Shiraz and Merlot at 12 weeks after planting. Bars with the same letter do not differ significantly ($p \leq 0.05$).

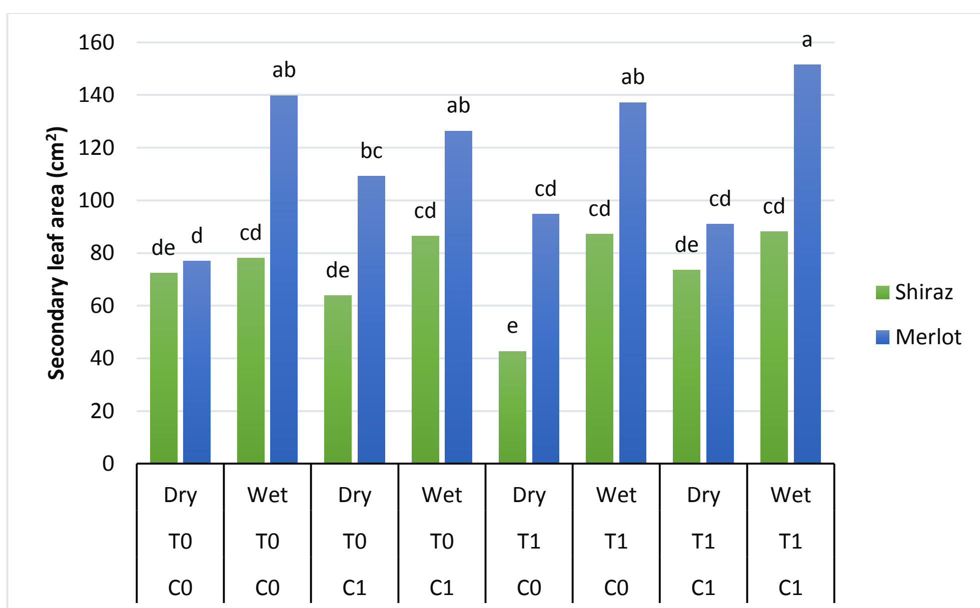

Fig. 4.2 Total secondary leaf area per primary shoot of Shiraz and Merlot at 12 weeks after planting. Bars with the same letter do not differ significantly ($p \leq 0.05$).

Table 4.3 Indication of significance levels of main treatment factors and basic interaction effects.

	Cv	Weeks after planting	CO_2	Temp	H_2O	Cv x Weeks	Cv x CO_2	Cv x Temp	Cv x H_2O	Weeks x CO_2	Weeks x Temp	Weeks x H_2O	CO_2 x Temp	CO_2 x H_2O	Temp x H_2O
1° shoot L	*** 5 %	*** 55 %	*** 2 %	*	*** 13 %	***	NS	NS	**	NS	**	***	NS	NS	NS
1° shoot M	NS	*** 48 %	*** 4 %	NS	*** 20 %	NS	NS	NS	NS	***	***	*** 2 %	NS	NS	NS
2° shoot L	*** 2 %	*** 22 %	*** 3 %	** 1 %	*** 12 %	*** 2 %	NS	NS	** 1 %	NS	*	*** 1 %	NS	NS	*
1° leaf A/shoot	*** 2 %	*** 70 %	***	NS	*** 8 %	***	NS	NS	NS	NS	***	*** 1 %	NS	NS	NS
2° leaf A/shoot	**	*** 47 %	** 1 %	NS	*** 8 %	*** 3 %	NS	NS	NS	NS	NS	*** 2 %	NS	NS	NS
1° leaf M/shoot	*** 5 %	*** 68 %	*** 1 %	**	*** 8 %	***	***	*	*	**	***	*** 2 %	NS	NS	NS
1° leaf A	*** 6 %	*** 65 %	**	*** 1 %	*** 5 %	***	NS	NS	*	NS	*** 1 %	**	*	NS	NS
1° leaf M/A	**	*** 26 %	*** 9 %	NS	**	NS	***	**	NS	*** 3 %	*	** 1 %	NS	*	*
New medium root mass	*** 10 %	*** 21 %	*** 1 %	NS	*** 5 %	NS	*	***	***	** 1 %	NS	*** 2 %	*	NS	NS
New fine root mass	NS	*** 62 %	*** 3 %	NS	*** 9 %	**	NS	**	***	*** 1 %	*	*** 3 %	*	*	***

(*, ** and *** indicate significance at $p \leq 0.05$, 0.01 and 0.001, respectively. NS indicates no significant difference ($p > 0.05$).
The percentage values included in the table indicate the contribution by the specific factor or interaction to the total variance of each parameter measured (values only included if contribution is > 1 %).
1°: Primary; 2°: Secondary; L: Length; M: Mass; A: Area; Cv: Cultivar; Temp: Temperature

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4.3.2 Primary shoot growth

Primary shoot length in all treatment combinations and for both cultivars increased at a fast rate during the first 8 weeks after planting where after growth decreased sharply (Fig. 4.3). Although the cessation of shoot growth occurred earlier than expected after only 8 weeks (bud burst occurred soon after planting), this pattern of shoot growth is consistent with the description of Coombe (1992) for mature vines. Under field conditions in a mature, balanced vineyard, shoot growth should ideally stop at véraison (Archer, 1988), which generally occurs between 120 and 140 days (17-20 weeks) after bud burst, depending on the cultivar and growth conditions (Bates et al. 2002; Malheiro et al. 2013). The growth period in glasshouses is normally shorter than under field conditions (Poorter et al. 2016). Since it is known that high temperatures result in a compressed growth (and ripening) season (Duchêne et al. 2010; Caffara & Eccel, 2011), the high temperatures applied in this study (maximum ranges of 27-31 °C compared to 30-34 °C) could also have contributed to the short vegetative growth period.

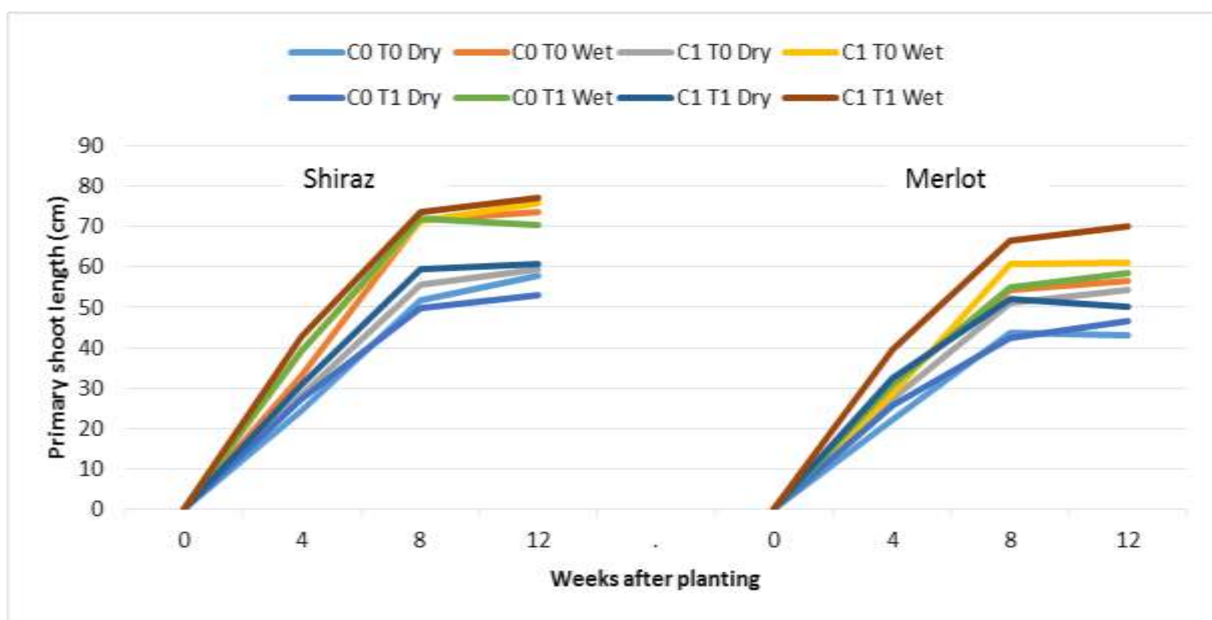

Fig. 4.3 Primary shoot length of Shiraz and Merlot at 4, 8 and 12 weeks after planting. Significant differences ($p \leq 0.05$) are indicated in **Fig. 4.4**.

Primary shoot growth in Shiraz seemed stronger than that of Merlot with longer shoots in all treatment combinations (Figs 4.3 & 4.4). This could be ascribed to inherent genotype vigour differences (Goussard, 2008), since the vigour of Shiraz is described as strong compared to the moderate vigour of Merlot.

At 12 weeks after planting the primary Shiraz shoots in the (wet) treatments were the longest, irrespective of the CO_2 or temperature levels, with no significant differences between them, except for the C0T0(dry) treatment (Fig. 4.4). It seems that shoot growth in Shiraz was mostly dependent on water supply. The

growth of Merlot shoots was also clearly enhanced by water supply, but the higher CO_2 levels (especially in combination with water supply) seemed to enhance growth of Merlot more than that of Shiraz. The effect of temperature as a single factor on primary shoot length was not as clear, although shoot growth in the C1T1(wet) treatment seemed stronger than in the C1T0(wet) treatment, particularly for Merlot (Figs 4.3-4.4). It is often stated that shoot growth and biomass are enhanced by an increase in temperature (Galat Giorgi et al. 2013; Sadras & Moran, 2013; Torregrosa et al. 2017), although there seems to be an optimum temperature range for vegetative growth. Hochberg et al. (2015) found a decreased growth rate under day/night temperatures of 35/30 °C, which could indicate that these temperatures exceeded the optimum temperature range for vegetative growth.

Fig. 4.4 Primary shoot length of Shiraz and Merlot at 4, 8 and 12 weeks after planting. Bars with the same letter within the same week do not differ significantly ($p \leq 0.05$).

The two cultivars reacted similarly to the growth conditions in the sense that water supply and elevated CO_2 resulted in longer shoots (Figs 4.3-4.4) and a higher number and average length of internodes (data not shown). This is in accordance with Palliotti et al. (2008) who reported significant reductions in shoot and internode length with an increase in water stress. Kriedemann et al. (1976) found a significant increase in grapevine growth rate under elevated CO_2 conditions, while the total vegetative growth was enhanced in free-air CO_2 enrichment (FACE) studies (Bindi et al. 2001; Long et al. 2004).

Primary shoot mass accumulation followed a similar pattern than shoot length (Fig. 4.5) and was also significantly enhanced by water supply and increased CO_2 levels. There was no difference between the shoot mass of the cultivars (Fig. 4.5; Table 4.3), which indicates a higher relative biomass accumulation in

Merlot shoots compared to Shiraz. This may be an indication of thicker shoots, but shoot diameters were not measured in this study. Temperature treatments did not affect the fresh shoot mass of either cultivar (Table 4.3), which indicates that the temperature regimes applied in this study did not result in any abnormal behaviour with regards to shoot biomass accumulation.

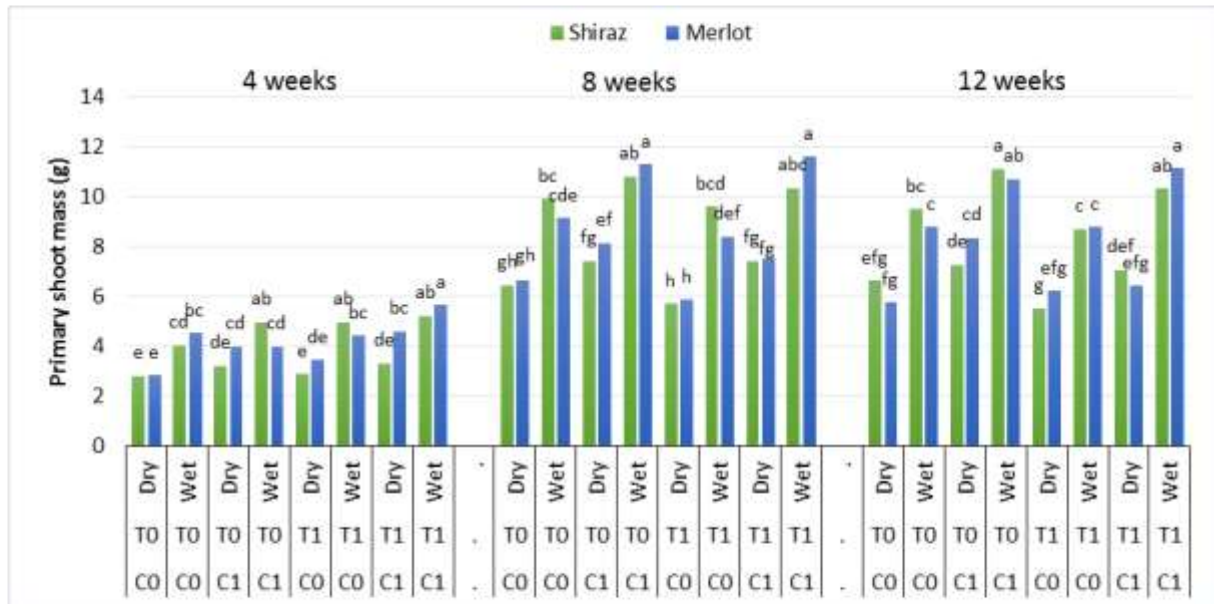

Fig. 4.5 Primary shoot fresh mass of Shiraz and Merlot at 4, 8 and 12 weeks after planting. Bars with the same letters within the same week do not differ significantly ($p \leq 0.05$).

4.3.3 Primary leaf growth

Individual primary leaves had a similar growth pattern than primary shoots with a fast growth rate during the first 8 weeks after planting, followed by a decline (or cessation in the case of Shiraz) in growth (Fig. 4.6). Merlot leaves were larger than those of Shiraz in all treatments, with regards to individual area (Figs 4.6-4.7) as well as mass per leaf (Fig. 4.8). The effect of the environmental variables on leaf growth was the same in both cultivars. Leaf expansion (and thus area) was enhanced by water supply, while the T0 conditions were more conducive to leaf growth than T1. The level of CO_2 did not seem to have a large effect on the individual leaf area, or its effect was masked by the stronger water and temperature effects. Leaf mass was also higher in the T0 than T1 treatments and when vines were well-supplied with water. Higher CO_2 levels resulted in heavier leaves, especially in the case of Merlot (Fig. 4.8).

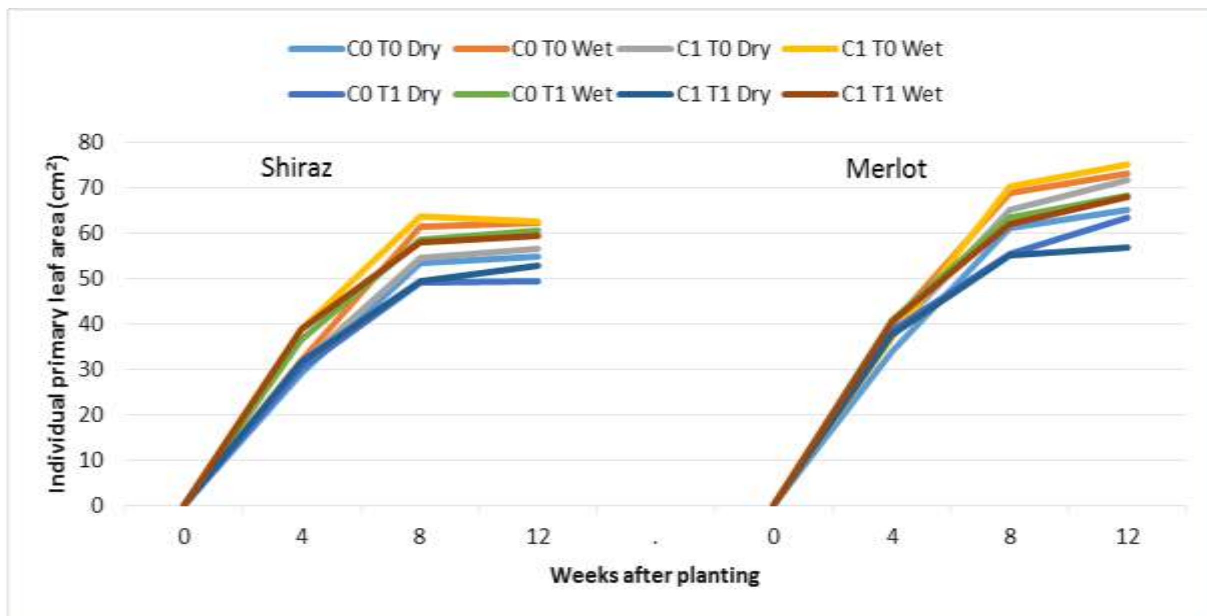

Fig. 4.6 Individual primary leaf area of Shiraz and Merlot at 4, 8 and 12 weeks after planting. Significant differences ($p \leq 0.05$) at week 12 are indicated in **Fig. 4.7**.

The individual primary leaf areas (Fig. 4.6) are the average values over the whole length of the primary shoot at the specific time in the various treatment combinations. The increase in leaf size between 4 and 8 weeks after planting is thus mainly ascribed to growth of leaves situated more towards the middle and apical ends of the shoots, since basal leaves should already have attained their full size by four weeks after unfolding (Hale & Weaver, 1962; Kriedemann et al. 1970).

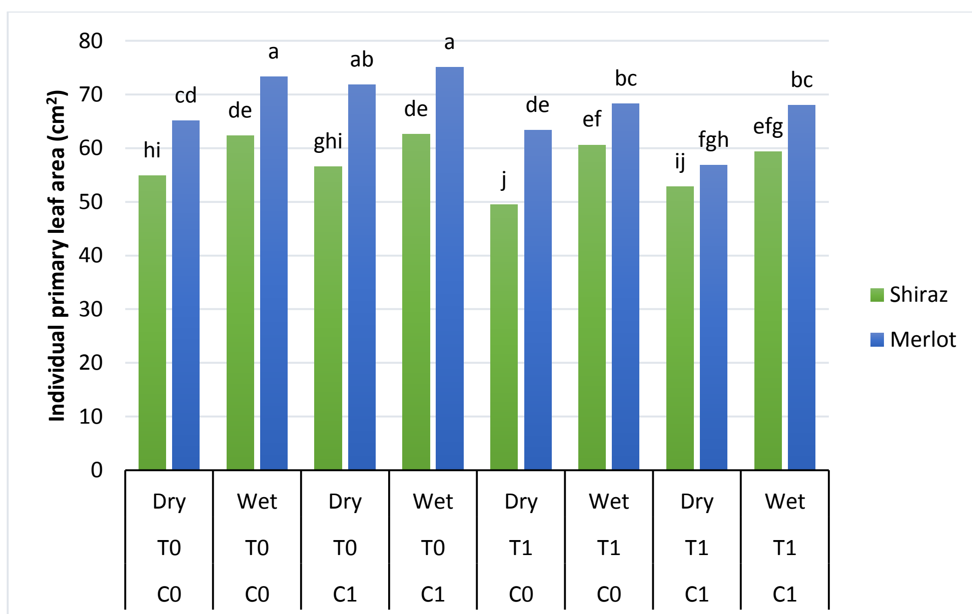

Fig. 4.7 Individual primary leaf area of Shiraz and Merlot at 12 weeks after planting. Bars with the same letter do not differ significantly ($p \leq 0.05$).

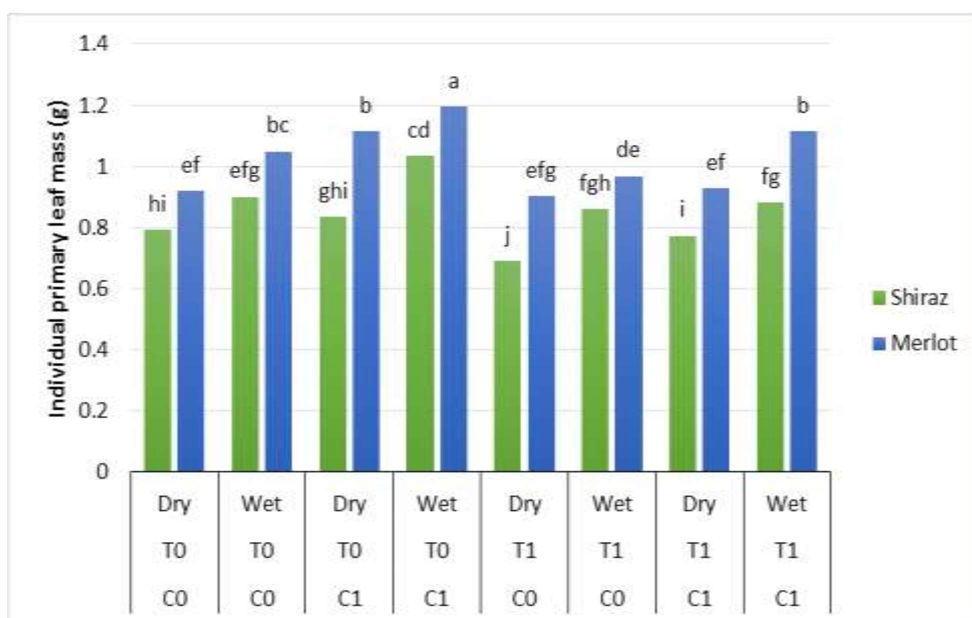

Fig. 4.8 Individual primary leaf mass of Shiraz and Merlot at 12 weeks after planting. Bars with the same letter do not differ significantly ($p \leq 0.05$).

The leaf fresh mass per unit leaf area may possibly be linked with the anatomical structure of the leaf, since an increase may be associated with an increase in structural components, such as cell walls (Mullins et al. 1992) and possibly a denser mesophyll structure. Robertson & Leech (1995) found no major changes in chloroplast structure or leaf anatomy of wheat plants when grown under elevated CO_2. According to Moutinho-Pereira et al. (2009), grapevines produced thicker leaves (higher leaf mass per unit area) in higher ambient CO_2 and ascribed it to an increase in palisade and/or spongy parenchyma and specifically an increase in the cell volumes of those tissues. A higher specific leaf mass may increase the CO_2 pathway to the chloroplasts and may partially explain the higher mesophyll resistance found in leaves under elevated CO_2 conditions (Aranjuelo et al. 2015). The higher CO_2 treatments seemed to increase the specific leaf mass in both cultivars (Figs 4.9-4.10), although the effect on Merlot was more apparent. According to Poorter et al. (2009), an increased specific leaf mass may provide a stronger defence against physical hazards, result in higher starch accumulation in leaf tissues, while it may also increase the longevity of the leaf.

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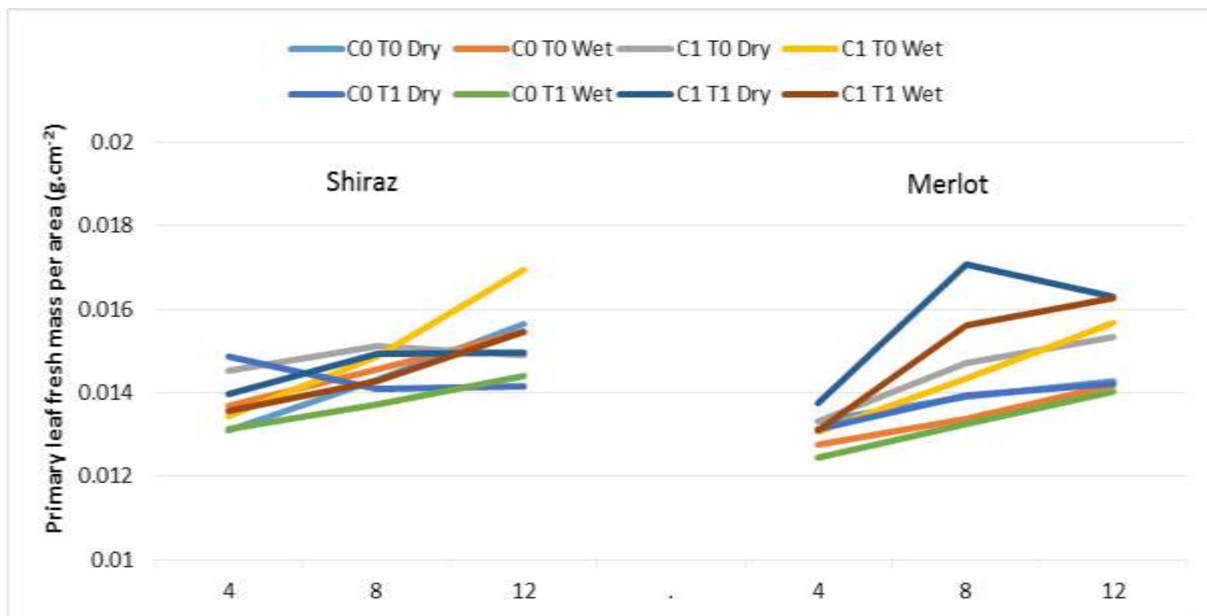

Fig. 4.9 Primary leaf fresh mass per area of Shiraz and Merlot at 4, 8 and 12 weeks after planting. Significant differences ($p \leq 0.05$) at week 12 are indicated in **Fig. 4.10**.

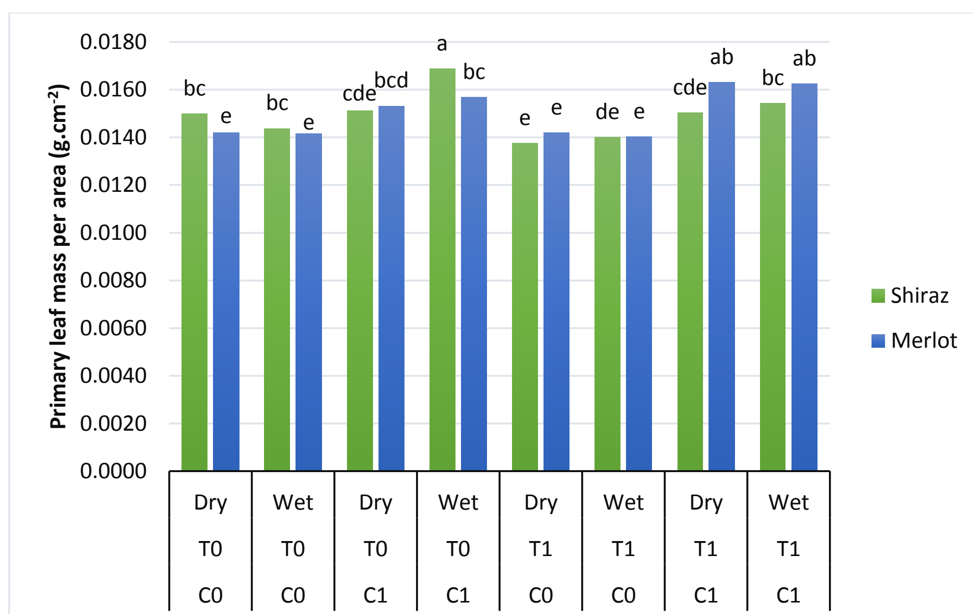

Fig. 4.10 Primary leaf fresh mass per area of Shiraz and Merlot at 12 weeks after planting. Bars with the same letter do not differ significantly ($p \leq 0.05$).

Primary leaf area per shoot followed an almost sigmoidal growth curve during the growth period (Fig. 4.11; Table 4.4). The enhancing effect of water supply on leaf area was apparent in both cultivars, while increased CO_2 levels also seemed to slightly enhance it. The temperature treatments of this study had no significant effect on the primary leaf area per shoot (Table 4.3).

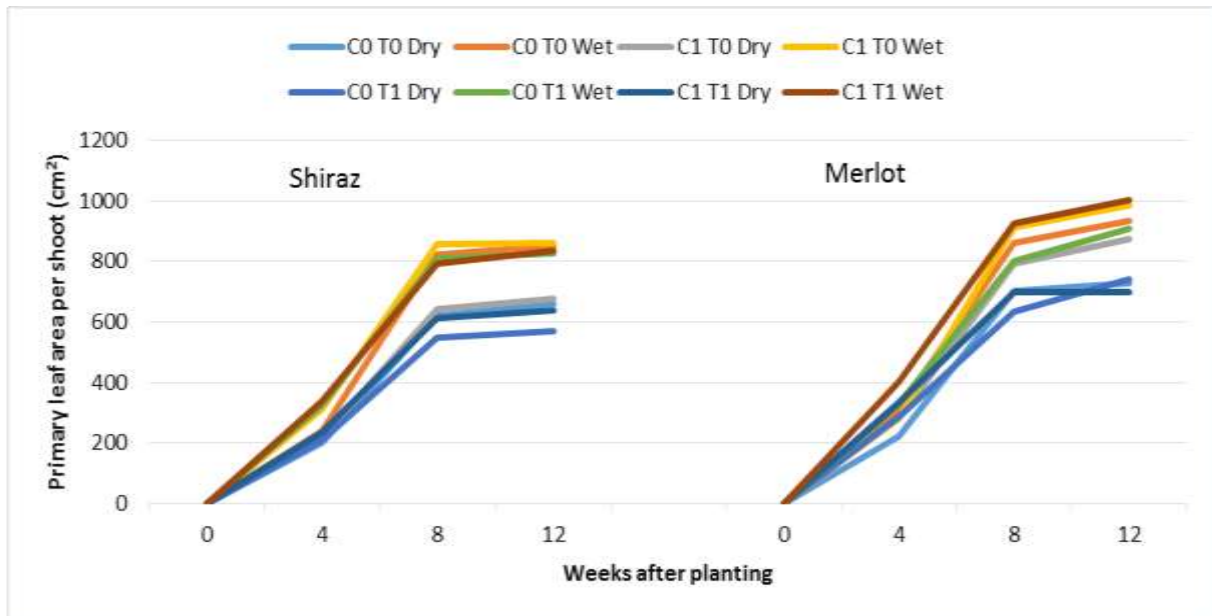

Fig. 4.11 Primary leaf area per shoot of Shiraz and Merlot at 4, 8 and 12 weeks after planting. Significant differences ($p \leq 0.05$) between treatments and cultivars at 12 weeks are indicated in **Table 4.4**.

Table 4.4 Primary leaf area per shoot of Shiraz and Merlot at 12 weeks after planting.

Primary leaf area per shoot (cm^2)	Shiraz		Merlot	
C0T0(dry)	659.68	fgh	727.05	fg
C0T0(wet)	853.65	cd	934.82	abc
C1T0(dry)	676.80	fg	876.28	cd
C1T0(wet)	861.37	cd	984.02	ab
C0T1(dry)	570.72	h	743.56	ef
C0T1(wet)	828.17	de	910.00	bcd
C1T1(dry)	638.89	gh	700.59	fg
C1T1(wet)	836.57	d	1001.19	a

Values provided with the same letter do not differ significantly ($p \leq 0.05$).

Of the three environmental variables, water supply had the largest effect on leaf mass per shoot in both cultivars (Fig. 4.12; Table 4.5). Merlot reacted more strongly with the elevated CO_2 and there was for example a clear distinction between C1(wet) and C0(wet), which seemed not to be the case for Shiraz.

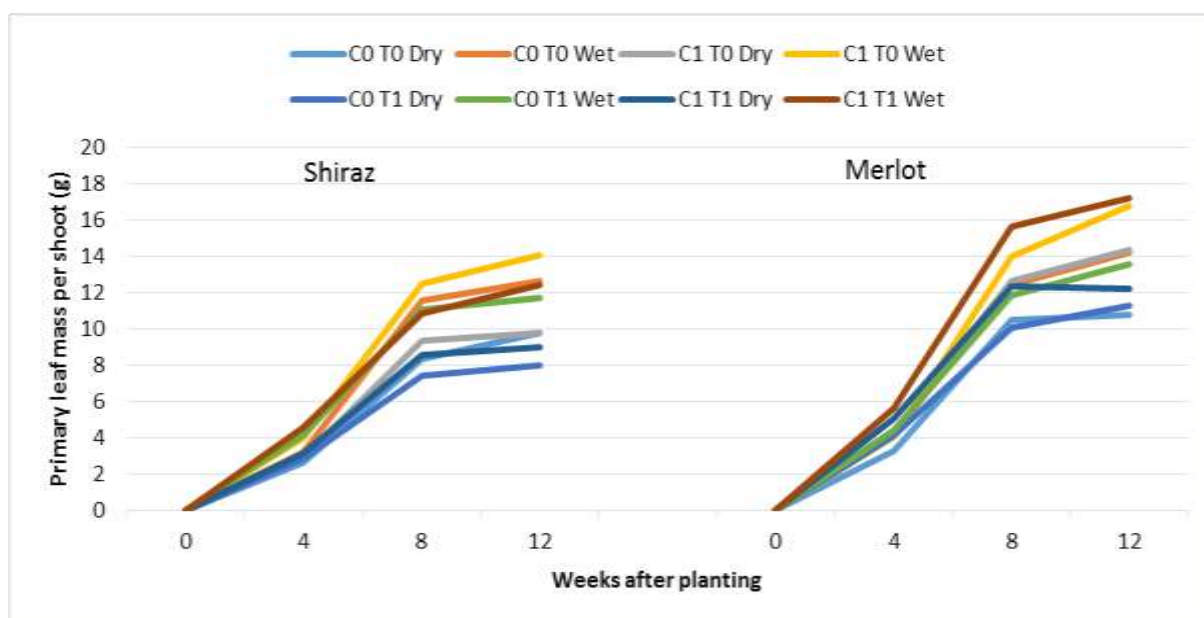

Fig. 4.12 Primary leaf mass per shoot of Shiraz and Merlot at 4, 8 and 12 weeks after planting. Significant differences ($p \leq 0.05$) between treatments and cultivars at 12 weeks are indicated in **Table 4.5**.

Table 4.5 Primary leaf mass per shoot of Shiraz and Merlot at 12 weeks after planting.

Primary leaf mass per shoot (g)	Shiraz		Merlot	
C0T0(dry)	9.78	gh	10.81	fg
C0T0(wet)	12.65	cde	14.22	b
C1T0(dry)	9.77	gh	14.33	b
C1T0(wet)	14.10	bc	16.80	a
C0T1(dry)	8.00	i	11.30	ef
C0T1(wet)	11.71	ef	13.57	bcd
C1T1(dry)	9.01	hi	12.18	def
C1T1(wet)	12.44	de	17.25	a

Values provided with the same letter do not differ significantly ($p \leq 0.05$).

The growth patterns of primary shoots and primary leaves were very similar (Figs 4.3 & 4.6). At 4 weeks after planting, the fresh mass of these showed an approximate 1:1 ratio (Fig. 4.13). During the following four weeks, biomass accumulation in the leaves were higher compared to that of shoots, which is in accordance with Buttrose (1968) who found increasingly more dry mass in leaves and correspondingly less in shoots with an increase in temperature between 20 °C and 30 °C. This increase in leaf mass relative to shoot mass continued (to a much lesser extent) between 8 and 12 weeks after planting so that at the end of the growth period the leaf mass:shoot mass ratio was about 60:40. These ratios were the same during the growth period for all treatment combinations, which implies that the pattern of carbon allocation between shoots and leaves is a constant, irrespective of the growth environment.

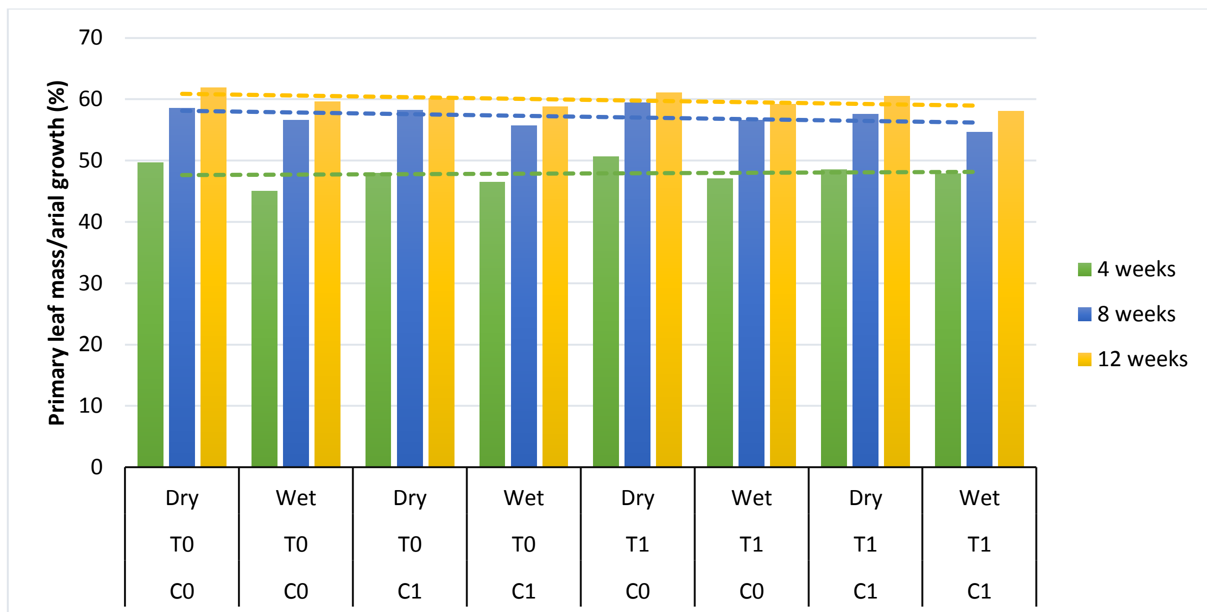

Fig. 4.13 Fresh mass of primary leaves as percentage of aerial growth (shoots plus leaves) at 4, 8 and 12 weeks after planting.

Vegetative growth of aerial vine organs occurred at high rates during the first 8 weeks after planting, whereafter the growth rate significantly decreased towards the end of the growth period. This translates into a sharp decrease in sink strength during the final 4 weeks of the period monitored. It was therefore expected that photosynthetates would either be redirected towards root growth or to perennial structures for reserve accumulation; or the rate of photosynthesis would decrease due to a lower sink strength, decreased export rates and feedback inhibition (Hunter et al. 1994; Keller, 2010; Salazar-Parra et al. 2012; 2015).

4.3.4 Root growth

In contrast to primary shoot and leaf growth, new root growth continued throughout the growth period with strong growth between 8 and 12 weeks after planting (Fig. 4.14) when shoot and leaf growth rate already decreased. This is in accordance with Araujo and Williams (1988) who found a large increase in young vine root biomass during the last part of the growth season under field conditions when the canopy is already well-developed. In mature vineyards, the ripening bunches are strong sinks for assimilates after véraison (Hunter & Visser, 1988). Since bunches are normally removed during young vine training (Archer & Hunter, 2010), the excess available photosynthetates in the leaves may be translocated to the roots for growth and reserve accumulation in the absence of strong above-ground vegetative sinks. The development of a well-distributed and strong root system during the first year after planting is required for optimal young vine functioning and for longevity of the vine (Archer & Hunter, 2010).

It has previously been found in glasshouse studies that root growth is inhibited by the size of the container in which the vines are kept for long periods. Even containers with volumes larger than 9 L would physically restrict root growth (Ainsworth et al. 2002). The pots used in this study had a volume of approximately 7.2 L, which could have restricted root growth. However, due to the strong and continuous increase in new root mass without any decrease in growth rate, it was assumed that root growth of the vines was not impaired by the size of the planting pots used.

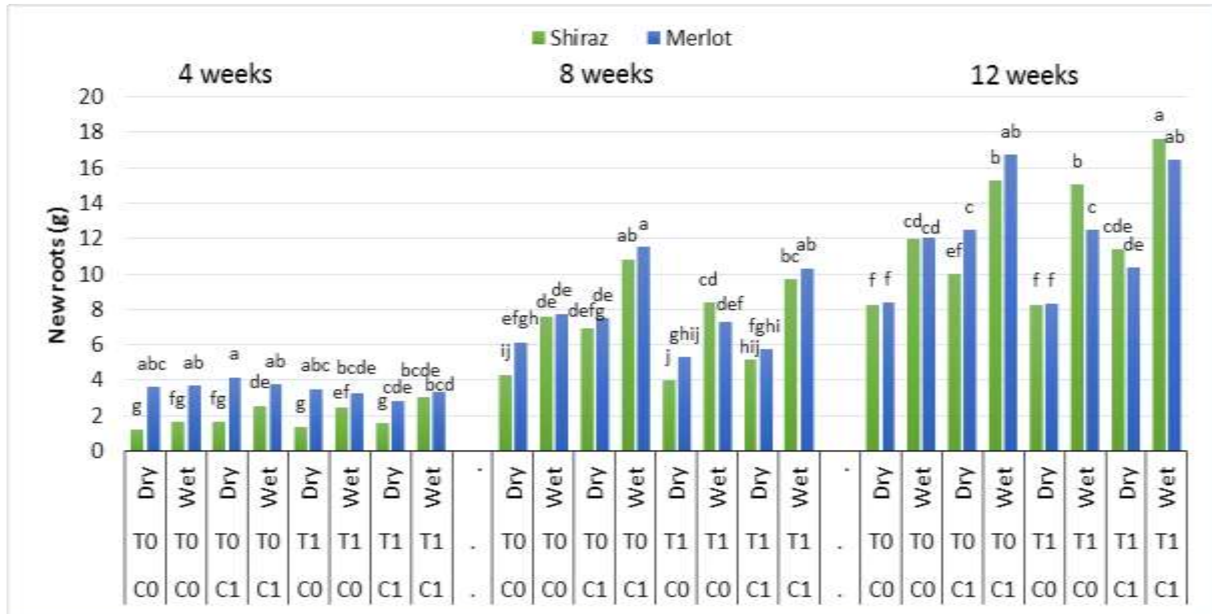

Fig. 4.14 New root mass per vine for Shiraz and Merlot at 4, 8 and 12 weeks after planting. Bars with the same letters within the same week do not differ significantly (p ≤ 0.05).

At 4 weeks after planting, root growth of Merlot was significantly stronger than Shiraz, with the exception of the C1T1(wet) treatment (Fig. 4.14). This may be explained by the vine characteristics at planting, where root systems of the Merlot vines were denser with a higher number of medium sized roots than Shiraz (Table 4.2). This apparent advantage of Merlot was already negated four weeks later, when new root growth of Shiraz was comparable to that of Merlot, which is an indication of the strong effect that the growth conditions during the season has on new root growth. Root growth for the two cultivars reacted similarly to the treatment combinations, which seems to indicate that the rootstock genotype may be a stronger determinant than the scion cultivar with regards to root growth patterns and reactions to environmental conditions.

New root growth in both cultivars was strongly stimulated by water supply, while elevated CO_2 levels also increased root growth. The development of new roots was not as sensitive to the temperature conditions as shoot and leaf growth and the different temperature treatments had no effect on new root growth (Table 4.3). The clayey soil used in this study could have, especially directly after irrigation, acted as

temperature buffer so that the temperature immediately surrounding the roots was lower than the applied air temperature in the glasshouse compartments.

The total new root growth consisted of mainly fine roots (Fig. 4.15) with a diameter of less than < 0.5 mm that may strongly affect the density of the root system and thus the efficiency of soil utilisation for the uptake of water and minerals. Both medium and fine root growth were stronger in well-watered treatments as well as in elevated CO_2 conditions, which should result in higher water and nutrient uptake by the vines. Due to the interaction between the rootstock and scion (Serra et al. 2014), stronger growth of the scion is expected under these conditions. It was found in this study (refer to 4.3.2-4.3.3).

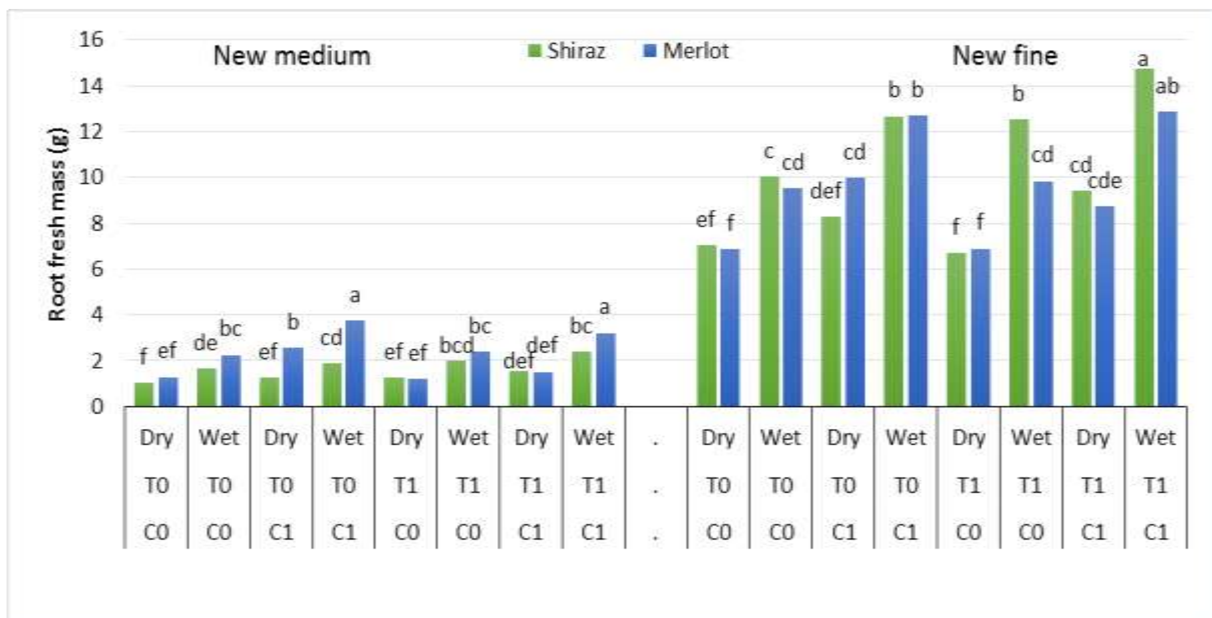

Fig. 4.15 New medium and fine fresh root mass per vine of Shiraz and Merlot at 12 weeks after planting. Bars with the same letters within the same root class do not differ significantly ($p \leq 0.05$).

During the first 12 weeks after planting, water supply was the strongest factor determining root growth of the young grapevines (Table 4.3). Adequate water supply during the initial stage of vineyard establishment is therefore critical for the development of the well-distributed and strong root system required for optimal grapevine functioning and longevity (Archer & Hunter, 2010).

4.3.5 Vegetative growth balances within the young vine

Shoot (Figs 4.3 & 4.4) and leaf growth (Figs 4.6 & 4.12) mainly occurred during the first 8 weeks after planting, whereafter the rate decreased with almost no visual growth activity. Between 8 and 12 weeks after planting, biomass accumulation in these organs continued, indicating a low, but constant, sink activity and therefore translocation to these organs (Figs 4.9, 4.16 & 4.17). The ratio between leaf and shoot fresh mass increased during the growth period with no significant differences between treatment combinations (Fig. 4.13). In contrast to the aerial growth, root growth occurred during the whole growth period, with

apparent enhanced growth when the sink strength of the aerial growth started to decline around 8 weeks after planting (Fig. 4.14). It would therefore seem that the inherent vegetative growth balances within the grapevine were not altered by any of the treatments.

It is further illustrated in Figs 4.16 & 4.17 that show the strong positive and significant correlation between new growths (aerial *vs* roots) during the growth period at 8 and 12 weeks, respectively, across treatment combinations.

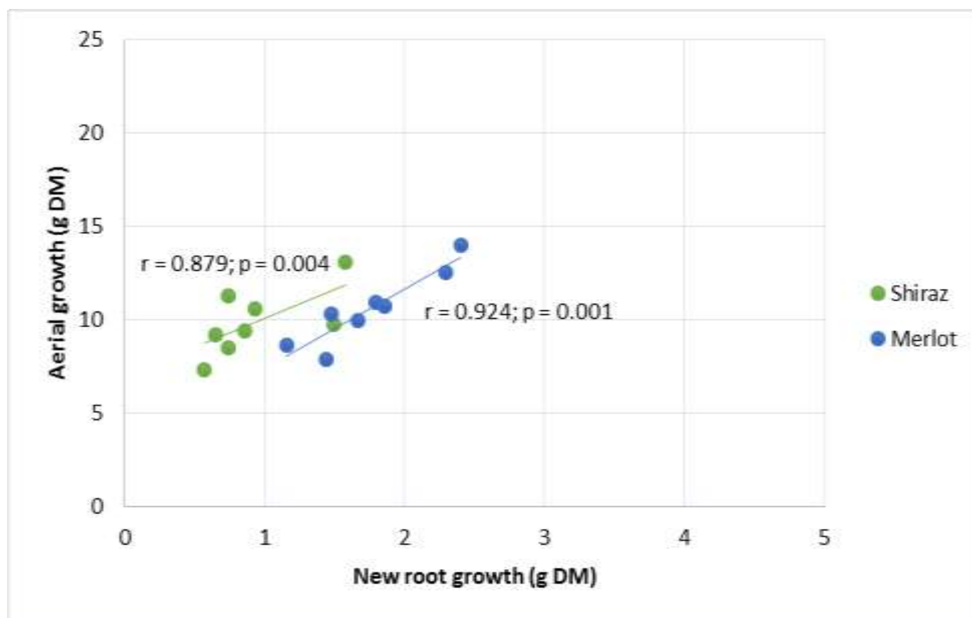

Fig. 4.16 Relation between aerial growth (dry mass) and new root growth (dry mass) for Shiraz and Merlot at 8 weeks after planting.

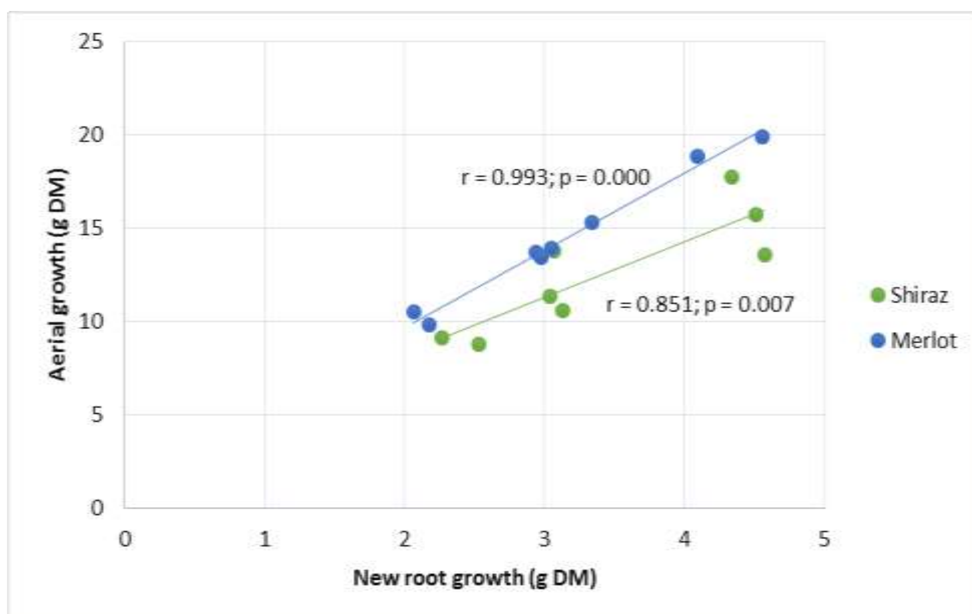

Fig. 4.17 Relation between aerial growth (dry mass) and new root growth (dry mass) for Shiraz and Merlot at 12 weeks after planting.

The ratio between aerial and root growth declined during the course of the growth period in both cultivars. This reflects the respective growth patterns (already discussed), with shoot growth relatively stronger than root growth during the first few weeks and higher relative root growth during subsequent weeks. Interestingly, with the exception of Shiraz in the T0 and the C1T1 treatments at 4 weeks after planting (Fig. 4.18) and C1T0 for Merlot at 12 weeks (Fig. 4.18 & 4.19), the (dry) treatments did not result in lower ratios as was expected. According to Sharp & Davies (1989), root growth is less affected by water deficit than shoot growth (which would decrease the aerial:root growth ratio) that enables the vine to optimise water and nutrient absorption and transport to the shoots. However, it might be that the ability of the vine to withstand drier conditions under field conditions is more related to the penetration depth of the root system than any insensitivity of root growth to limited soil water (Chaves et al. 2010). The results of this study may partially be explained by the physical limitation enforced by the planting pots with regards to deep root growth. It may also be that vines were not sufficient water-stressed to induce the switch in the direction of translocation; or that the overall stimulatory effect of elevated CO_2 on vegetative growth masked any small shifts in aerial:root growth that did occur.

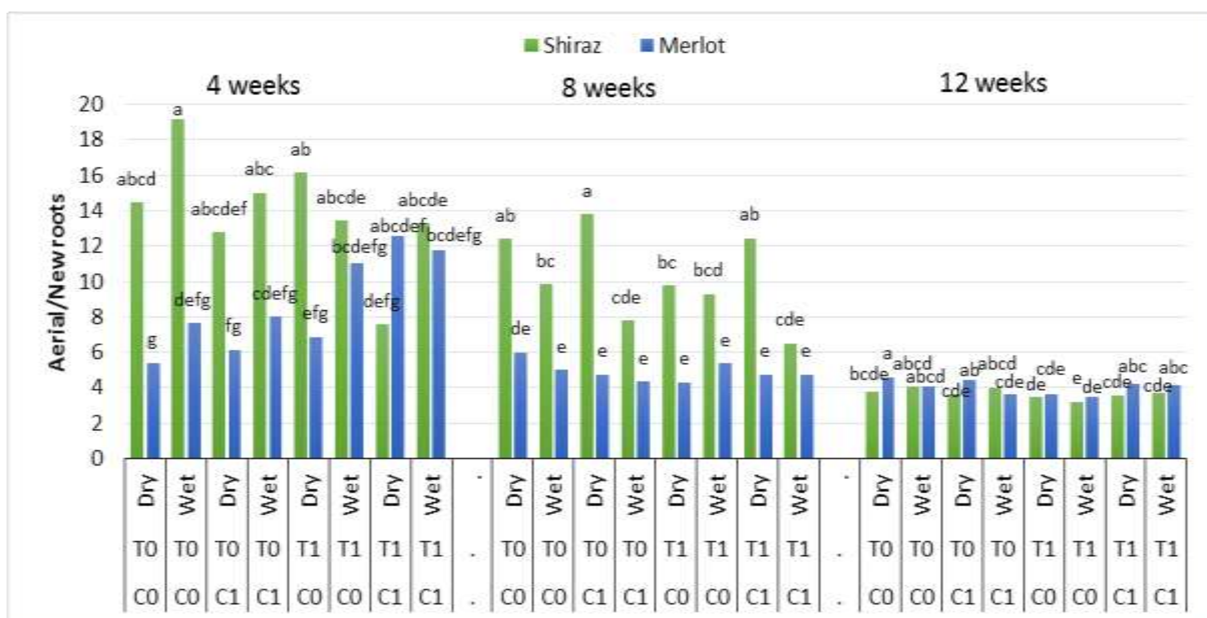

Fig. 4.18 Relation between aerial growth (dry mass) and new root growth (dry mass) for Shiraz and Merlot at 4, 8 and 12 weeks after planting. Bars with the same letters within the same week do not differ significantly ($p \leq 0.05$).

At the end of the 12 weeks, differences between cultivars were observed with the ratios in Shiraz varying between 3.2 and 4.0, and in Merlot a little higher at 3.5 - 4.6 (Fig. 4.19). No clear effect of the treatment combinations could be discerned, which could indicate strong control exercised by the grapevine on inherent growth patterns and balances and the large natural adaptive capacity (plasticity) of the grapevine (Jones & Alves, 2013; Seguin & Garcia de Cortazar, 2015).

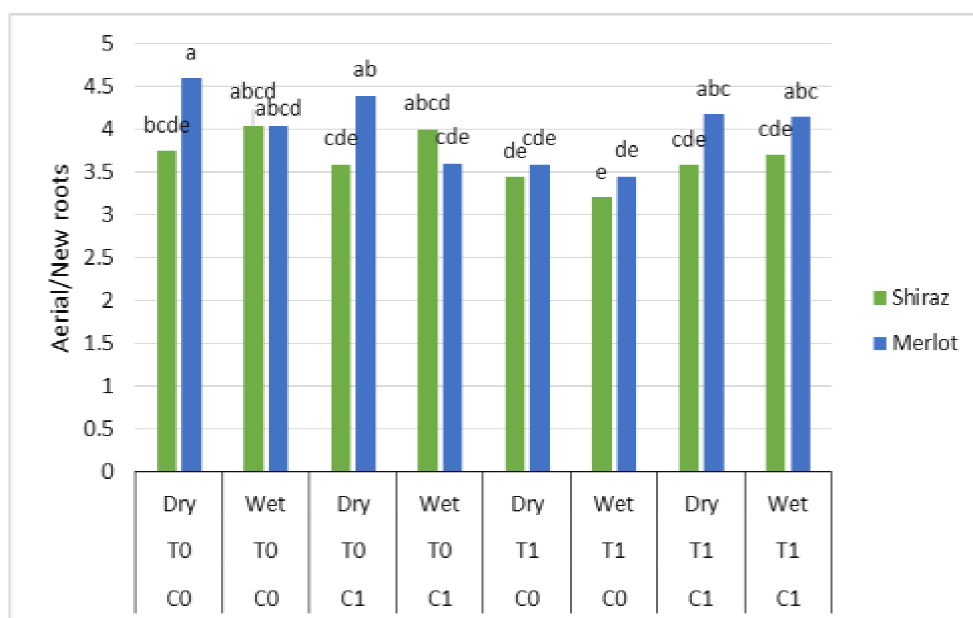

Fig. 4.19 Relation between aerial growth (dry mass) and new root growth (dry mass) for Shiraz and Merlot at 12 weeks after planting. Bars with the same letters do not differ significantly ($p \leq 0.05$).

4.4 CONCLUSIONS

Elevated CO_2 resulted in stronger overall vegetative growth of the grapevine, while the growth response was strongly dependent on water supply. The temperatures applied in the study did not seem to affect growth, although aerial growth seemed to decrease a little in the higher temperature treatments. Inherent growth patterns in the vine during the course of the growth period remained unchanged for all treatments, with relative stronger aerial growth during the first few weeks, followed by relatively stronger root growth after the decrease in shoot growth rate around 8 weeks after planting.

Continued growth and biomass accumulation confirmed that vegetative sinks existed for the duration of the three-month growth period monitored, especially when vines were well-supplied with water and/or were grown in elevated CO_2 conditions. This is in accordance with the results discussed in Chapter 3, where photosynthetic activity was sustained until the end of the growth period under these conditions.

The occurrence of strong vegetative growth during the first year after planting is imperative for optimal vineyard establishment. A strong positive relationship was found between new aerial and root growth (dry mass), irrespective of the treatment combinations or the scion cultivar. Under future climatic conditions, the higher atmospheric CO_2 levels are expected to stimulate vegetative growth (both aerial and roots) in young vines and facilitate the process of establishing a vineyard. Water supply, especially during the first few months, would be critical for initial root development. After that, judicious water management would be required to optimally exploit the stimulatory growth effect of the higher CO_2 on roots. It is advised that more water is then applied at a lower frequency (Myburgh, 2018), should the soil

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type allow it. This should encourage deeper root penetration to the subsoil, which would increase the resilience of the vine to limited water availability.

Since water availability for irrigation is expected to decrease in future, correct soil preparation, planting and young vine training would become even more important. All practices should be aimed at providing the roots with optimum growth conditions during the first year of vineyard establishment, in order to maximise the buffer capacity of the vineyards against adverse environmental (soil and climate) conditions.

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CHAPTER 5: INTEGRATIVE EFFECTS OF CLIMATE CHANGE FACTORS (TEMPERATURE, CO_2 AND WATER) ON THE NUTRIENT CONTENT IN YOUNG, GRAFTED GRAPEVINES

ABSTRACT:

The nutrient content of young, potted grapevines was measured at four week intervals during the first 12 weeks after planting. The effect of different combinations of ambient temperature (maximum ranges of 27-31 °C, compared to 30-34 °C), ambient CO_2 (400 ppm *vs* 800 ppm) and soil water (irrigation to water holding capacity and 50 % thereof), applied immediately after planting, on the uptake of nutrients by young vines was investigated under glasshouse conditions. Two scion cultivars (Shiraz and Merlot), both grafted onto 101-14 Mgt, were used in this study. Nutrient concentration and content varied between leaves and roots, as well as among the different root classes. The effect of environmental factors on the concentration of a nutrient depended on the particular nutrient, the scion cultivar, and the applicable tissue. Nutrient concentrations (except for Cu) were higher in current (400 ppm) CO_2 levels and under water-deficit conditions. Stronger vegetative growth of well-watered vines and those grown in elevated CO_2 compensated for the lower nutrient concentrations. No nutrient deficiencies were observed in any of the treatments.

Current nutrient guidelines used for the development of fertilisation programmes will possibly need adjustment within the context of higher CO_2 levels and lower water availability. Many potential problems regarding vine nutrition may be avoided by using the rootstock-scion genotype best suited to the environment and soil conditions.

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5.1 INTRODUCTION

Over the last few decades, the average temperature during the grapevine growing season has increased in most of the global wine producing regions, with a higher frequency of temperature extremes (Jones, 2007). Atmospheric CO_2 is continually on the rise with current levels at 410 ppm, compared to about 340 ppm in 1980 (NOAA-ESRL, 2019). This is considered to be the main cause of warming (IPCC, 2014) and higher CO_2 and temperature would thus be an inseparable combination in future climates. In many winegrowing regions of the world, the general water requirement of vineyards (300-700 mm) is already higher than the annual mean precipitation (Medrano et al. 2015), while higher ambient temperatures would further increase evapotranspiration. Vineyards will therefore be exposed to elevated CO_2, increased temperature and limited water availability in future (Morales et al. 2016).

Elevated levels of ambient CO_2 increased the rate of photosynthesis in grapevines (Moutinho-Pereira et al. 2009) and enhanced both vegetative and reproductive growth (Bindi et al. 2001; Long et al. 2004). However, acclimation of photosynthesis is often mentioned with longer term vine exposure to high CO_2 levels (Leibar et al. 2015; Salazar-Parra et al. 2012, 2015; Martínez-Lüscher et al. 2015). This decrease in stimulation of photosynthesis in high CO_2 environments is commonly associated with the inability of plants to acquire sufficient nitrogen (N) (Alonso et al. 2009). According to Rogers et al. (2006), a sustained increase in growth response to higher CO_2 would require acquisition of additional N by the plant in proportion to the higher amount of fixed carbon to prevent a weakening of the CO_2 stimulatory effect. Grapevine leaf nitrogen concentration levels decreased in elevated CO_2 environments (Morales et al. 2016), resulting in increased carbon (C)/N, potassium (K)/N and magnesium (Mg)/N ratios (Moutinho-Pereira et al. 2009). Rogers et al. (2006) found that the initial decrease in soybean leaf N concentration in response to elevated CO_2 was eradicated later in the season due to the N fixing ability of the crop. Irigoyen et al. (2014) also observed avoidance of photosynthetic acclimation in legumes and also possibly in cases where arbuscular mycorrhizal fungi colonise the root system, increasing its absorption surface. Sufficient N in the vine is critical for growth, as it is a structural component of proteins and nucleic acids (Mullins et al. 1992; Saayman, 2016). Nitrogen also forms part of chlorophyll, and deficiency resulted in decreased photosynthesis and chlorophyll content (Girardin et al. 1985). Leaf chlorosis as deficiency symptom mostly occurs in basal leaves (Mullins et al. 1992), as N compounds in less active, older leaves are hydrolysed and translocated to younger, more active plant parts (Conradie, 1986). The accepted norm for leaf N concentration is between 1.6 % and 2.7 % (Saayman, 2016). Norby et al. (1986) cautiously compared experimental or field data directly with published “critical nutrient concentration” values, especially since they found an increase in growth of 85 % in severely N deficit white oak seedlings under elevated CO_2 conditions. Increased CO_2 also markedly decreased the concentrations of phosphorous (P), K, calcium (Ca),

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Mg, sulphur (S), iron (Fe), manganese (Mn), boron (B) and molybdenum (Mo) in grapevine leaves of fruit-bearing cuttings (Morales et al. 2016).

The effect of limited water availability and scion genotype on leaf and petiole nutrient concentrations was investigated by Shellie & Brown (2012). Lower concentrations of N, K and copper (Cu) were found in leaf blades of vines under deficit irrigation, while the scion cultivar genotype had a larger effect on P, Ca, Mg, sodium (Na), zink (Zn), Fe and Mn concentrations than additional irrigation. In a study on cv. Sibera, high temperatures and rainfall resulted in higher leaf B and Cu concentrations, while Fe, Mn, Zn, Mo and Na decreased (Gastol & Domagala-Swiatkiewicz, 2014). It seems clear that the level of water availability as well as the scion cultivar affect petiole and leaf blade nutrient concentrations (Shellie & Brown, 2012). Raath (2012) found seasonal variation in petiole mineral content and recommended that soil analysis should be done in combination with plant tissue analysis to ascertain nutrient availability and uptake by the vine.

The efficient absorption of essential nutrients from the soil is critical for optimal physiological functioning, growth and reproduction of the grapevine. The uptake of soil nutrients by the vine roots depends on the degree of root development and distribution in the available soil volume, which in turn affects aerial growth of the grapevine (Swanepoel & Southey, 1989). Large and dense root systems with a high number of medium and fine roots are required to maximise water and nutrient absorption capacity (Hunter et al. 1995; Hunter, 1998a), while increasing the buffer capacity of the vine against environmental stress (Hunter et al. 2016). Soil preparation (Van Huyssteen, 1988; Conradie et al. 1996), vineyard establishment (Archer et al. 1988; Myburgh et al. 1996; Hunter, 1998a; 1998b) and cultivation practices (Hunter et al. 1995; Hunter & Volschenk, 2001) should therefore be focussed on eliminating any barriers to root growth in order to obtain maximal utilisation of the available soil volume.

The rate of water and nutrient absorption between suberized and non-suberized roots may differ (Kramer & Bullock, 1966; Mullins et al. 1992), while root order, age, location in the soil (Volder et al. 2005) and root diameter (Kramer & Bullock, 1966) may also affect uptake. The total uptake of nutrients by the roots depends on both the size and activity of the root system. The latter may be improved by canopy management practices, such as partial defoliation (Hunter & Le Roux, 1992), or increasing the demand on the root system by altering the canopy size (Hunter & Volschenk, 2001).

It is thus imperative that a large, deep penetrating and dense root system that consists of different root classes starts to develop from the first year to optimise soil utilisation, water and nutrient uptake and maximise nutrient storage capacity (Hunter et al. 2016). Such a root system would contribute to buffer the vine against adverse climate conditions or environmental stress for the rest of its productive life (Archer & Hunter, 2010).

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In this part of the study the combined effect of projected climate change conditions (high ambient temperature, elevated CO_2 and water deficit) on the nutrient uptake and translocation by young, grafted grapevines during the first 12 weeks after planting was measured under controlled conditions in glasshouse compartments. This is a novel approach to gain a better understanding of how young vines would function and grow (at leaf, root and whole-plant level) under future climates during the very important young vineyard establishment stage.

5.2 MATERIALS AND METHODS

5.2.1 Study location and glasshouse compartments

Four glasshouse rooms situated at ARC Infruitec-Nietvoorbij, Stellenbosch, were used to accommodate the different treatments. The rooms were 2.4 m X 6.0 m each and prepared according to the treatment criteria explained in detail in Chapter 3 and summarised in Table 5.1. The experiment comprised of five consecutive growth cycles (planting times during the first week of February and the first week of September), using Shiraz (SH 470) as scion cultivar for the first three, and Merlot noir (MO 348) for the other two. Both scions were grafted onto rootstock 101-14 Mgt. The potted vines were randomly allocated per glasshouse compartment in a randomised complete block design. There were 108 vines per room (54 per irrigation treatment) and thus 432 vines were used for each growth cycle.

Table 5.1 Treatment combinations randomly allocated in four glasshouse compartments for five growth cycles.

PARAMETER	TREATMENTS											
	C0T0			C1T0			C0T1			C1T1		
Vine age (weeks)	4	8	12	4	8	12	4	8	12	4	8	12
CO_2 levels (ppm)	400	400	400	800	800	800	400	400	400	800	800	800
Temperature (°C)	15/27	15.5/29	16/31	15/27	15.5/29	16/31	18/30	18.5/32	19/34	18/30	18.5/32	19/34
Water treatments	Wet: WC Dry: $\frac{1}{2}$WC	Wet: WC Dry: $\frac{1}{2}$WC	Wet: WC Dry: $\frac{1}{2}$FWC	Wet: WC Dry: $\frac{1}{2}$WC	Wet: WC Dry: $\frac{1}{2}$WC	Wet: WC Dry: $\frac{1}{2}$WC	Wet: WC Dry: $\frac{1}{2}$WC	Wet: WC Dry: $\frac{1}{2}$WC	Wet: WC Dry: $\frac{1}{2}$WC	Wet: WC Dry: $\frac{1}{2}$WC	Wet: WC Dry: $\frac{1}{2}$WC	Wet: WC Dry: $\frac{1}{2}$WC

C0: Lower CO_2 (400 ppm); C1: Higher CO_2 (800 ppm); T0: Lower temperature; T1: Higher temperature (T0 max + 3°C); WC: Water-holding capacity

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5.2.1.1 Potting soil

The soil used for each growth cycle was obtained from the same fallow vineyard site in Robertson, South Africa, and transported to the experiment location. Each plastic planting pot (25 cm diameter; roughly 7.2 L) was provided with a Bidim-layer at the bottom before the vine was planted in 6.50 kg of soil. The soil was a sandy clay loam with a high pH (Chapter 3). Soils were analysed for their macro- and micro-nutrient content before each planting date by a SANAS Accredited Testing Laboratory (in accordance with ISO 17025:2005). No additional nutrients were provided to the young, growing vines for the duration of the study.

5.2.1.2 Grafted vines

Vines were obtained from a SAPO (South African Plant Improvement Organisation) accredited nursery in the Wellington/Paarl region. Shiraz (SH 470), grafted onto rootstock 101-14 Mgt, was used for the first three growth cycles and Merlot noir (MO 348), grafted onto the same rootstock, for the last two cycles.

Before planting, vines were pruned back to two buds and roots (only those originating from the basal node were kept) cut to a length of 10 cm. A sample of 18 prepared vines was taken and the fresh mass (Chapter 4, Table 4.2) and nutrient levels of the various vine tissues was separately determined. Shoot removal and weed control were continuously done during the growth cycles to ensure optimal growth of the vines under the respective growth conditions. Primary shoot tips were not removed and all developing secondary shoots were allowed to grow.

5.2.2 Measurements

The plant material of six vines was combined for each of the three replications of each treatment combination at 12 weeks after planting. All primary leaves (from the basal to the apical part of the shoot) were combined before analysis. Roots were separated into old (dark in colour and suberized) and new (soft and light brown to white in colour) roots. Each group was further separated into thick (> 2.0 mm in diameter), medium (0.5 - 2.0 mm) and fine (< 0.5 mm) roots before analysis. Primary leaves and root classes were analysed for their mineral concentration (in $g.100\ g^{-1}$ for macro-nutrients and $mg.kg^{-1}$ for micro-nutrients) by the same accredited analytical laboratory that performed the soil analysis. The respective total nutrient contents per old thick (OT), old medium-fine (OMF) and new medium-fine (NMF) root classes and primary leaves were also calculated by using the average dry mass per tissue fraction for each.

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5.2.2.1 Laboratory analysis

Soil analysis

Soil texture - chemical dispersion was done using sodium hexametaphosphate (calgon) and three sand fractions were determined through sieving, as described by The Non-Affiliated Soil Analysis Work Committee (TNASAWC, 1990). Silt and clay percentages were then determined using sedimentation rates at 20 ^{o}C, using an ASTM E100 (152H-TP) hydrometer.

Macro-nutrients - the soil was air dried, sieved through a 2 mm sieve for determination of stone fraction (weight/weight basis) and analysed for pH (1.0 M KCl), P (Bray II) and total extractable cations, namely K, Ca, Mg and Na (extracted at pH = 7 with 0.2 M ammonium acetate) and organic carbon by means of the Walkley-Black method (TNASAWC, 1990).

Micro-nutrients - Zn, Mn, Cu & Fe were extracted with Di-ammonium EDTA (0.02 M) and boron (B) by using a 1:2 hot water ratio (TNASAWC, 1990). Sulphur (S) was extracted with concentrated phosphoric acid (at pH = 4) according to an adapted method as described in Pansu & Gautheyrou (2006). The extracted solutions were analysed with a Varian ICP-OES optical emission spectrometer. Salinity was determined by measuring the resistance of a saturated paste in an electrode cup (TNASAWC, 1990).

Leaf and root analysis

Each sample (2 g fresh mass) was washed with a Teepol solution, rinsed with de-ionised water and dried overnight at 70 ^{o}C in an oven. The dried material was then milled and ashed at 480 ^{o}C, shaken up in a 50:50 HCl (32 %) solution for extraction through filter paper (Campbell & Plank, 1998; Miller, 1998). The macro- (K, Na, Ca, Mg) and micro-nutrient (Cu, Zn, Mn, B, Fe) content of the extract was measured with a Varian ICP-OES optical emission spectrometer. Total N content of the ground material was determined through total combustion in a Leco Truspec® CN N-analyser (Leco Africa, Kempton Park, South Africa).

5.2.3 Statistical layout of project

The data was subjected to analysis of variance (ANOVA) using the General Linear Models Procedure (PROC GLM) of SAS software (Version 9.2; SAS Institute Inc., Cary, USA). The ANOVA was performed in order to evaluate the main influences/effects of cultivar, CO_2, temperature and H_2O, as well as to detect interaction effects among these factors. Measurements over sampling times were included in a split-plot analysis of variance with sampling times as sub-plot factor (Little & Hills, 1978) where applicable. The Shapiro-Wilk test was performed on the standardised residuals from the model to verify normality (Shapiro & Wilk, 1965). Levene's test showed dissimilarity of cultivar variances (Levene, 1960). To correct for variance differences between cultivars, a weight was included in the ANOVA. The weight was the inverse of the experimental error of each cultivar (John & Quenouille, 1977). Fisher's least significant difference was

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calculated at the 5 % level to compare means of the factors (main effects) and factor interaction means (Ott & Longnecker, 2001). A probability level of 5 % was considered significant for all significance tests. The Pearson product moment (Pearson) correlation tests were performed using XLSTAT (Version 2015.1.03.15485, Addinsoft, Paris).

5.3 RESULTS AND DISCUSSION

5.3.1 Before planting

5.3.1.1 Soil

A complete soil analysis was done before each planting (Table 5.2). The cation exchange capacity (CEC) of the soil was high due to the high pH. The percentage organic material was moderate and considered within the norm of soils from the Robertson region. The K content was however extremely high, even for a soil from the Breede River where high K levels are common. According to Treeby et al. (2004), an oversupply of K in the soil does not directly affect growth and yield negatively, but it may result in lower Ca and Mg concentrations in plant tissues. Calcium is a component of cell walls and membranes and therefore plays an important role in membrane permeability, while Mg may regulate enzyme activity and has a structural role in chlorophyll (Etchebarne et al. 2009). The uptake of K by the roots to the detriment of Ca and Mg could thus indirectly impair photosynthesis and internal transport, should the levels of these nutrients decrease to below-minimum levels. However, the base saturation of K was at the optimum of 4 %, while the K:Mg ratio was within the range required for sustainable grapevine production (Lanyon et al. 2004).

Table 5.2. Composition of the soil used in this study.

Soil analysis before planting		Interpretation*
Clay (%) Silt (%) Sand (%)	26.07 15.60 58.33	The soil was classified as a sandy clay loam with good water holding capacity, but with a relatively high percentage of sand that improved soil aeration.
pH_{KCl}	7.29	High
C (%)	0.74	Within the norm for Breede River
P_{Olsen} (mg.kg^{-1})	14.11	Not deficient (norm of 15 in clay with pH > 7.0)
$P_{Bray\ II}$ (mg.kg^{-1})	65.73	Not deficient (norm of 21 in clay with pH > 7.0)
K (mg.kg^{-1})	368.00	Excessive (> 120 for Breede River soils) (Conradie, 1994) Not growth limiting
Na (cmol(+).kg^{-1})	0.26	Base saturation of 1 % (norm < 7 %)
K (cmol(+).kg^{-1})	0.94	Base saturation of 4 % (optimal)

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Ca (cmol (+).kg^{-1})	18.36	Not deficient (> 2.50) Base saturation 80 %
Mg (cmol (+).kg^{-1})	3.29	Not deficient (> 1.00) Base saturation 14 %
Ca:Mg	5.6	Meets criteria (4-6)
K:Mg	0.3	Meets criteria (0.1-0.4) (Lanyon et al. 2004)
Cu_{EDTA} (mg.kg^{-1})	4.95	Not deficient (> 0.5)
Zn_{EDTA} (mg.kg^{-1})	1.87	Not deficient (> 0.5)
Mn_{EDTA} (mg.kg^{-1})	26.94	Not deficient (> 2.0)
B_{water} (mg.kg^{-1})	0.80	Not deficient (> 0.3)
Fe (mg.kg^{-1})	3.75	Not common in SA; (Australian norm > 4.5)
Soluble S (mg.kg^{-1})	21.62	High (> 12). Not deficient.

*Interpretation of results was based on the soil norms published in Fertiliser Guidelines for the Wine Industry (Raath, 2016), unless otherwise stated.

None of the other mineral levels indicate any deficiency and the various ratios between the exchangeable cations are all within the respective ranges. The soluble S is a little high, which could possibly be ascribed to the application of fungicides and fertilisers since the soil originates from a previous commercial vineyard.

Based on Table 5.2, the physiological functioning and growth of the newly planted grapevines should not have been negatively affected by the soil and the vine performance should reflect the manipulated growth conditions within the glasshouse rooms.

5.3.1.2 Vines

The Merlot and Shiraz vines were relatively similar in size when they were planted, with comparable scion, graft union and rootstock mass (Table 5.3). The thick root mass was also the same for the two cultivars, but Merlot vines had denser root systems with especially more medium-size roots than Shiraz. This could be ascribed to the scion-rootstock genotype interaction (since the cultivars were both grafted onto 101-14 Mgt) or to the nursery environment in which the plants were grown after grafting. The root-soil contact area and thus initial water and nutrient uptake by Merlot might therefore have been higher than that of Shiraz. If root nutrient concentrations of the cultivars have been similar, the higher root mass of Merlot would have provided a higher pool of reserve nutrients for budding and initial growth.

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Table 5.3. Average mass distribution (expressed as fresh mass per vine) and nutrient levels in young Shiraz and Merlot vines before planting.

Parameter		Shiraz	Merlot
Scion fresh mass (g)		11.74 a	10.90 a
Graft union fresh mass (g)		7.58 a	8.92 a
Rootstock mass (g)		24.01 a	24.58 a
Thick root mass (g)		4.14 a	4.29 a
Medium root mass (g)		3.05 b	7.33 a
Fine root mass (g)		0.86 a	0.65 b
N (%)	RS	0.89 b	1.19 a
	OT	1.40 a	1.15 b
	OMF	1.74 a	1.42 b
P (%)	RS	0.12 a	0.13 a
	OT	0.20 a	0.20 a
	OMF	0.22 a	0.19 a
K (%)	RS	0.26 a	0.28 a
	OT	0.25 a	0.24 a
	OMF	0.34 a	0.36 a
Ca (%)	RS	0.53 b	0.70 a
	OT	0.69 a	0.81 a
	OMF	0.67 b	0.77 a
Mg (%)	RS	0.10 a	0.10 a
	OT	0.10 a	0.10 a
	OMF	0.10 a	0.11 a
Na (mg.kg^{-1})	RS	180.83 b	370.50 a
	OT	153.50 b	271.75 a
	OMF	184.17 b	310.25 a
Mn (mg.kg^{-1})	RS	48.17 a	36.75 b
	OT	46.17 a	33.50 b
	OMF	38.67 a	25.25 b
Fe (mg.kg^{-1})	RS	833.00 a	907.00 a
	OT	523.00 a	391.75 b
	OMF	1440.80 a	630.00 b
Cu (mg.kg^{-1})	RS	12.33 a	12.25 a
	OT	13.50 a	15.50 a
	OMF	16.83 a	17.25 a
Zn (mg.kg^{-1})	RS	50.33 a	41.75 b

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	OT	50.17 a	43.75 a
	OMF	61.50 a	40.25 b
B ($mg.kg^{-1}$)	RS	8.83 b	11.00 a
	OT	8.67 b	10.00 a
	OMF	8.83 b	10.75 a

Values in rows followed by the same letter do not differ significantly ($p \leq 0.05$).
RS: Rootstock trunk; OT: Old thick roots; OM: Old medium roots; OF: Old fine roots; OMF: Combination of OM and OF

Differences in the root nutrient concentrations were found, depending on the scion cultivar used (Table 5.3). Shiraz vines contained higher concentrations of N, Mn, Fe and Zn in their roots, while Ca, Na and B were higher in Merlot. The levels of the other nutrients tested (P, K, Mg and Cu) did not differ between cultivars. It seems possible that the scion genotypes might have affected nutrient uptake and storage dynamics in the roots already at an early stage.

The N concentration is the lowest in the RS, followed by OT roots and the highest in the OMF roots. Although the concentration is lower, the mass of the RS part is much higher than that of the OT and OMF roots and it would therefore contain a high total N content. Both Ca and P were present at lower levels in the RS, with higher, comparable levels in the OT and OMF roots. The K, Mn and Cu concentrations of the RS part and OT roots were similar, while the OMF roots contained higher K and Cu and lower Mn levels in comparison. Sodium (Na) and Fe levels were the lowest in the OT roots, and higher and at comparable levels in the RS and OMF roots. The concentrations of Mg, Zn and B in RS, OT and OMF roots were similar.

There is limited data available on the mineral concentration and content in grapevine roots and most data is based on the root system as a whole with no distinction between root classes (Araujo & Williams, 1988; Conradie, 1988; Hunter & Ruffner, 1997). In work done on nitrogen uptake by roots, Volder et al. (2005) distinguished between fine roots based on their age (in days) rather than their size. Bates et al. (2002) separated roots based on their diameter into fine (< 2 mm), thin (2 – 5 mm) and thick (> 5 mm) classes, but focussed mainly on starch and nitrogen content without reporting on other nutrients. The low number of quantitative root studies under field conditions may be ascribed to the amount of labour involved, while root biomass measurements are often inaccurate because of the loss of fine roots during excavation (Mullins et al. 1992).

The data in Table 5.3 indicates heterogeneous nutrient distribution among the different root classes, which emphasises the importance of separating roots to improve understanding of nutrient dynamics within the root system and the contribution of various root classes to overall grapevine growth and functioning. It appears that the scion cultivar has no effect on the distribution patterns of the respective nutrients among

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the rootstock and different root classes (only on the levels) and that nutrient distribution is probably determined by the rootstock cultivar.

5.3.2 After planting

5.3.2.1 General nutrient distribution trends

Roots:

The decrease in N concentration levels in OT and OMF roots between planting (Table 5.3) and sampling (after 12 weeks of growth) (Table 5.4) may be ascribed to the translocation of stored N to sites of new growth. According to Conradie (1992) reserve N is translocated and utilised over the course of the whole season. Between bud break and flowering, the roots primarily export N to growing tissues, with the result that the concentration in these tissues sharply declines (Conradie, 1992; Bates et al. 2002). After flowering, N reserves start to accumulate in the roots, while they remain a source of N too. Simultaneous import and export of N therefore occur in most vine organs (including roots), which makes it difficult to quantify the contribution of root N reserves to grapevine growth during the season (Conradie, 1992). Hunter & Ruffner (1997) found relative stable N concentrations in vine roots from berry set during the season which they ascribed to the effective control of this import/export relationship. In two-year-old vines that were pruned back to two buds prior to bud burst, the root N concentration initially decreases until relatively late in the season before it stabilised at about 1.2 % (Araujo & Williams, 1988). This value is higher than what was found for OT (0.51-0.75 %) and OMF (0.59-0.92 %) roots in this study, but is similar to the concentration found in the NMF roots (0.84 – 1.34 %) (Appendix 1a).

Table 5.4 Comparative macro- and micro-nutrient concentrations in the three respective root classes and primary leaves of Shiraz and Merlot at 12 weeks after planting.

	N (g.100 g^{-1})	P (g.100 g^{-1})	K (g.100 g^{-1})	Ca (g.100 g^{-1})	Mg (g.100 g^{-1})	Na (mg.kg^{-1})	Mn (mg.kg^{-1})	Fe (mg.kg^{-1}	Cu (mg.kg^{-1})	Zn (mg.kg^{-1})	B (mg.kg^{-1})
OT:S	0.64a	0.12a	0.41a	0.94a	0.10a	427.52a	19.72a	*544.85a*	11.05a	37.29a	17.91a
OT:M	0.56b	0.87b	0.33b	0.74b	0.09b	389.31b	13.09b	*517.12a*	9.42b	22.65b	16.59b
OMF:S	0.84a	0.14a	*0.46a*	1.28a	0.13a	531.55a	19.74a	974.75a	17.36a	36.20a	23.48a
OMF:M	0.64b	0.11b	*0.45a*	1.00b	0.12b	434.43b	15.97b	640.10b	15.58b	28.33b	20.98b
NMF:S	1.06a	0.21a	1.32a	1.73a	0.24a	685.36a	26.51b	*1580.5a*	64.38a	21.04b	26.09a
NMF:M	1.00b	0.17b	1.07b	1.33b	0.21b	638.23b	31.34a	*1635.4a*	53.37b	23.87a	23.90b
LEAVES:S	1.60b	*0.14a*	1.83b	1.14b	0.31a	107.41b	*39.91a*	*216.15a*	4.99b	39.32b	50.59a
LEAVES:M	1.78a	*0.15a*	1.97a	1.22a	0.27b	194.06a	*39.63a*	*203.88a*	5.90a	51.06a	48.27b

Italic value pairs do not differ significantly ($p \leq 0.05$). OT: Old thick roots; OMF: Old medium and fine roots; NMF: New medium and fine roots; S: Shiraz; M: Merlot

Later during the season, especially after harvest, root N levels increased significantly (Araujo & Williams, 1988; Conradie, 1988; Bates et al. 2002). However, in this study the young vines grew only for the first 12 weeks after planting and it is assumed that the sampling at the end of this period occurred during the

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stable root N content stage before the normal increase in root N reserves that occur between harvest and leaf fall.

There is not much information available (with the exception of N) about the concentration and content of macro- and micro-nutrients in grapevine roots. When comparing the values in Table 5.3 with those in Table 5.4, the P, Mn and Zn concentration levels in OT and OMF roots decreased between planting and sampling, while K, Na and B concentrations were higher and Cu and Fe concentrations were seemingly not affected. In the thick roots, Ca and Mg levels remained approximately the same, but were higher in the OMF roots at sampling, compared to planting.

The lower concentrations of P and Zn in old roots after 12 weeks of growth may indicate net export to sites of active growth or metabolic activity. Zinc is an important micro-nutrient that increases chlorophyll synthesis and plays a structural role to stabilise protein (Aravind & Prasad, 2004). Phosphorous deficiency negatively affects photosynthesis by disrupting electron transport in photosystem I, while the production of ATP is also restricted (Carstensen et al. 2018). A decrease in Mn concentration may be explained by a relatively slower rate of uptake compared to root growth. The constant levels of Cu and Fe in the OT and OMF roots could indicate a balance between the rates of nutrient uptake and that of growth and export. The higher concentrations in K, Na and B in the suberized roots at the end of the growth period may be because of a faster uptake/less utilisation of nutrients, while import into the roots in the case of Na and K is also a possibility - the remobilisation rate of B in plants is very low (Treeby et al. 2004). The comparative levels of the respective nutrients therefore vary during the season and differed between the root classes as well as between cultivars.

At 12 weeks after planting, the distribution patterns of all the macro- and micro-nutrients in the root system were similar, meaning that the lowest concentrations were in the OT roots, followed by the OMF roots. The highest nutrient concentrations in the root system were located in the NMF roots, indicating the partitioning of nutrients to sites of new growth. The only exception was Zn that was present in higher concentration in old (OT and OMF) than in new roots (Table 5.4). These patterns applied to both Shiraz and Merlot cultivars across all treatment combinations, which means that the distribution of nutrients between the various root classes is mainly determined by the rootstock genotype and not the scion or the environmental growth conditions. In all three root classes, Shiraz roots contained higher macro- and micro-nutrient concentrations than Merlot. The only exceptions were Mn, Fe and Zn in the NMF roots that occurred in higher concentrations in Merlot than in Shiraz.

Regarding macro-nutrients, the Ca concentration was the highest in all three root classes, followed by N and K with P and Mg in the lowest concentrations at comparable levels (Table 5.4). This is in accordance with Conradie et al. (1996) who stressed the high demand for Ca by young, non-bearing vines and who

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mentioned that this nutrient should receive more attention in fertilisation programmes for these vines. The micro-nutrient present in the highest concentrations in all root classes was Fe (Table 5.4), followed by Na, Zn, and then Mn and B with similar concentrations in all three of the root classes and at much lower concentrations than Fe and Na. The Cu concentration in roots varied the most between the root classes. In OT roots, Cu was present in the lowest concentration of all the micro-nutrients. It increased to levels comparable to those of Mn and B in OMF roots and surpassed them in NMF roots. Once again, the specific concentrations of the nutrients differed between the cultivars, but the patterns were the same regardless of the scion cultivar used. According to Kodur et al. (2010) the distribution of K in the grapevine is determined by the scion-rootstock interaction, with the rootstock genotype (and root system morphology) determining the amount of uptake. This may in principle be true for most soil nutrients and would explain the similar distribution of nutrients within the root system and between root classes, since 101-14 Mgt was the only rootstock used during this study.

The scion-rootstock interaction also affects both aerial and subterranean growth parameters (Tandonnet et al. 2010). The rootstock affects the growth of the scion by the uptake of water and nutrients (Serra et al. 2014) and may buffer the vine against edaphic stresses (Stevens et al. 2008). Since the amount and rate of nutrient uptake by the roots are determined by the total root length, total root surface area and percentage of fine roots (Kodur et al. 2010), it might be useful to calculate the content of the respective nutrients (in mg per tissue/tissue class or plant) as well to allow for the effect of vegetative growth and determine the total uptake.

Leaves:

Leaves are often used to identify nutrient deficiency or toxicity (Treeby et al. 2004; Raath, 2016), but foliar symptoms are not always visible and therefore tissue and soil analyses are used for better accuracy. Higher concentrations of N, K, Mg, Mn, Zn and B occurred in leaves than in any of the root classes, while the roots contained higher Na, Fe and Cu (Table 5.4). The Ca and P concentrations in the leaves were only surpassed by the concentrations in the NMF roots. Nutrient requirement and accumulation are therefore different between various vine organs within the same vine. Contrary to that found in the roots, nutrient concentrations were generally higher in Merlot than in Shiraz leaves. The only exceptions were Mg and B, while P, Mn and Fe levels did not differ significantly between the cultivars. This could indicate differences in the respective sink strengths of the roots and shoots between the cultivars which would determine the direction of nutrient partitioning within the vines. It seems as if allocation to the leaves is higher in Merlot than in Shiraz and *vice versa* regarding the roots.

The average leaf analyses of the two cultivars at the end of the growth period did not indicate any serious nutrient deficiency. Leaf N and K concentrations were low, with those of Shiraz being at the lower

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recommended limit of 1.6 % for N and both cultivars just higher than 0.13 % for P (Saayman, 2016). Since P deficiency symptoms are rarely observed in grapevines and poor growth mostly being the only symptom (Saayman, 2016), soil analyses are predominantly used to determine the P status of the vine. The analysis of the soil did not indicate any P deficiency (Table 5.2) and it was therefore assumed that a general inhibition of growth or photosynthesis as result of P deprivation (Thuynsma et al. 2016) did not occur in any of the treatment combinations.

The K concentration in the leaves indicated an excess with levels higher than 1.3 % (Raath & Schutte, 2001; Saayman, 2016), which may be ascribed to the high K levels in the soil. Another contributing factor could be the antagonism between P and K in both leaf blades and petioles as found by Conradie & Saayman (1989). An oversupply of K to vines is not directly detrimental to vine growth and productivity, but may result in lower Ca and Mg concentrations in tissues (Treeby et al. 2004), which might explain the low leaf Ca concentrations in both cultivars. According to the minimum norm for leaf Ca of 1.2 % (Raath & Schutte, 2001), Shiraz may have experienced some Ca deficiency in some of the treatment combinations in this study. However, Ca deficiency is yet to be observed in grapevines under field conditions (Saayman, 2016). It would appear that the high K levels did not affect the Mg levels, since the leaves of both cultivars contained levels between 0.2 % and 0.6 % (Raath & Schutte, 2001), which is deemed sufficient for normal grapevine functioning. All of the micro-nutrient levels were within the accepted range, with no deficiencies or toxicities indicated.

5.3.2.2 Treatment effects on nutrient distribution

In this study potted vines were exposed to combinations of ambient CO_2, temperature and water supply conditions and both the nutrient concentration and content in the different root classes as well as the primary leaves were compared.

The treatments affected the concentration of all the nutrients tested in some or all of the tissues analysed, although the older, suberized roots often showed lower degrees of response compared to NMF roots and especially leaves (Table 5.5). The following is a short summary of the information in Table 5.5 with the focus on results with significance levels $p \leq 0.001$. [Refer to Appendices 1a (macro-nutrients) & b (micro-nutrients) for comprehensive results of both Merlot and Shiraz in the various treatment combinations].

Table 5.5 Significance level of main treatment factors and interaction effects on macro- and micro-nutrient concentration in root classes and leaf blades at 12 weeks after planting.

	Cultivar	CO_2	Temp	H_2O	Cultivar x CO_2	Cultivar x Temp	Cultivar x H_2O	CO_2 x Temp	CO_2 x H_2O	Temp x H_2O	Cultivar x CO_2 x Temp	Cultivar x CO_2 x H_2O	Cultivar x Temp x H_2O	CO_2 x Temp x H_2O	Cultivar x CO_2 x Temp x H_2O
N (%)															
OT	*** 11 %	** 3 %	NS	*** 10 %	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
OMF	*** 55 %	*** 5 %	NS	*** 6 %	NS	NS	NS	NS	*	NS	NS	NS	NS	NS	NS
NMF	*** 2 %	*** 10 %	*** 5 %	*** 8 %	NS	NS	NS	**	NS	*** 2 %	*** 2 %	*	*	*** 3 %	*** 2 %
LEAF	*** 3 %	*** 52 %	*** 2 %	*** 20 %	**	NS	NS	NS	*** 1 %	*	*** 2 %	NS	NS	NS	NS
P (%)															
OT	*** 33 %	NS	* 1 %	*** 6 %	NS	NS	NS	*	NS	NS	NS	NS	NS	NS	NS
OMF	*** 32 %	*** 2 %	*	*** 4 %	*	*	** 1 %	*** 5 %	*	NS	*** 2 %	NS	*	NS	*
NMF	*** 7 %	*** 2 %	*	NS	NS	NS	NS	NS	** 2 %	NS	NS	NS	NS	NS	NS
LEAF	*	*** 49 %	**	*** 15 %	NS	NS	NS	*** 2 %	NS	*	NS	NS	*	NS	NS
K (%)															
OT	*** 32 %	NS	NS	*** 8 %	NS	NS	NS	** 2 %	NS	NS	NS	NS	NS	NS	NS
OMF	*	*** 6 %	NS	NS	NS	NS	* 1 %	*** 4 %	NS	** 2 %	*** 3 %	NS	NS	NS	** 2 %
NMF	*** 12 %	*** 36 %	*** 3 %	*** 8 %	NS	NS	NS	* 1 %	NS	NS	NS	NS	NS	NS	NS
LEAF	*** 1 %	*** 12 %	NS	***	**	*** 1 %	NS	NS	NS	***	***	NS	**	NS	NS

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	Cultivar	CO_2	Temp	H_2O	Cultivar x CO_2	Cultivar x Temp	Cultivar x H_2O	CO_2 x Temp	CO_2 x H_2O	Temp x H_2O	Cultivar x CO_2 x Temp	Cultivar x CO_2 x H_2O	Cultivar x Temp x H_2O	CO_2 x Temp x H_2O	Cultivar x CO_2 x Temp x H_2O
Ca (%)															
OT	*** 20 %	NS	** 2 %	NS	NS	NS	NS	* 1 %	NS	NS	NS	NS	NS	* 1 %	NS
OMF	*** 31 %	*** 1 %	** 1 %	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	*
NMF	*** 14 %	*** 6 %	* 1 %	NS	NS	NS	*	NS	NS	NS	NS	NS	NS	NS	NS
LEAF	**	*** 39 %	NS	*** 4 %	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Mg (%)															
OT	*** 15 %	NS	*** 5 %	* 1 %	** 2 %	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
OMF	*** 13 %	*** 8 %	** 2 %	* 1 %	NS	NS	NS	NS	NS	NS	NS	NS	NS	** 2 %	** 2 %
NMF	*** 6 %	*** 18 %	** 3 %	** 3 %	NS	NS	* 1 %	NS	* 1 %	NS	NS	NS	NS	*** 5 %	NS
LEAF	*** 2 %	*** 44 %	*** 5 %	*** 9 %	NS	**	NS	**	***	NS	NS	NS	NS	NS	NS
Na ($mg.kg^{-1}$)															
OT	*** 6 %	*** 8 %	*** 5 %	* 2 %	NS	NS	NS	NS	NS	NS	NS	NS	NS	* 2 %	NS
OMF	*** 21 %	*** 15 %	*** 3 %	*** 4 %	NS	NS	* 1 %	NS	*** 3 %	*** 2 %	NS	NS	NS	* 1 %	NS
NMF	* 1 %	*** 25 %	** 2 %	*** 21 %	NS	NS	NS	NS	* 1 %	NS	NS	NS	NS	** 2 %	NS
LEAF	*** 24 %	*** 16 %	NS	NS	NS	NS	NS	*	*	NS	**	NS	NS	NS	NS

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	Cultivar	CO_2	Temp	H_2O	Cultivar x CO_2	Cultivar x Temp	Cultivar x H_2O	CO_2 x Temp	CO_2 x H_2O	Temp x H_2O	Cultivar x CO_2 x Temp	Cultivar x CO_2 x H_2O	Cultivar x Temp x H_2O	CO_2 x Temp x H_2O	Cultivar x CO_2 x Temp x H_2O
Mn (mg.kg^{-1})															
OT	*** 24 %	** 1 %	** 1 %	*** 4 %	NS	NS	*	NS	*	NS	*	*	NS	NS	NS
OMF	*** 11 %	*** 3 %	** 2 %	* 1 %	NS	* 1 %	NS	NS	NS	NS	NS	NS	NS	NS	NS
NMF	*** 3 %	** 1 %	NS	* 1 %	*	NS	NS	*	NS	NS	** 1 %	NS	NS	*	NS
LEAF	NS	*** 29 %	*** 2 %	*** 15 %	*	NS	NS	NS	*	NS	NS	NS	NS	NS	NS
Fe (mg.kg^{-1})															
OT	NS	NS	** 2 %	NS	*	NS	NS	*** 3 %	NS	*	NS	NS	NS	NS	NS
OMF	*** 10 %	*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
NMF	NS	**	NS	**	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
LEAF	NS	*** 22 %	***	*** 2 %	**	NS	*	*** 5 %	***	NS	***	NS	*	NS	NS
Cu (mg.kg^{-1})															
OT	*** 18 %	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
OMF	** 3 %	NS	NS	* 1 %	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
NMF	*** 8 %	NS	NS	*** 8 %	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
LEAF	*** 2 %	*** 59 %	*** 1 %	*** 6 %	NS	*	NS	*** 10 %	***	***	*** 1 %	NS	NS	NS	NS

	Cultivar	CO_2	Temp	H_2O	Cultivar x CO_2	Cultivar x Temp	Cultivar x H_2O	CO_2 x Temp	CO_2 x H_2O	Temp x H_2O	Cultivar x CO_2 x Temp	Cultivar x CO_2 x H_2O	Cultivar x Temp x H_2O	CO_2 x Temp x H_2O	Cultivar x CO_2 x Temp x H_2O
Zn ($mg.kg^{-1}$)															
OT	*** 31 %	** 2 %	NS	* 1 %	NS	NS	NS	* 1 %	NS	NS	* 1 %	NS	NS	NS	NS
OMF	*** 9 %	*** 5 %	** 1 %	*** 3 %	NS	NS	NS	NS	**	NS	*	NS	NS	*** 2 %	NS
NMF	** 2 %	*	NS	* 1 %	NS	NS	NS	NS	NS	NS	NS	NS	NS	*	NS
LEAF	*** 4 %	*** 29 %	*** 1 %	*** 2 %	*** 3 %	*** 3 %	NS	* 2 %	NS	NS	*** 3 %	NS	NS	NS	NS
B ($mg.kg^{-1}$)															
OT	** 5 %	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	* 2 %	* 2 %	NS
OMF	*** 16 %	*** 3 %	** 2 %	*** 4 %	NS	NS	** 2 %	NS	NS	NS	NS	NS	NS	NS	NS
NMF	** 2 %	*** 5 %	NS	*** 4 %	** 2 %	NS	NS	NS	NS	NS	NS	NS	NS	* 1 %	NS
LEAF	*	*** 17 %	*** 17 %	*** 3 %	NS	NS	NS	NS	NS	NS	NS	NS	*	NS	NS

*, ** and *** indicate significance at $p \leq 0.05$, 0.01 and 0.001, respectively. NS indicates no significant difference ($p > 0.05$).
The percentage values included in the table indicate the contribution by the specific factor or interaction to the total variance of each parameter measured (values only included if contribution is > 1 %).
Temp: Temperature; OT: Old thick roots; OMF: Old medium and fine roots; NMF: New medium and fine roots

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Old thick roots:

Except for Fe that was not affected, the scion cultivar strongly and significantly affected the concentrations of all nutrients tested in these roots, with Shiraz containing higher concentrations than Merlot. This may be due to the differences in genotype combinations, but also to the different responses that the cultivars might have on the environmental growth conditions. The cultivar effect was stronger than any of the environmental effects in determining the concentrations of N, P, K, Ca, Mg, Mn, Cu, Zn and B. Ambient CO_2 did not affect P, K, Ca, Mg, Fe, Cu or B in old thick roots, but resulted in decreased Na. The temperature treatments also had no effect on N, K, Cu, Zn or B, but increased temperature resulted in higher Mg, Na and Fe concentration. Adequate water supply decreased the concentration levels of N, P, K and Mn and was probably due to an attenuation effect. It did not affect Fe, Ca, Cu and B. When comparing only the three climate variables, CO_2 affected Na and Zn the strongest; temperature had the largest effect on Ca, Mg and Fe; and N, P, K and Mn were affected by water supply. The concentrations of Cu and B in old, thick roots were not affected by the environmental factors.

Old medium-fine roots:

The effect of the cultivar used was stronger than any of the environmental effects on the concentrations of all the nutrients analysed, with the exception of K that was most strongly affected by the ambient CO_2 level. Elevated CO_2 did not affect Cu, but decreased the concentrations of N, P, K, Ca, Mg, Na, Mn, Zn and B in OMF roots. The higher temperature conditions decreased Na, Zn and B, while the concentrations of N, K, Fe and Cu were not affected by ambient temperature. Water supply had no effect on K, Ca and Fe levels in OMF roots, but decreased the concentrations of N, P, Na, Zn and seem to increase B. Comparison between the climatic factors indicated that K, Ca, Mg, Na, Mn, Fe and Zn were the most affected by CO_2. Water played the most important role in the respective concentrations of N, P, Cu, Zn and B in OMF roots. Temperature did not affect nutrient concentration in OMF roots to the same degree than the other two factors.

New medium-fine roots:

The effect of cultivar was stronger than the environmental variables with regards to P, Ca and Zn concentration in the NMF roots. Ambient CO_2 decreased N, P, K, Ca, Mg, Na, Mn, Fe, Zn and B concentrations, while the Cu levels in NMF roots were unaffected by CO_2. Higher temperature had no effect on Mn, Fe, Cu, Zn and B, but increased N, P, K, Ca, Mg, and Na levels. The Mn, Fe, Zn, Cu and B levels were unaffected by temperature conditions. Adequate water supply did not affect P or Ca concentration in the NMF roots, but decreased the concentrations of N, K, Mg, Na, Mn, Fe, Zn and B. The Cu concentration in NMF roots was increased by water supply and decreased under water deficit conditions. The CO_2 level had a stronger effect on N, P, K, Ca, Mg, Na and B than water, although the latter also affected

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N, Na and B strongly. The effect of temperature on nutrient concentrations in NMF roots was not as strong compared to CO_2 and water.

Leaves:

The environmental conditions strongly affected nutrient concentrations in the leaves, and (with the exception of Na) were stronger than the cultivar effect, which is in agreement with the findings of Rogers et al. 2006). Elevated CO_2 significantly decreased the leaf concentrations of all of the nutrients analysed. The temperature treatment had no effect on K, Ca, and Na, while higher concentrations of N, P, Mg, and especially B were associated with an increase in temperature. The enhancing effect of high temperatures on leaf B levels was also reported by Gastol et al. (2014). Water supply did not affect the Na concentrations in leaves, but it decreased the concentrations of all the other nutrients tested. This is contrary to the findings of Shellie & Brown (2012) who found decreased leaf N, K and Cu concentrations in vines under deficit irrigation. When the effects of CO_2 and water were compared, CO_2 generally had a larger effect on leaf nutrient concentrations. When these factors were combined, they accounted for more than 50 % of the total variance calculated for N, P, Mg and Cu.

The nutrient level of the already existing, suberized roots was largely dependent on the cultivar itself as well as the reigning conditions in the nursery. The environmental growth conditions had a much larger effect on the new growth of the season and directly affected the nutrient concentrations in the NMF roots and leaves. Ambient CO_2 levels and level of water supply were the environmental factors that affected nutrient uptake and accumulation the most. The effect of CO_2, temperature and water (and the relative importance of each variable) depended on the specific nutrient as well as on the vine organ/tissue concerned. More studies are required to better understand the intricate mechanisms involved in grapevine nutrition and the expected effects a changing climate might have.

5.3.2.3 Nitrogen

Significantly lower N concentrations were found in leaf and root tissues of vines grown in elevated CO_2 levels (Fig. 5.1) and those well-supplied with water, while the higher temperature conditions seem more conducive to N accumulation. The CO_2 level particularly affected the leaf N concentration and contributed up to 52 % of the total variation found (Table 5.4). The decreasing effect of elevated CO_2 levels on leaf N concentration (Norby et al. 1986; Moutinho-Pereira et al. 2009; Morales et al. 2016) is often given as the reason why stimulated photosynthesis by ambient CO_2 elevation is not sustained over the longer term (Alonso et al. 2009).

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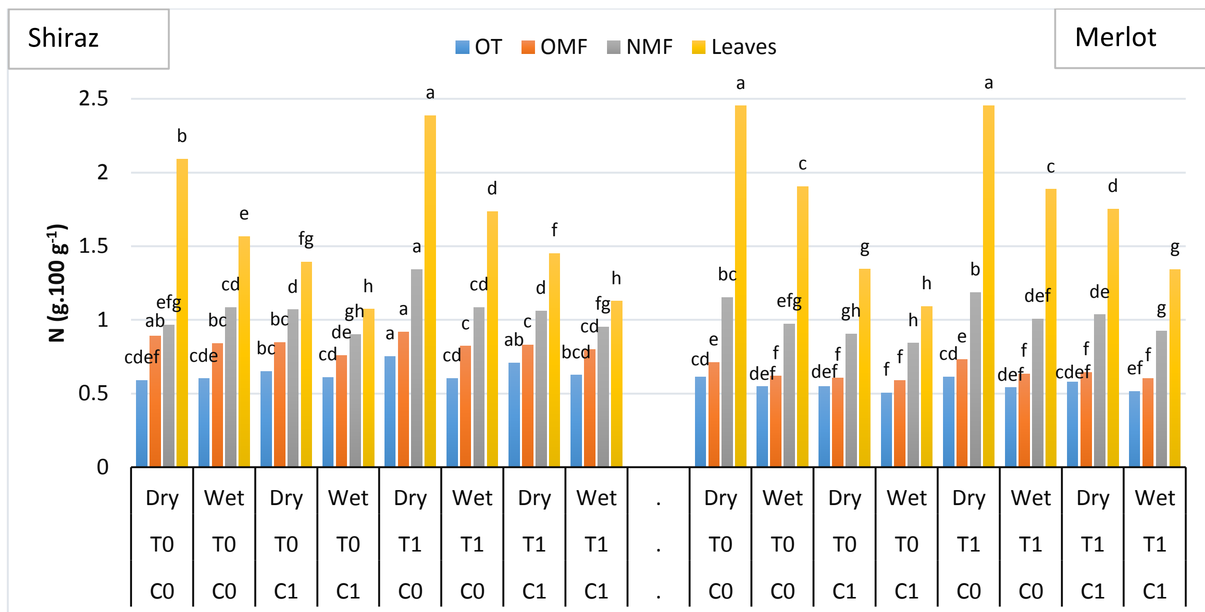

Fig. 5.1 Comparative N concentration in various root classes and leaves of Shiraz and Merlot in the different treatment combinations. Values in the same series followed by the same letter do not differ significantly ($p \leq 0.05$).

According to the minimum leaf N guidelines of 1.6 % (Raath & Schutte, 2001; Saayman, 2016), all the vines in the C1 treatments experienced N deficiency at 12 weeks after planting (Fig. 5.1). It may therefore have been assumed that both photosynthetic activity and vegetative growth of these vines were detrimentally affected. However, the rate of photosynthesis and the vegetative growth (both shoots and roots) were higher in the C1 than the C0 treatments for both cultivars, with a further increment when water was supplied (Chapters 3 & 4). Therefore, no physiological or growth limitation was noticeable as a result of the indicated N deficiency. Despite the fact that the vines used in this glasshouse study were young and non-bearing and exposed to different environmental conditions pertaining soil, nutrients and climate, the accuracy/applicability of existing guidelines and nutrient concentration norms (for petioles and leaf blades) for fertilisation programmes under increased CO_2 conditions may thus be questioned. Shellie & Brown (2012) already found limited diagnostic and prescriptive usefulness of petiole and leaf blade nutrient analyses when vines are grown with limited water supply. It might very well be that current threshold values will have to change in future when expected ambient CO_2 is higher and water availability lower to avoid over-fertilisation of grapevines.

It is interesting that the highest N concentrations were found in vines growing under the less than ideal circumstances (namely lower CO_2 levels and water deficit conditions) with regards to both physiological activity and vegetative growth. Therefore, the total N content (in mg) per tissue type was calculated to determine how the combination of concentration and growth affected the total N content (Fig. 5.2). Lateral shoots and leaves, leaf petioles and primary shoots were not analysed for their nutrient content in this study. Since they are also potential pools of nutrient accumulation, the graphs do not represent total

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nutrient levels per vine and were only used to facilitate comparison between the tissues fractions that were analysed.

The total N content in the leaves of Merlot was higher than that of Shiraz, which was ascribed to both the higher comparative concentrations (Fig. 5.1) and stronger leaf growth (Chapter 4). However, within each cultivar, when CO_2, water supply and temperature were considered as single factors, no significant difference in the leaf N content was found, except for the C1(dry) treatment in Shiraz that was lower than the T0(wet) and C0T1(wet) treatments (Fig. 5.2). Therefore, the rate and amount of N uptake by the roots were generally not much affected by the respective environmental variables. The lower leaf N concentration in the C1 and (wet) treatments was thus possibly due to the attenuating effect of the stronger vegetative growth.

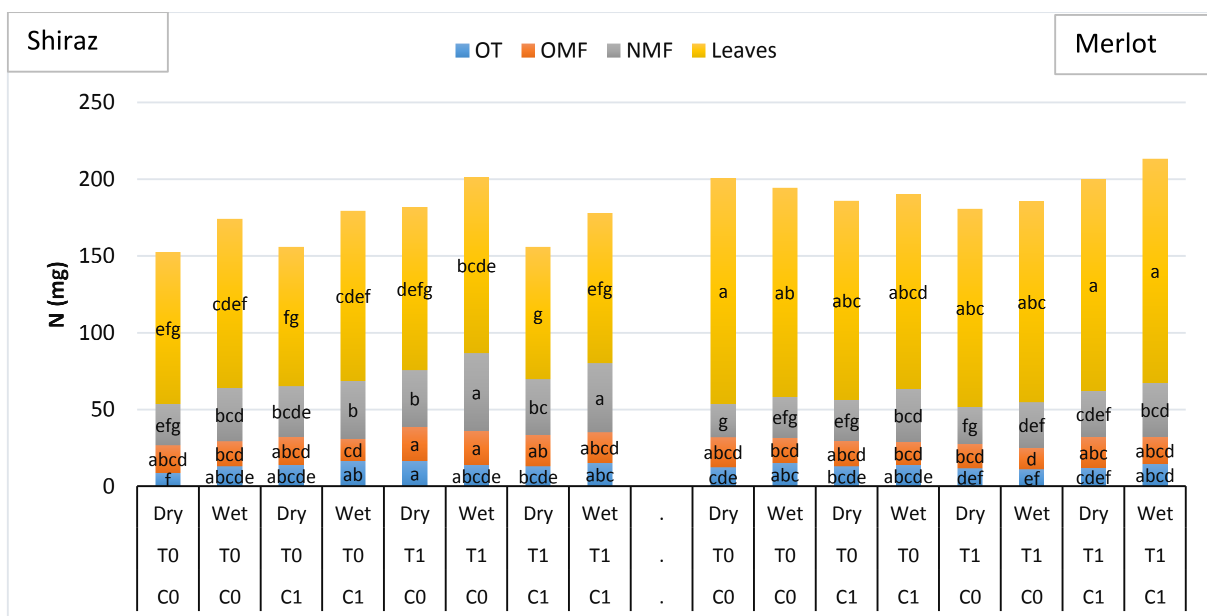

Fig. 5.2 N content in various root classes and leaves of Shiraz and Merlot in the different treatment combinations. Values in the same series followed by the same letter do not differ significantly ($p \leq 0.05$).

Very strong positive and highly significant correlations were found between leaf N concentration and chlorophyll levels per mass unit leaf in both cultivars (Chapter 3). It may possibly be assumed that the lower chlorophyll concentration in the C1 treatments (Chapter 3) would also translate into comparable chlorophyll contents per canopy per vine due to the difference in vegetative growth. As already mentioned, photosynthetic rate per unit leaf area in the C1 treatments did not decrease compared to the C0 treatments, despite the significantly lower N (and thus chlorophyll) levels (Chapter 3). It seems that the activity of the chlorophyll and photosynthetic system was enhanced by the elevated CO_2 and decreased the level of N concentration requirement in the leaves. Plants appear to have strong inherent control in the distribution direction of N (Norby et al. 1986), and it would appear that elevated CO_2 decreases the minimum required N concentration in leaves for efficient photosynthesis. This would enable

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the vine to allocate N to other vine organs than the leaves to be used for other purposes such as growth (Drake et al. 1997; Leakey et al. 2009).

Based on the N concentration and content differences in Figs 5.1 & 5.2, it seems that the N partitioning in the two cultivars was prioritised to the leaves and active roots. However, Shiraz allocated relatively more N to the roots and Merlot to the leaves. When the N content of each tissue class was expressed as a percentage of their combined N pool, the difference in allocation became more clear (Fig. 5.3). Shiraz seemed to invest more N in the OMF and NMF roots, while relatively more N in Merlot was contained in the leaves.

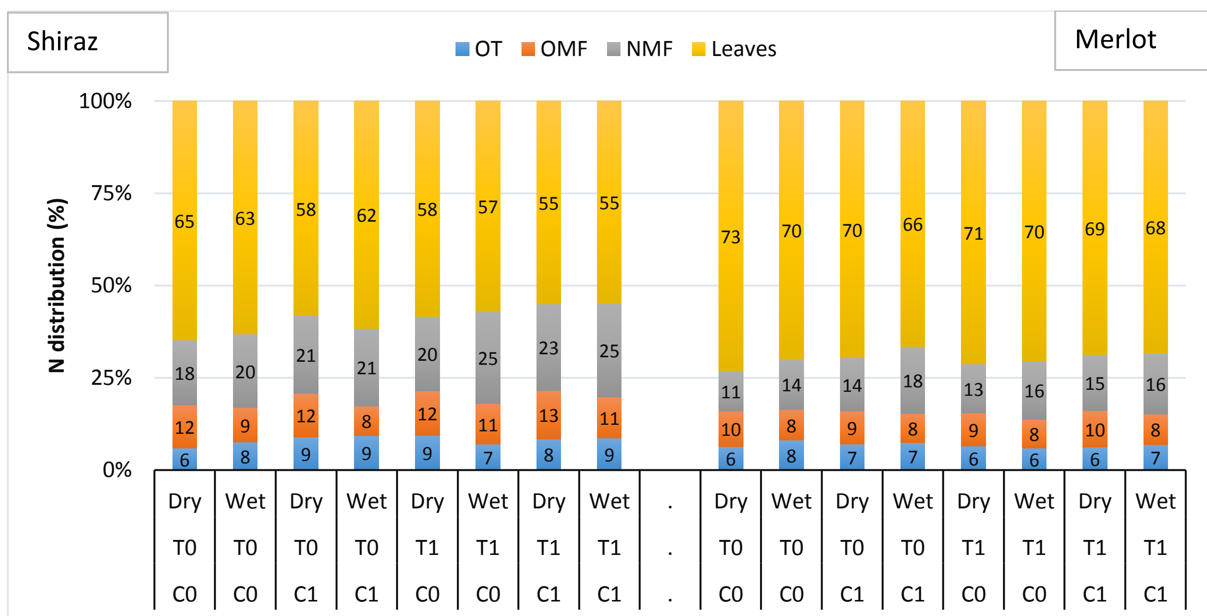

Fig. 5.3 Percentage distribution of N among various root classes and leaves in Shiraz and Merlot in the different treatment combinations.

This was also reflected in the respective dry mass of the leaves and root classes (Fig. 5.4). Merlot accumulated more biomass in the leaves, while the dry mass of the NMF roots in Shiraz was either higher or comparable to that of Merlot.

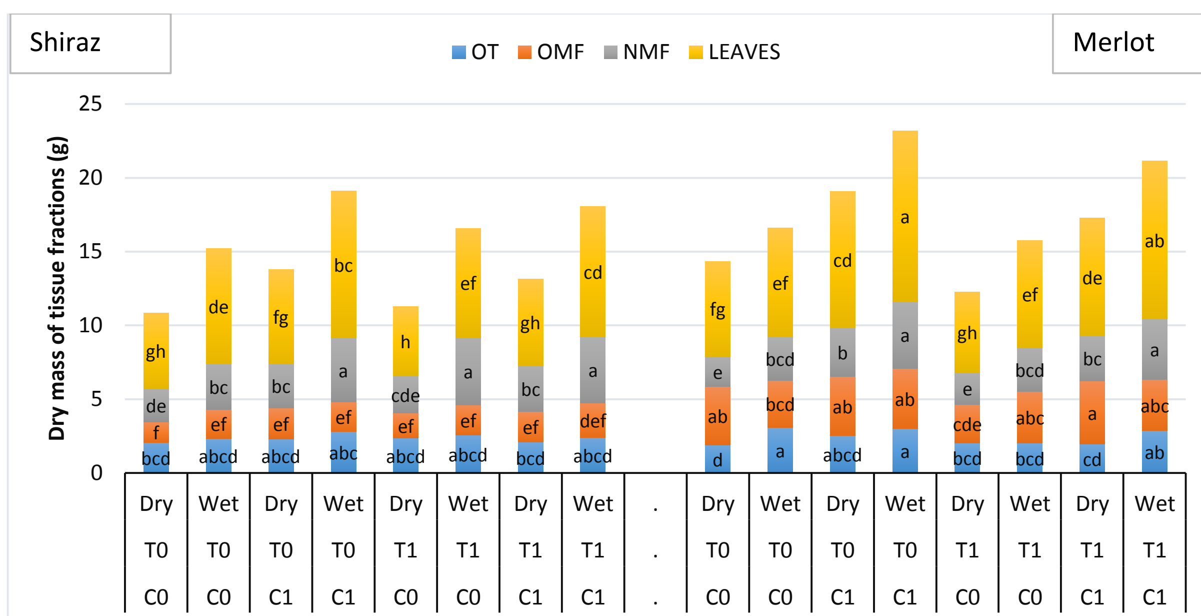

Fig. 5.4 Respective dry masses of various root classes and leaves of Shiraz and Merlot in the different treatment combinations. Values in the same series followed by the same letter do not differ significantly ($p \leq 0.05$).

Young, newly planted vines are generally provided with ample water and nutrients (especially N) to obtain maximum vegetative growth and bringing vines into full production as soon as possible (Myburgh et al. 1996). However, Conradie et al. (1996) found in a study on Glenrosa soil that soil-derived N should be able to meet the N requirement of the young vine for the first three years after soil preparation under current climatic conditions. Therefore, the needless addition of N could be an over-supply (especially on heavier soils) and would only be wasted, since N uptake, shoot growth and yields seem to reach plateaus in response to increasing N in the soil (Spayd et al. 1993; Keller & Koblet, 1995). The unutilised NO_3 in the soil could be leached out of the soil profile and may also result in the eutrophication of nearby water sources and rivers (Good & Beatty, 2011). In the Western Cape with predominantly acid soils (Conradie, 1988), an overabundance of N in the presence of adequate soil water would increase the production of N_2O, thereby further contributing to GHG emissions (Good & Beatty, 2011; Saayman, 2016).

Keller (2005) discussed the interaction between nitrate and sucrose translocation in the grapevine and concluded that excess N availability and resultant high leaf nitrate levels may result in the inhibition of root growth and a decreased root:shoot ratio. Since a strong, well-developed root system is required from the start to buffer the vine against adverse climatic conditions or environmental stress (Archer & Hunter, 2010), over-fertilisation with N during vineyard establishment might even be detrimental for vine growth and productivity in the long term.

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5.3.2.4 Other macro-nutrients

Phosphorous

Phosphate concentration was relatively evenly distributed within the vines, with Shiraz containing higher P levels in all the root fractions than Merlot and similar concentrations in the leaves (Fig. 5.5). New growth (NMF roots and leaves) contained higher P concentrations than the old roots. When the P content was determined, the stronger vegetative growth in the well-watered vines and those in higher CO_2 conditions (Chapter 4) seemed to result in more P per tissue fraction (Fig. 5.6). The root system of Shiraz contained more P than that of Merlot, which is mainly due to the higher concentration and not the dry mass (Fig. 5.4). In Merlot P allocation to the leaves was relatively more than to the roots, while the opposite was found for Shiraz (Figs 5.6 & 5.7).

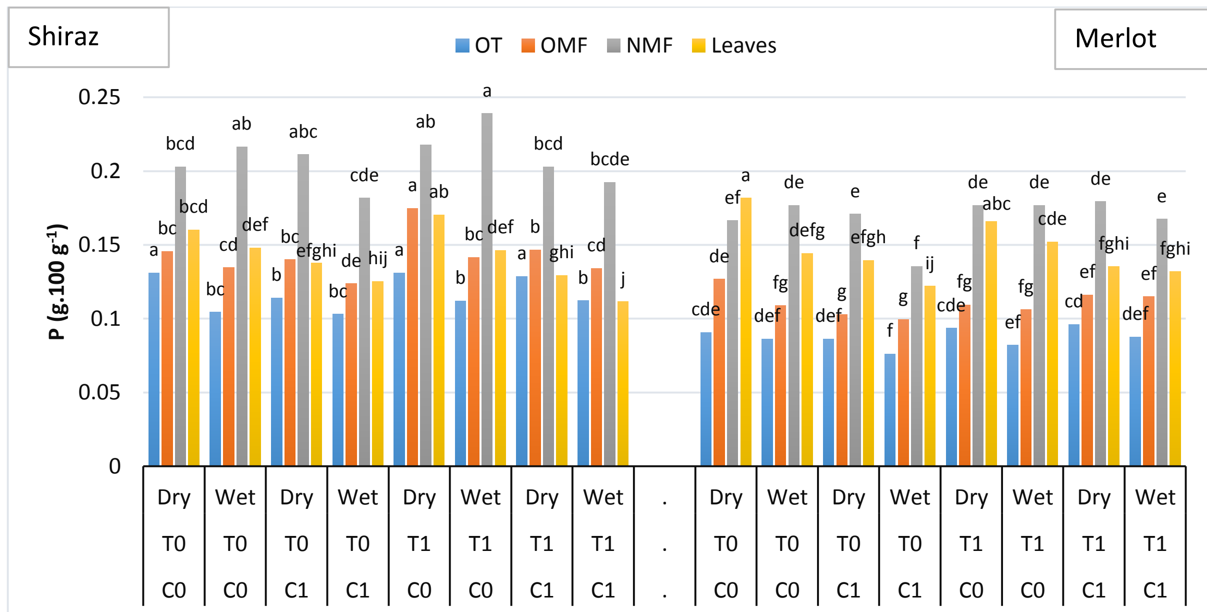

Fig. 5.5 Comparative P concentration in various root classes and leaves of Shiraz and Merlot in the different treatment combinations. Values in the same series followed by the same letter do not differ significantly ($p \leq 0.05$).

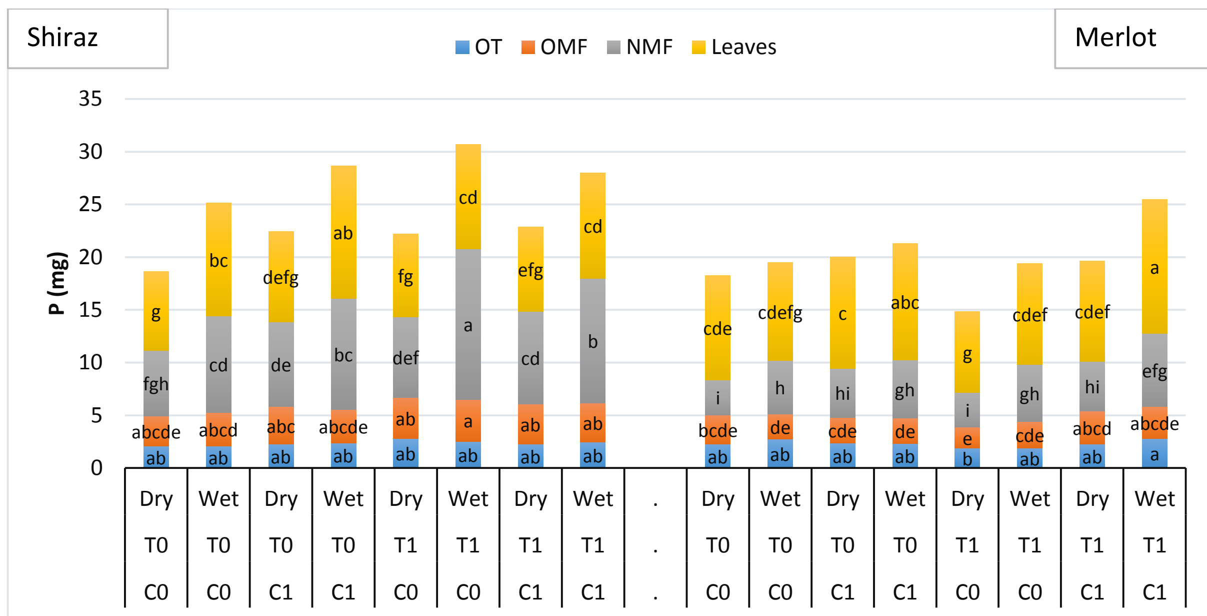

Fig. 5.6 P content in various root classes and leaves of Shiraz and Merlot in the different treatment combinations. Values in the same series followed by the same letter do not differ significantly (p ≤ 0.05).

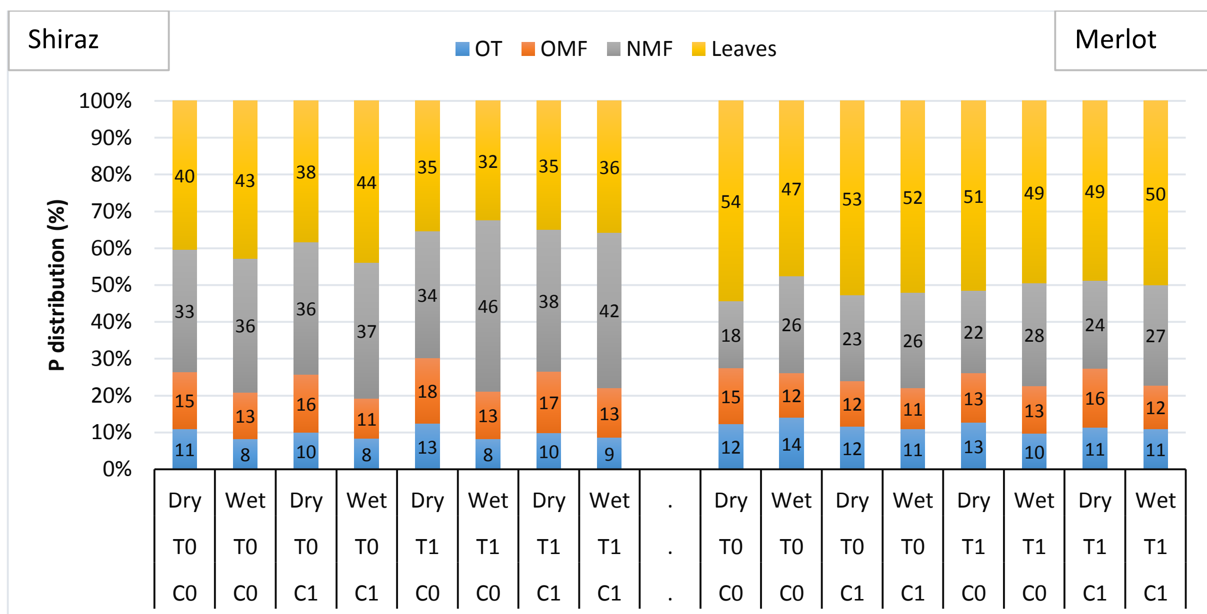

Fig. 5.7 Percentage distribution of P among various root classes and leaves in Shiraz and Merlot in the different treatment combinations.

Potassium

In both cultivars, K concentration (Fig. 5.8) and content (Fig. 5.9) were much lower in the old than in the new roots, with the highest levels accumulating in the leaves. In the NMF roots, higher K concentrations were linked with C0, T0 and (dry) treatments (Fig. 5.8), while the treatment variables as single factors had no effect on the K content (Fig. 5.9). Interestingly, K allocation to the leaves in response to the treatments differed between the cultivars (Fig. 5.8). In the T0 temperatures, C1 levels resulted in lower K

concentrations, while it also seemed to be the case in the well-watered treatments. However, in the higher temperatures, K concentrations in the Merlot leaves were at comparably high levels irrespective of the CO_2 or water treatment and this explains the high K content in the Merlot leaves in the C1T1 treatments (Fig. 5.9). A higher percentage of K was translocated to the leaves in Merlot vines relative to the roots, and in Shiraz more to the roots (Fig. 5.10).

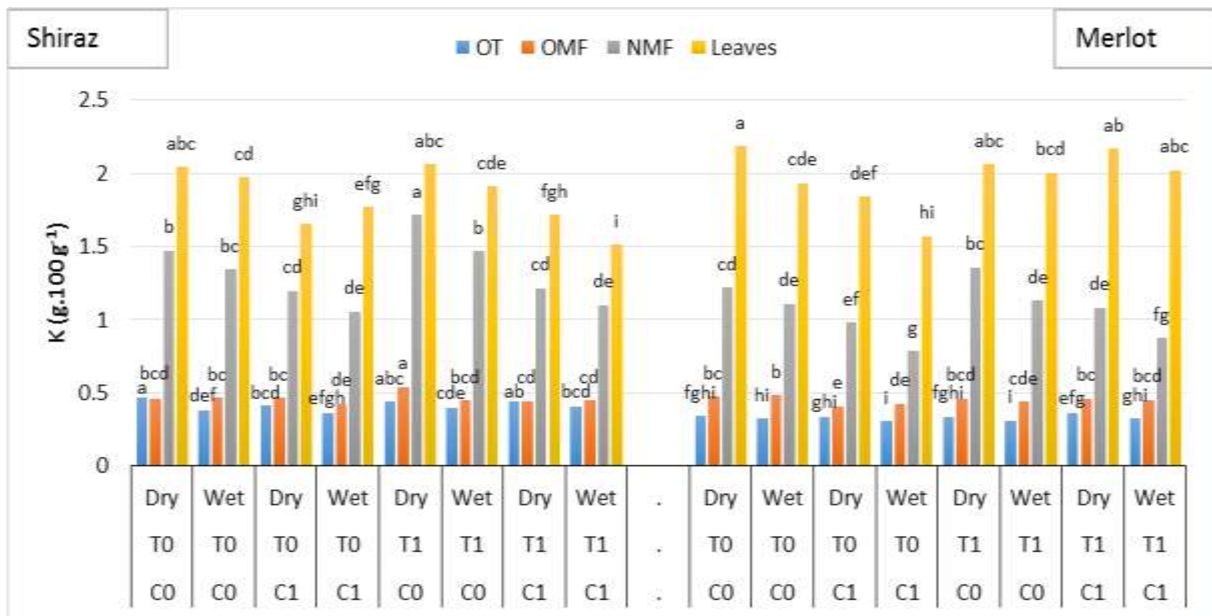

Fig. 5.8 Comparative K concentration in various root classes and leaves of Shiraz and Merlot in the different treatment combinations. Values in the same series followed by the same letter do not differ significantly ($p \leq 0.05$).

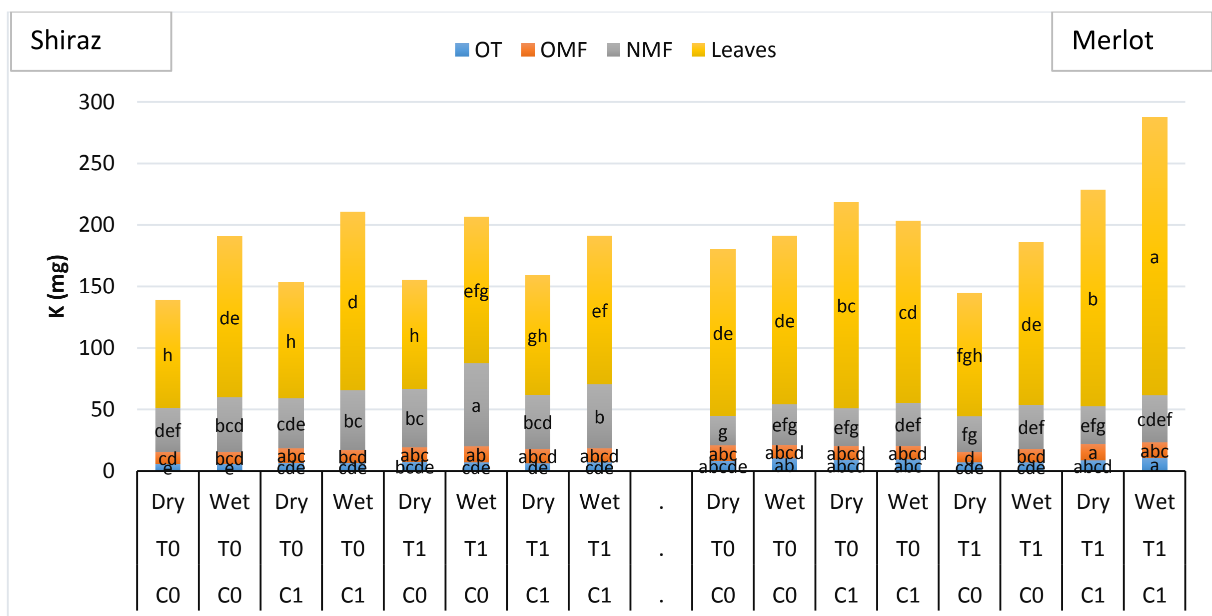

Fig. 5.9 K content in various root classes and leaves of Shiraz and Merlot in the different treatment combinations. Values in the same series followed by the same letter do not differ significantly ($p \leq 0.05$).

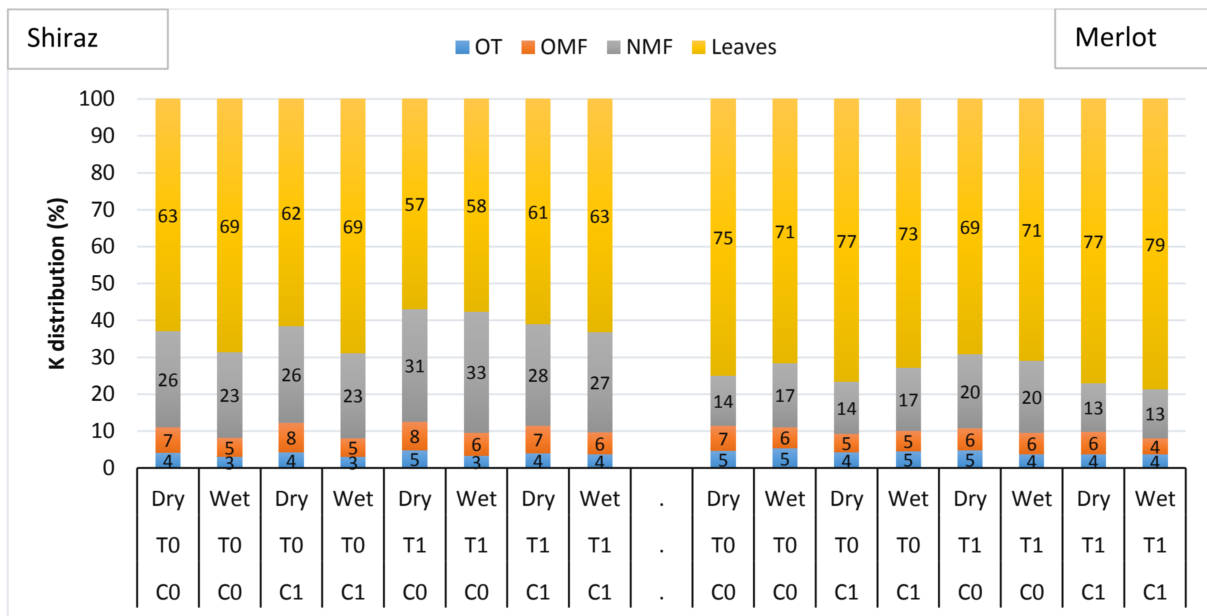

Fig. 5.10 Percentage distribution of K among various root classes and leaves in Shiraz and Merlot in the different treatment combinations.

Calcium

The Ca concentration was uniformly distributed between the older tissues (OT and OMF) and the new growth (NMF and leaves) (Fig. 5.11). The concentration in the Shiraz roots was significantly higher than that of Merlot in all three root fractions, which was also apparent in the content (Fig. 5.12). The Ca concentration in the Merlot leaves seemed higher than Shiraz (Fig. 5.11), but it was not statistically significant [except for C1T1(wet)]. The Ca levels in Shiraz leaves were often lower than in OMF roots, and comparable with the OT concentrations in the C1 treatments (Fig. 5.11). The leaf Ca content was more similar between the cultivars, with only C0T1(dry) and C1T1(wet) treatments where the content was higher in the Merlot leaves (Fig.5.12). Shiraz roots (OT, OMF and NMF) contained more Ca than Merlot roots. It was thus expected that the larger percentage of Ca in Shiraz was allocated to the roots, while Merlot leaves imported more Ca than the roots (Fig. 5.12).

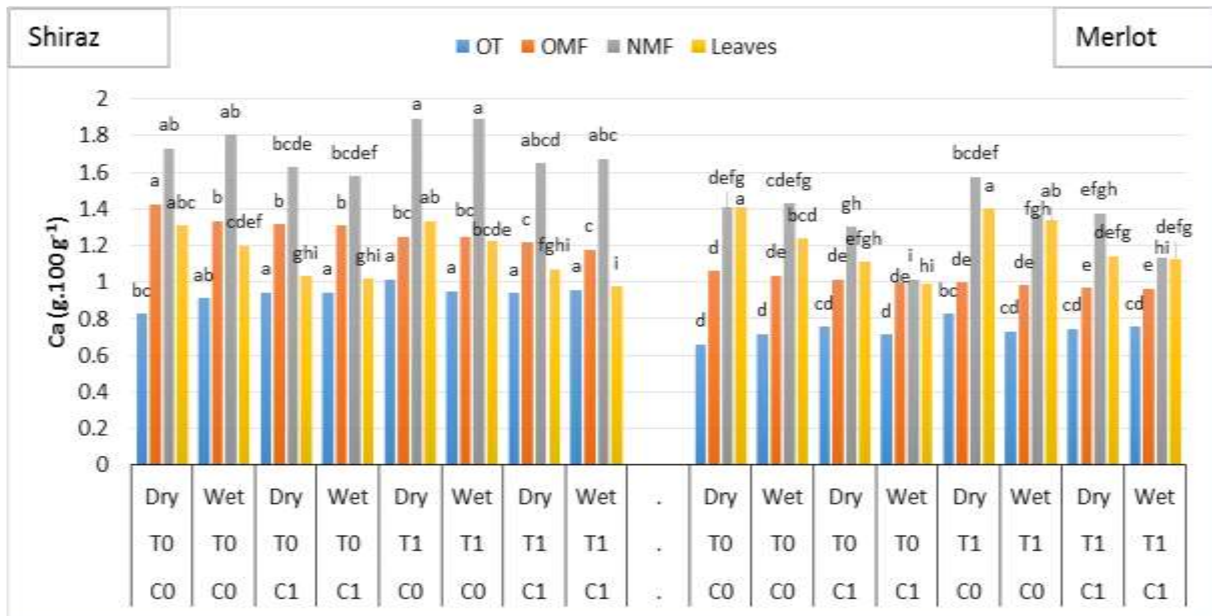

Fig. 5.11 Comparative Ca concentration in various root classes and leaves of Shiraz and Merlot in the different treatment combinations. Values in the same series followed by the same letter do not differ significantly (p ≤ 0.05).

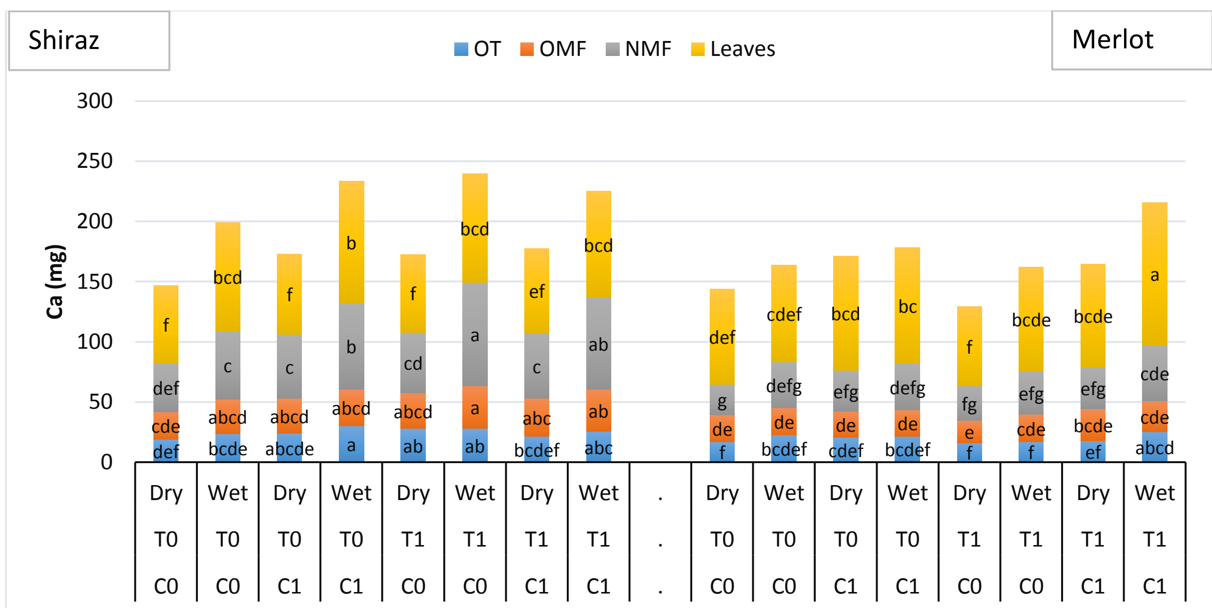

Fig. 5.12 Ca content in various root classes and leaves of Shiraz and Merlot in the different treatment combinations. Values in the same series followed by the same letter do not differ significantly (p ≤ 0.05).

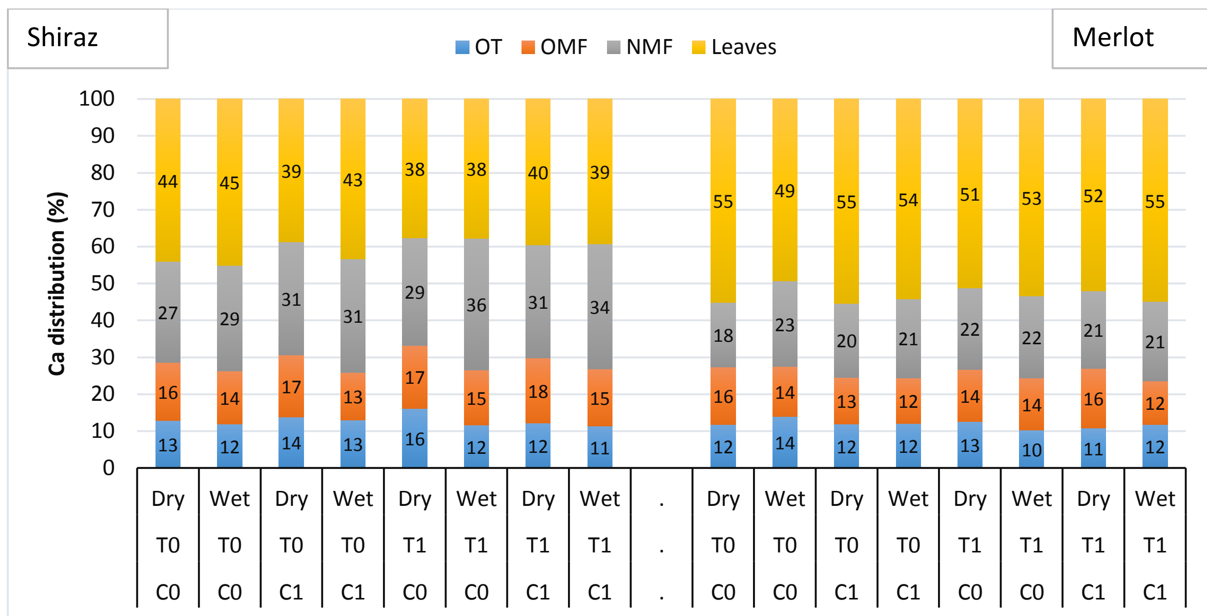

Fig. 5.13 Percentage distribution of Ca among various root classes and leaves in Shiraz and Merlot in the different treatment combinations.

Magnesium

In both cultivars, Mg was mainly translocated to the younger tissues (Fig. 5.14), with higher concentrations in the leaves than in the NMF roots. Concentrations in the NMF roots were similar between the cultivars (Fig. 5.14), while the content in the Shiraz roots was higher in the T1 treatments as well as in C1T0(wet) (Fig. 5.15). Magnesium is the only macro-nutrient where the concentrations in the Shiraz leaves seemed higher than in Merlot leaves and in the T0 treatments the difference was significant (Fig. 5.14). This did not translate into higher contents (Fig. 5.15), due to the stronger leaf growth found in Merlot (Chapter 4). The relative translocation of Mg to the Merlot leaves was higher than to the roots, while in Shiraz the roots were more favoured (Fig. 5.16).

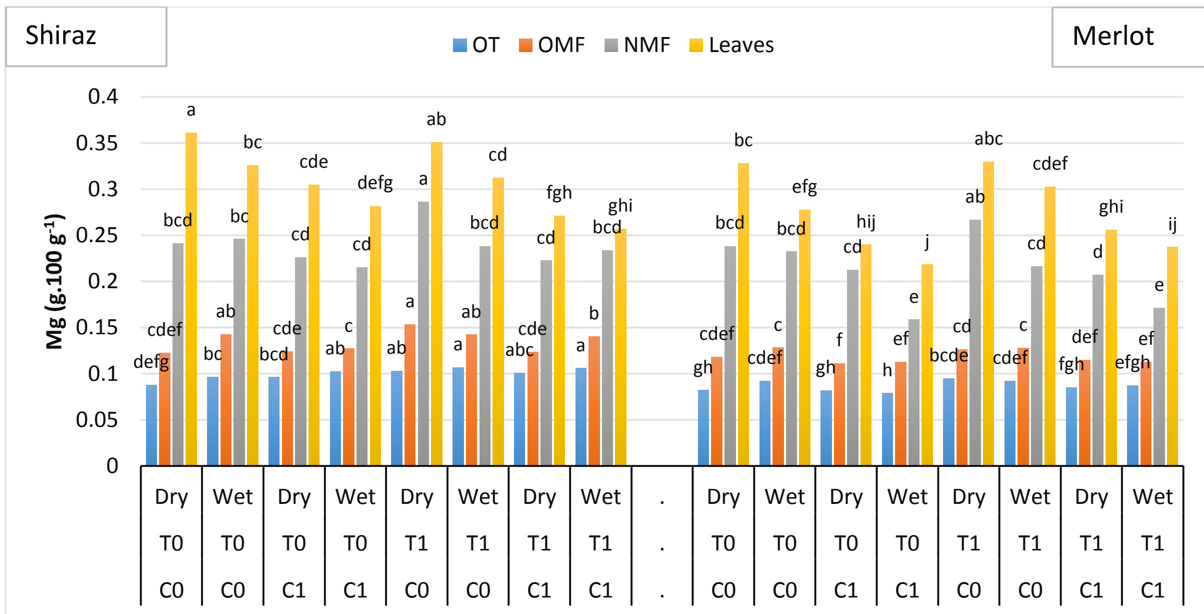

Fig. 5.14 Comparative Mg concentration in various root classes and leaves of Shiraz and Merlot in the different treatment combinations. Values in the same series followed by the same letter do not differ significantly (p ≤ 0.05).

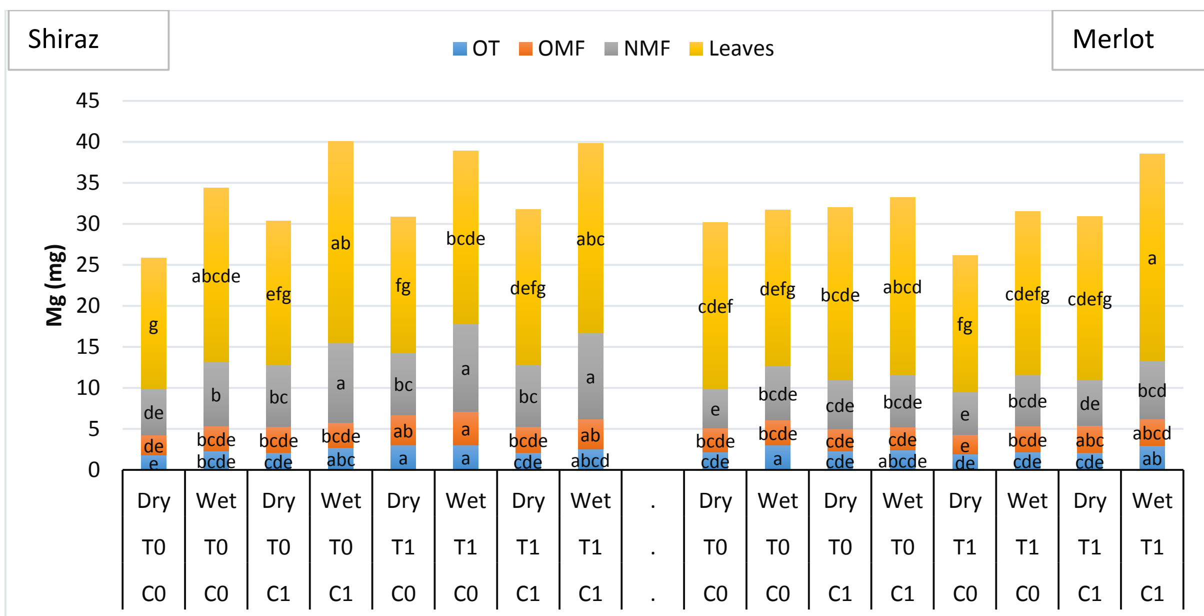

Fig. 5.15 Mg content in various root classes and leaves of Shiraz and Merlot in the different treatment combinations. Values in the same series followed by the same letter do not differ significantly (p ≤ 0.05).

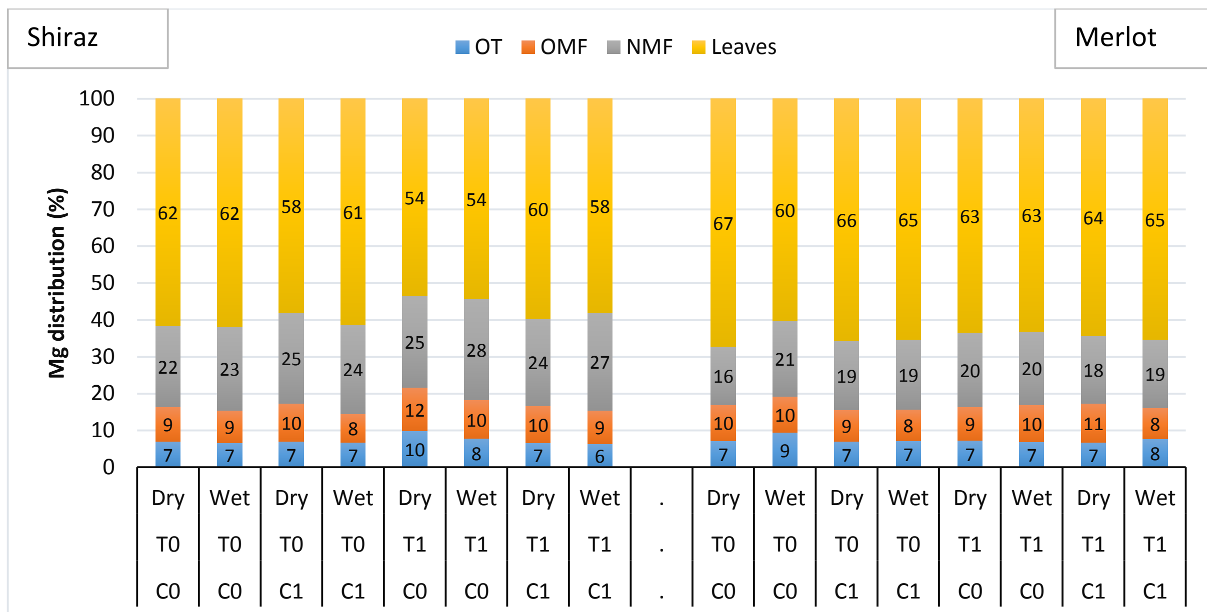

Fig. 5.16 Percentage distribution of Mg among various root classes and leaves in Shiraz and Merlot in the different treatment combinations.

As already discussed, leaf P levels were very low, but no deficiency of any macro-nutrient was found in any of the treatments. Generally speaking, the concentration levels of the remaining macro-nutrients analysed followed similar trends than N, with higher levels in the (dry) than (wet), and in the C0 than C1 treatments in both cultivars. Specific concentrations differed between Shiraz and Merlot and concentrations also varied according to the particular macro-nutrient or the tissue type analysed. Allocation to the leaves was relatively higher in Merlot, while in Shiraz a higher percentage of the available macro-nutrient pool was translocated to the roots.

It would seem that environmental growth conditions, particularly water supply and the CO_2 level (within the temperature range of this study), affected the concentrations and contents of macro nutrients in vines, while the scion cultivar played a more dominant role regarding the allocation of the nutrients between the leaves and the roots.

5.3.2.5 Micro-nutrients

From the results of the macro-nutrients, it is clear that the nutrient concentration alone only provides a partial picture and that the content (that takes the amount of vegetative growth into consideration) is an effective way to compare cultivars and treatment combinations.

Manganese

The highest Mn concentration (Fig. 5.17) and content (Fig. 5.18) were found in the leaves in both cultivars, followed by the NMF roots (Fig. 5.18). Leaf Mn concentrations strongly decreased when vines were well-

watered and under elevated CO_2 conditions. The stronger leaf growth obtained under these conditions resulted in similar Mn leaf content per vine (Fig. 5.18). The effect of the environmental variables on roots was not as clear as for the leaves. Shiraz contained higher Mn concentrations in the OMF and OT roots, while Mn in Merlot roots was more concentrated in the NMF roots (Fig. 5.17). Mn content in the old roots was higher in Shiraz than in Merlot and significantly higher in the NMF roots and leaves in the C0T1 treatments (Fig. 5.18).

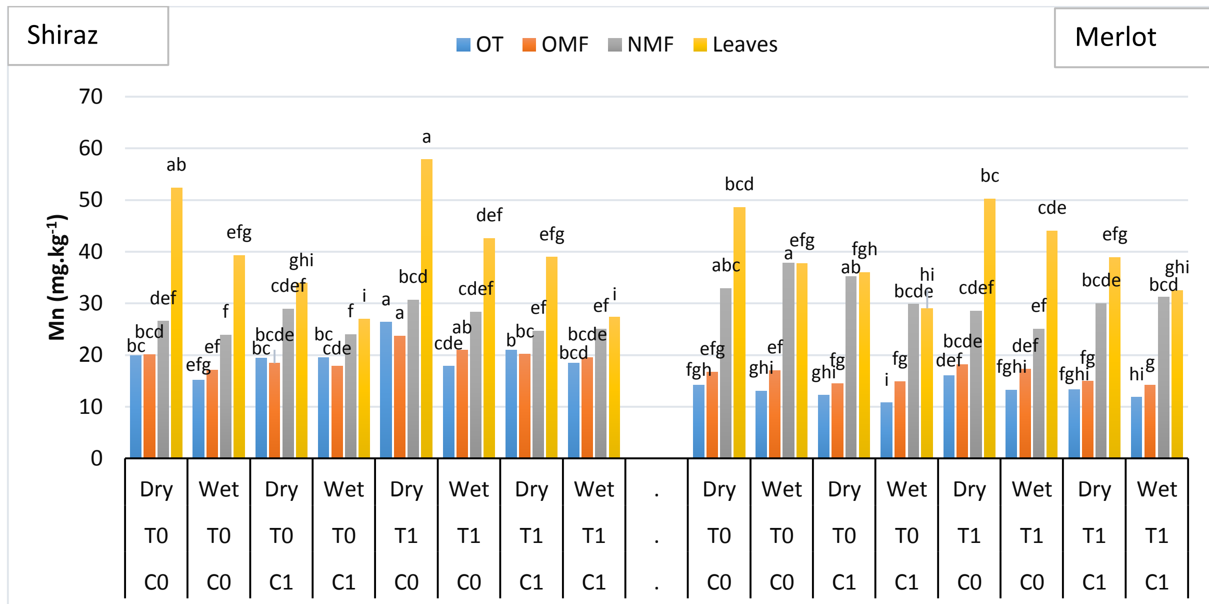

Fig. 5.17 Comparative Mn concentration in various root classes and leaves of Shiraz and Merlot in the different treatment combinations. Values in the same series followed by the same letter do not differ significantly ($p \leq 0.05$).

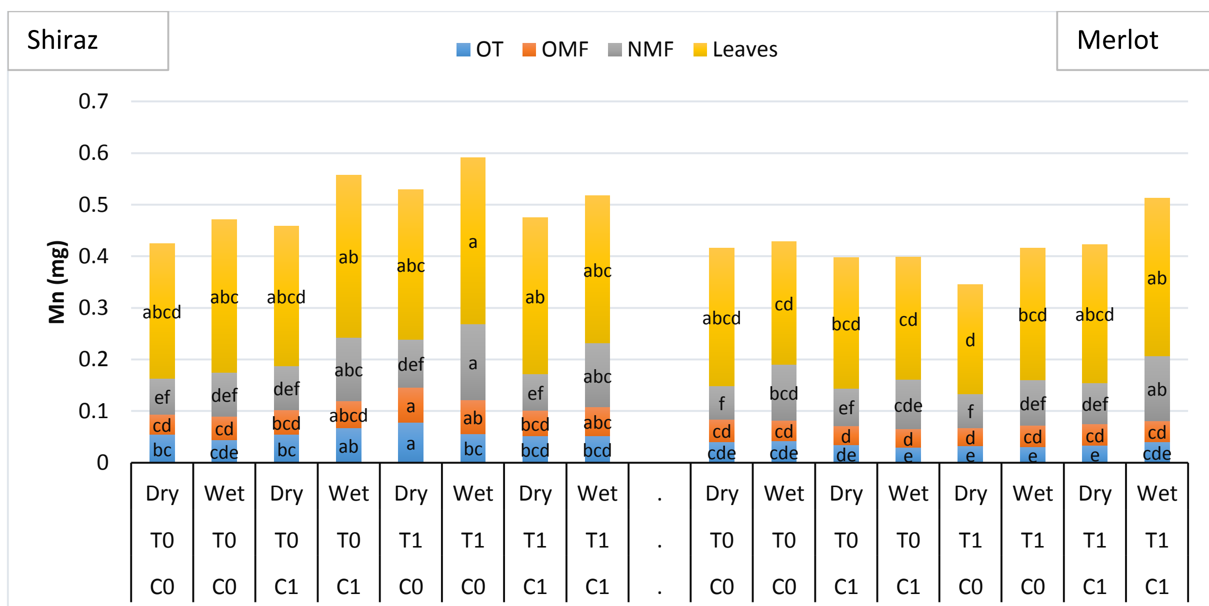

Fig. 5.18 Mn content in various root classes and leaves of Shiraz and Merlot in the different treatment combinations. Values in the same series followed by the same letter do not differ significantly ($p \leq 0.05$).

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Zinc

Zinc was found to be evenly distributed in the plants of both cultivars (Fig. 5.19). Leaves tended to contain the highest concentrations in the C0 treatments (especially Merlot), but their levels were significantly lower in the C1 treatments. The higher leaf concentrations in the water-limited treatments (Fig. 5.19) did not translate into higher Zn contents, due to the stronger vegetative growth of the well-watered vines (Fig. 5.20). The Zn concentration in the Shiraz vines was higher in the OT and OMF than in the NMF roots, while this was not the case for Merlot where the Zn concentration of the root classes was more comparable (Fig. 5.19).

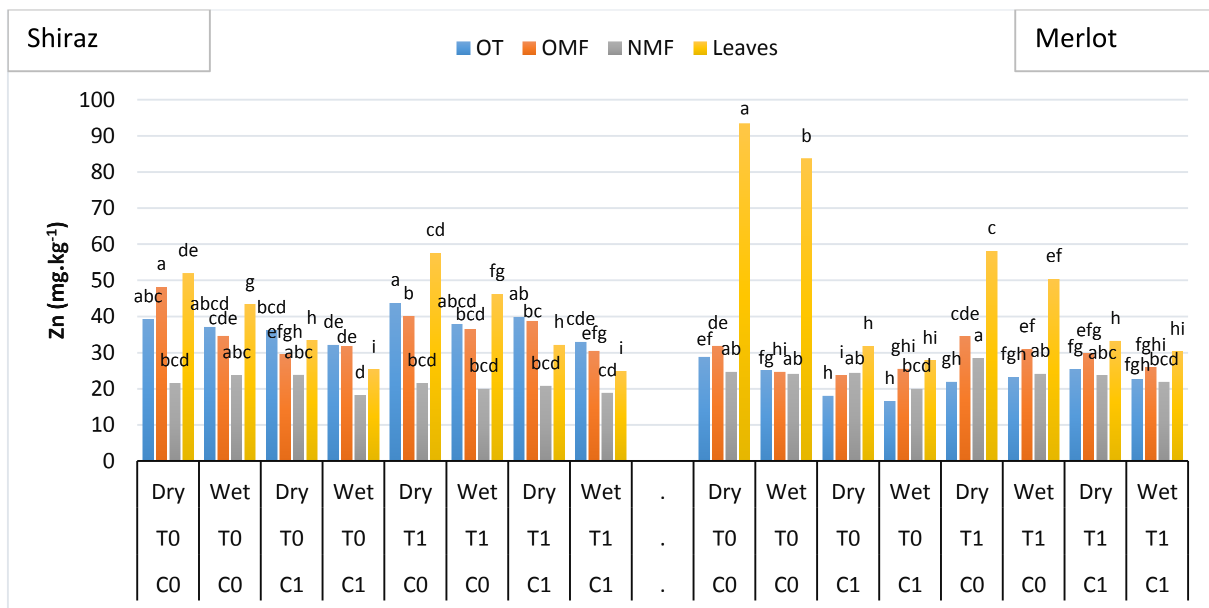

Fig. 5.19 Comparative Zn concentration in various root classes and leaves of Shiraz and Merlot in the different treatment combinations. Values in the same series followed by the same letter do not differ significantly ($p \leq 0.05$).

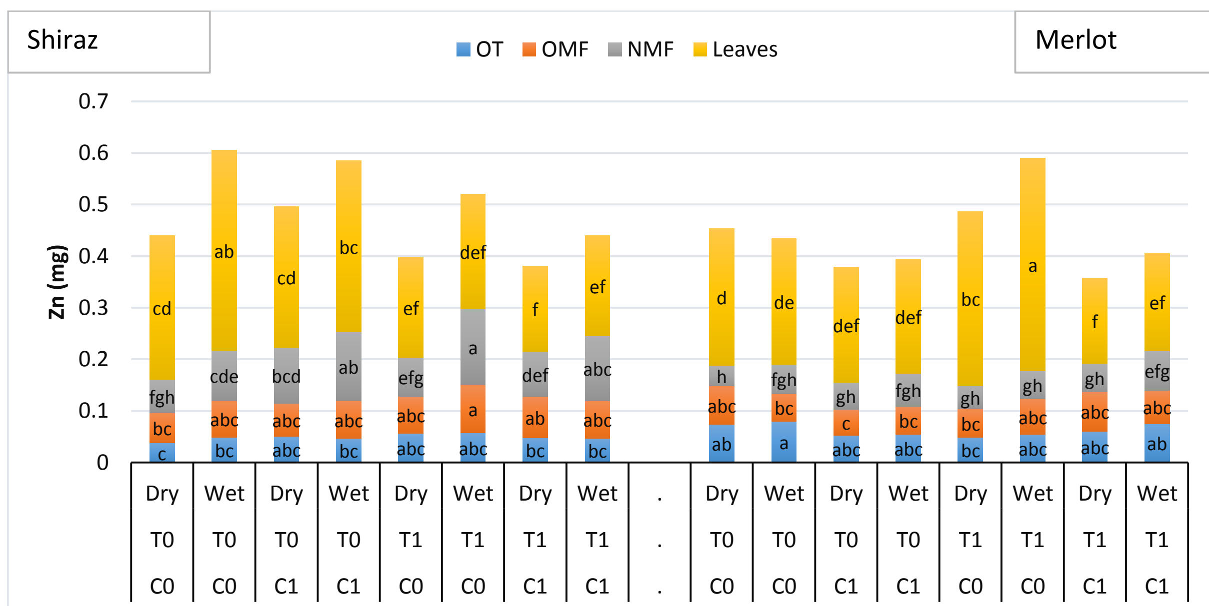

Fig. 5.20 Zn content in various root classes and leaves of Shiraz and Merlot in the different treatment combinations. Values in the same series followed by the same letter do not differ significantly ($p \leq 0.05$).

Boron

The highest B concentrations were found in the leaves (Fig. 5.21). The concentrations in the leaves tended to be lower in the well-watered vines, but seemed more strongly affected by the decreasing effect of the higher CO_2 levels. It is interesting that B seemed to be more temperature sensitive than most of the other nutrients, with significantly higher leaf concentrations in the T1 treatments. Root concentrations seemed to be similar between cultivars and treatments, with the exception of the C1(wet) vines where the concentrations in the NMF roots were significantly lower than in the other treatments. The B content in the Shiraz leaves and NMF roots was significantly higher compared to Merlot in all the treatment combinations, except for C0T0(dry) (Fig. 5.22).

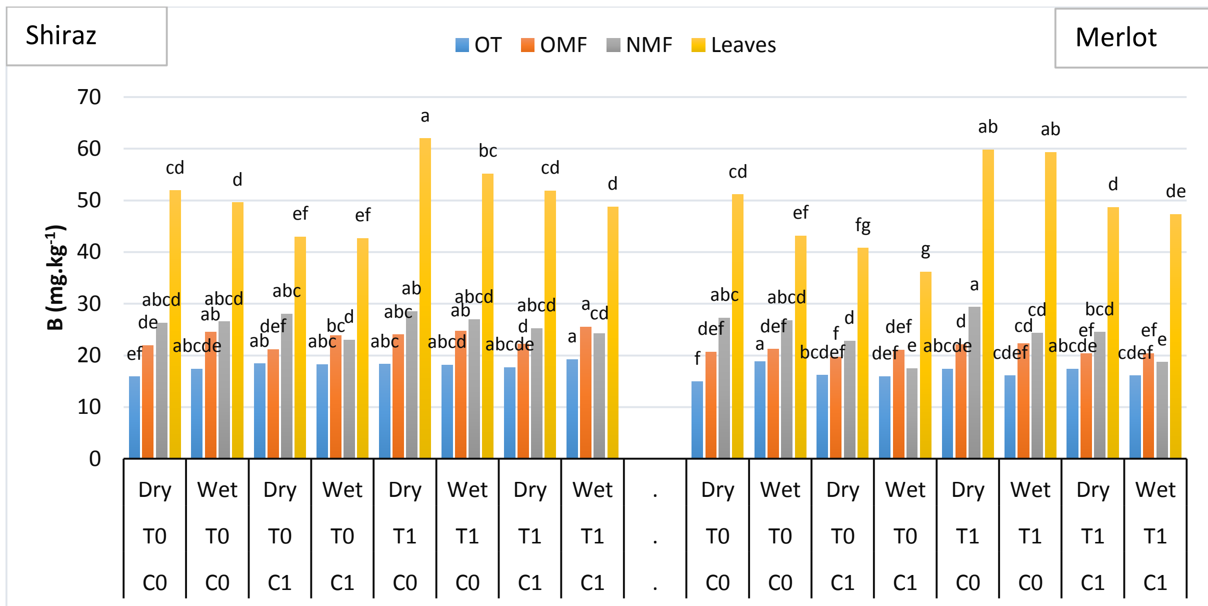

Fig. 5.21 Comparative B concentration in various root classes and leaves of Shiraz and Merlot in the different treatment combinations. Values in the same series followed by the same letter do not differ significantly (p ≤ 0.05).

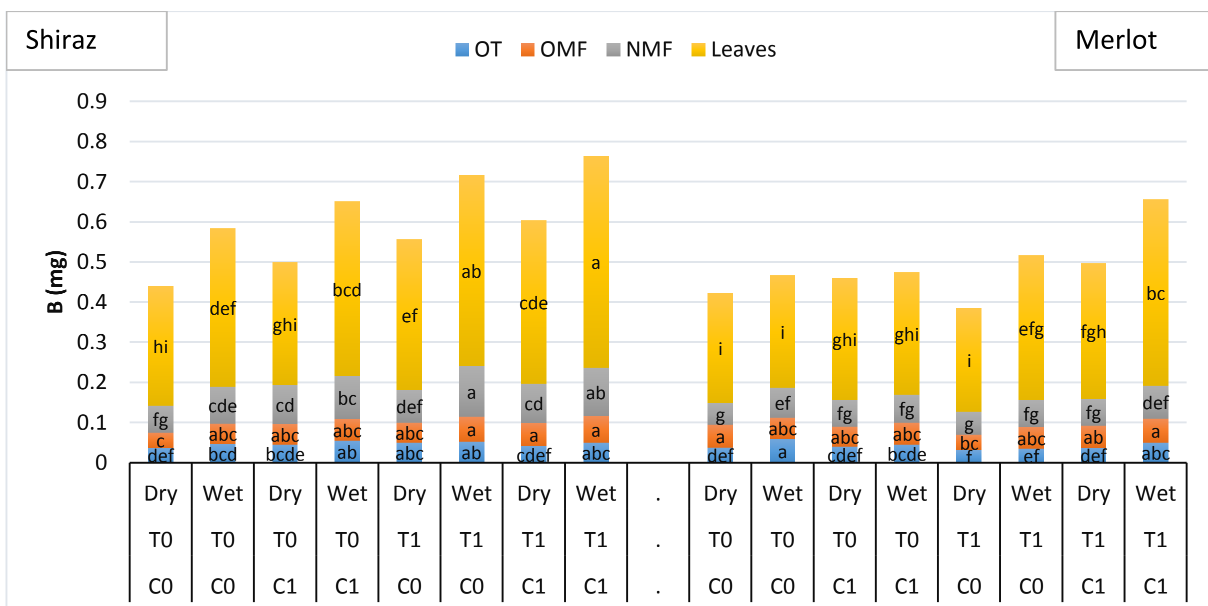

Fig. 5.22 B content in various root classes and leaves of Shiraz and Merlot in the different treatment combinations. Values in the same series followed by the same letter do not differ significantly (p ≤ 0.05).

Sodium

Sodium was clearly higher in the roots than in the leaves in both cultivars, with the highest concentrations in the NMF roots (Fig. 5.23). Concentrations were significantly lower in the C1 treatments for all the tissue types, while the Na concentrations were higher in the NMF roots where water was limited. The Na content of the NMF root class was higher in Shiraz than in Merlot in the T1 treatments, while the OMF roots of Merlot in the C0T0 contained more Na than Shiraz (Fig. 5.24). Both the leaf Na concentration and content were higher in Merlot than in Shiraz (Figs 5.23 & 5.24).

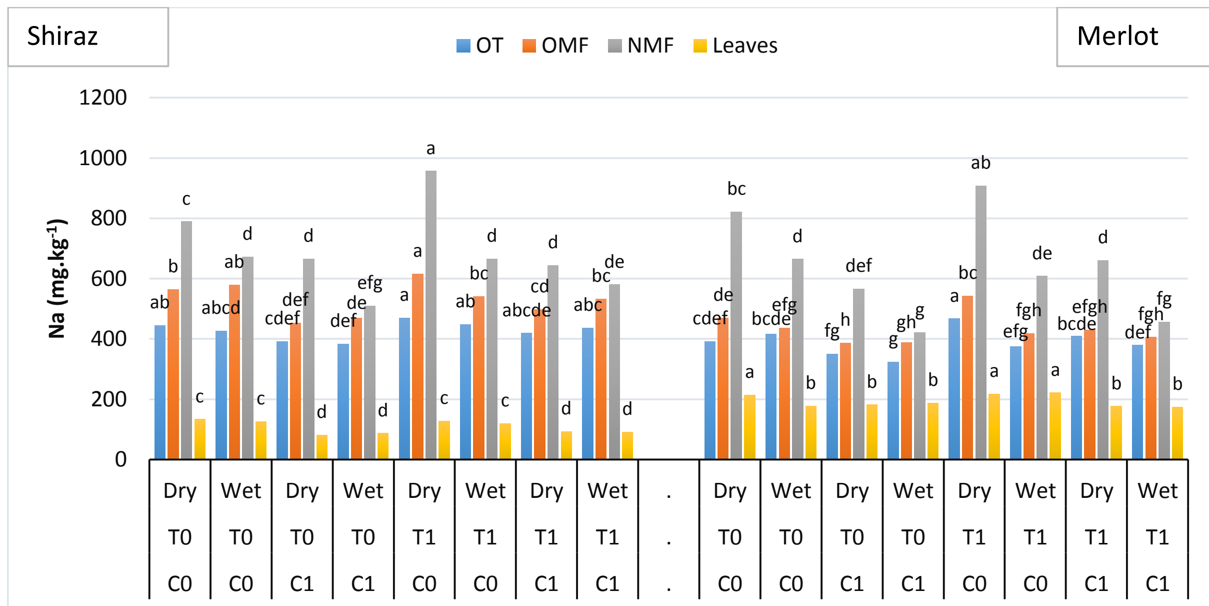

Fig. 5.23 Comparative Na concentration in various root classes and leaves of Shiraz and Merlot in the different treatment combinations. Values in the same series followed by the same letter do not differ significantly (p ≤ 0.05).

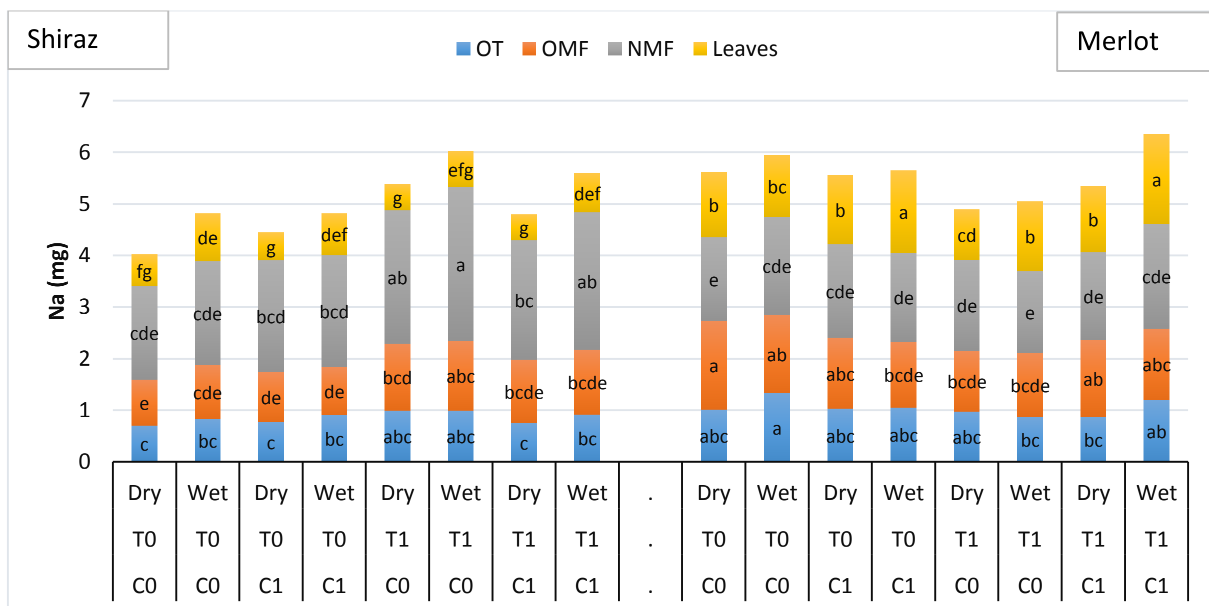

Fig. 5.24 Na content in various root classes and leaves of Shiraz and Merlot in the different treatment combinations. Values in the same series followed by the same letter do not differ significantly (p ≤ 0.05).

Iron

This micro-nutrient is also mostly concentrated in the NMF roots for both cultivars, with the lowest concentrations in the leaves (Fig. 5.25). The new roots of Merlot vines seemed more sensitive to environmental factors than those of Shiraz, with the lowest concentrations in the C1(wet) treatments. The latter treatment combination also resulted in the lowest Fe concentrations in Shiraz and Merlot leaves in T1 temperatures. The Fe concentration in the OMF roots of Shiraz was significantly higher than that of Merlot roots in all the treatment combinations (Fig. 5.25). All the root classes of Shiraz vines accumulated more Fe than those of Merlot, especially the OMF roots (Fig. 5.26).

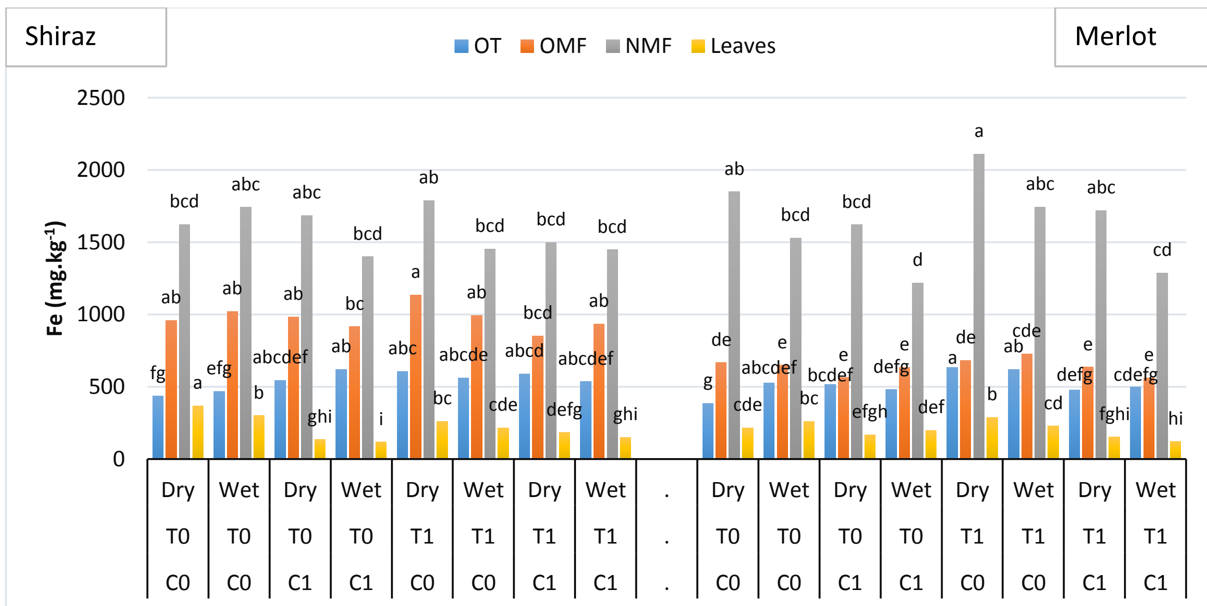

Fig. 5.25 Comparative Fe concentration in various root classes and leaves of Shiraz and Merlot in the different treatment combinations. Values in the same series followed by the same letter do not differ significantly (p ≤ 0.05).

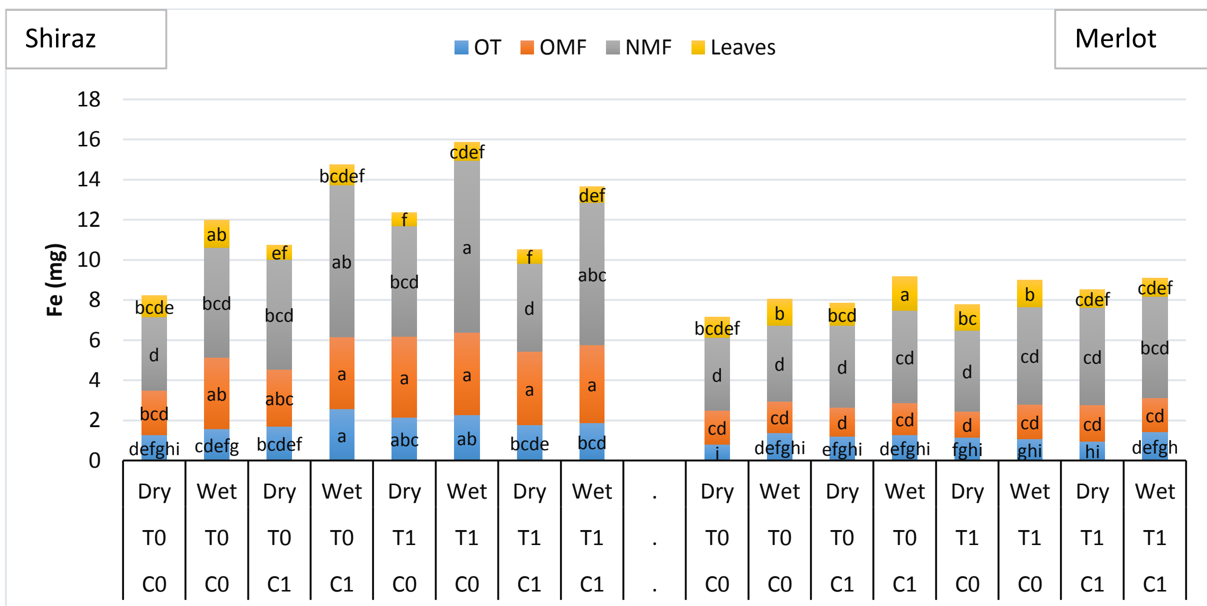

Fig. 5.26 Fe content in various root classes and leaves of Shiraz and Merlot in the different treatment combinations. Values in the same series followed by the same letter do not differ significantly (p ≤ 0.05).

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Copper

Copper mainly accumulated in the NMF roots of both cultivars (Fig. 5.27). The OMF roots contained higher Cu concentrations than the OT roots, while the concentrations in leaves were very low. Concentrations in the old roots did not seems to vary much between the treatments, while roots of Shiraz vines had higher levels than those of Merlot. In Shiraz, the Cu levels in the NMF roots and leaves seemed to both depend strongly on water availability, but with opposite effects. In the (wet) treatments, the Cu concentration was significantly higher in the NMF roots, but lower in the leaves. The reaction of the NMF roots in Merlot vines was similar but not as significant, while the Cu concentration in the leaves seemed to depend more on the CO_2 level than water availability. More Cu accumulated in Shiraz vines than in Merlot (Fig. 5.28), particularly in the NMF roots.

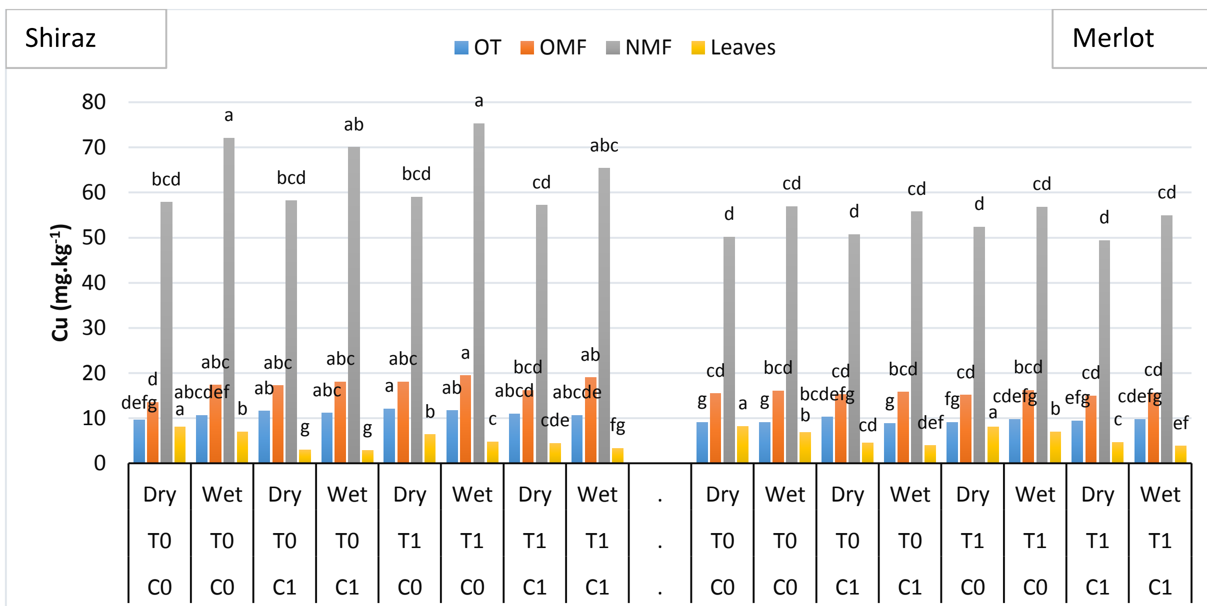

Fig. 5.27 Comparative Cu concentration in various root classes and leaves of Shiraz and Merlot in the different treatment combinations. Values in the same series followed by the same letter do not differ significantly (p ≤ 0.05).

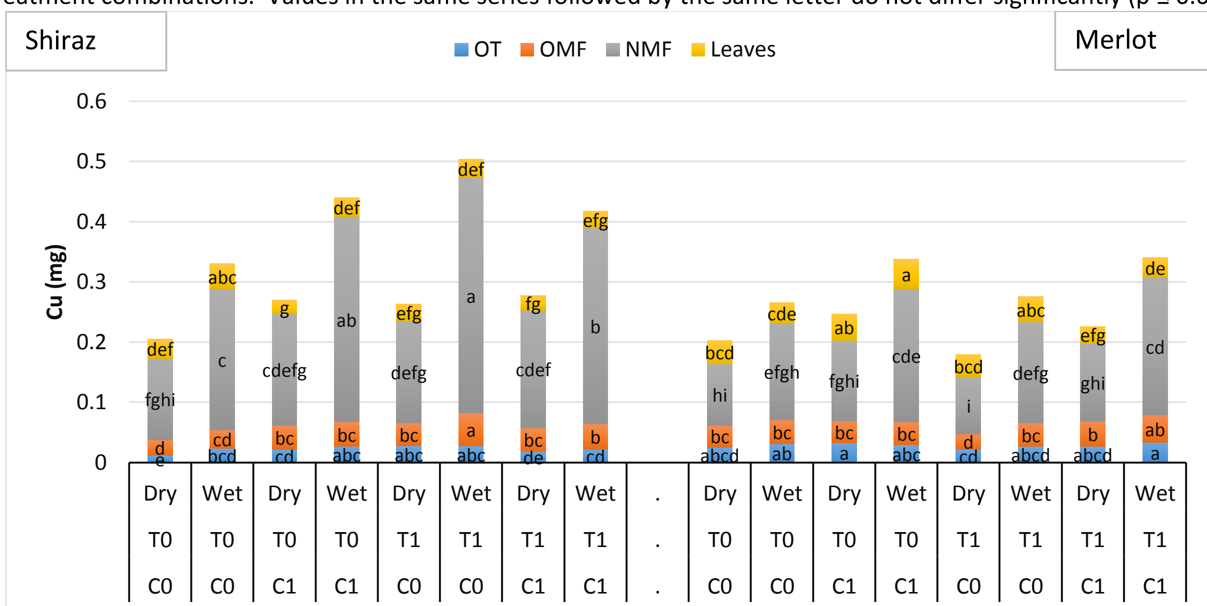

Fig. 5.28 Cu content in various root classes and leaves of Shiraz and Merlot in the different treatment combinations. Values in the same series followed by the same letter do not differ significantly (p ≤ 0.05).

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The distribution of the micro-nutrients (mg.kg^{-1}) within the vines had two distinct patterns: Na, Fe and Cu accumulated mainly in the roots, and Mn, Zn and B in the leaves (in both cultivars). The leaf-dominant micro-nutrients reacted similarly to the environmental variables than the macro-nutrients, in the sense that lower concentrations were found in the C1, well-watered treatments. The root-dominant micro-nutrients seemed more variable in their responses, although water supply and C1 levels were often linked with lower nutrient concentrations in the tissues.

5.4 CONCLUSIONS

The uptake, accumulation as well as distribution of macro- and micro-nutrients will be affected by a changing climate. Generally speaking, nutrient concentrations increased in water deficit conditions and decreased under elevated CO_2.

Although the reaction patterns in response to treatment variables were mostly similar, large differences between cultivars were found with regards to the nutrient concentration, the content, as well as the relative distribution within the vine between the leaves and the different root classes. Since Shiraz and Merlot was grafted onto the same rootstock (101-14 Mg), it would seem as if the scion cultivar itself together with the interaction between the scion and rootstock, plays an important role when it comes to vine nutrition. Potential problems regarding vine nutrition may be avoided by using the rootstock-scion genotype best suited to the environment and soil conditions.

Nutrient concentration is an easy measure for comparison, but does not take the degree of vine growth or the relative distribution patterns into account. The stronger vegetative growth obtained in the C1 and (wet) treatments often compensated for lower concentrations so that the final content per tissue per vine was similar between treatments.

Although leaf N levels in C1 treatments seemed to indicate deficiency, no physiological or vegetative symptoms were observed. This raises a question about the current guidelines used for the planning of fertilisation programmes. The results of the study indicate that existing guidelines may require re-evaluation and possible adjustment under future climatic conditions, particularly regarding N fertilisation in young vines where unnecessary fertilisation may be more harmful than beneficial.

APPENDIX 1a. Comparison of the macro-nutrient concentration and approximate content of Shiraz and Merlot vines at 12 weeks after planting.

			N (g.100 g-1)	N (mg.vine-1)	P (g.100 g-1)	P (mg.vine-1)	K (g.100 g-1)	K (mg.vine-1)	Ca (g.100 g-1)	Ca (mg.vine-1)	Mg (g.100 g-1)	Mg (mg.vine-1)
OT	C0T0(D)	Shiraz	0.589 cdef	8.890 f	0.131 a	2.065 ab	0.465 a	5.804 e	0.828 bc	18.825 def	0.088 defg	1.788 e
		Merlot	0.612 cd	12.666 cde	0.091 cde	2.248 ab	0.342 fghi	8.417 abcde	0.661 d	16.933 f	0.082 gh	2.175 cde
	C0T0(W)	Shiraz	0.601 cde	13.139 abcde	0.104 bc	2.070 ab	0.378 def	5.805 e	0.912 ab	23.634 bcde	0.096 bc	2.263 bcde
		Merlot	0.550 def	15.569 abc	0.086 def	2.741 ab	0.323 hi	10.318 ab	0.718 d	22.738 bcdef	0.092 cdef	2.990 a
	C1T0(D)	Shiraz	0.650 bc	13.930 abcde	0.114 b	2.249 ab	0.412 bcd	6.622 cde	0.942 a	23.955 abcde	0.096 bcd	2.113 cde
		Merlot	0.548 def	12.988 bcde	0.086 def	2.335 ab	0.335 ghi	9.230 abcd	0.756 cd	20.328 cdef	0.082 gh	2.248 cde
	C1T0(W)	Shiraz	0.611 cd	16.583 ab	0.103 bc	2.388 ab	0.359 efgh	6.614 cde	0.945 a	30.385 a	0.102 ab	2.706 abc
		Merlot	0.506 f	14.022 abcde	0.076 f	2.328 ab	0.306 i	9.466 abcd	0.714 d	21.596 bcdef	0.079 h	2.404 abcde
	C0T1(D)	Shiraz	0.753 a	16.804 a	0.131 a	2.779 ab	0.438 abc	7.609 bcde	1.02 a	27.911 ab	0.103 ab	3.025 a
		Merlot	0.613 cd	11.681 def	0.094 cde	1.877 b	0.339 fghi	6.902 cde	0.826 bc	16.281 f	0.095 bcde	1.899 de
	C0T1(W)	Shiraz	0.603 cd	14.037 abcde	0.112 b	2.533 ab	0.399 cde	7.100 cde	0.951 a	27.872 ab	0.107 a	3.043 a
		Merlot	0.544 def	11.053 ef	0.082 ef	1.893 ab	0.311 i	7.171 cde	0.731 cd	16.671 f	0.092 cdef	2.169 cde
	C1T1(D)	Shiraz	0.708 ab	13.106 bcde	0.129 a	2.263 ab	0.445 ab	6.571 de	0.941 a	21.544 bcdef	0.101 abc	2.093 cde
		Merlot	0.581 cdef	12.221 cdef	0.096 cd	2.241 ab	0.364 efg	8.715 abcd	0.745 cd	17.720 ef	0.085 fgh	2.083 cde
	C1T1(W)	Shiraz	0.624 bcd	15.400 abc	0.112 b	2.425 ab	0.408 bcd	7.075 cde	0.957 a	25.523 abc	0.106 a	2.524 abcd
		Merlot	0.515 ef	14.789 abcd	0.088 def	2.796 a	0.327 ghi	10.673 a	0.758 cd	25.345 abcd	0.087 efgh	2.958 ab

			N (g.100 g^{-1})	N (mg.vine^{-1})	P (g.100 g^{-1})	P (mg.vine^{-1})	K (g.100 g^{-1})	K (mg.vine^{-1})	Ca (g.100 g^{-1})	Ca (mg.vine^{-1})	Mg (g.100 g^{-1})	Mg (mg.vine^{-1})
OMF	C0T0(D)	Shiraz	0.891 ab	17.882 abcd	0.146 bc	2.876 abcde	0.460 bcd	9.652 cd	1.425 a	22.983 cde	0.122 cdef	2.442 de
		Merlot	0.711 e	19.394 abcd	0.127 de	2.784 bcde	0.474 bc	12.238 abc	1.059 d	22.379 de	0.118 cdef	2.929 bcde
	C0T0(W)	Shiraz	0.841 bc	16.235 bcd	0.135 cd	3.198 abcd	0.468 bc	9.864 bcd	1.333 b	28.622 abcd	0.143 ab	3.050 bcde
		Merlot	0.618 f	16.252 bcd	0.109 fg	2.371 de	0.483 b	10.981 abcd	1.037 de	22.229 de	0.128 c	3.086 bcde
	C1T0(D)	Shiraz	0.846 bc	18.410 abcd	0.140 bc	3.551 abc	0.465 bc	12.155 abc	1.317 b	28.927 abcd	0.124 cde	3.132 bcde
		Merlot	0.606 f	16.646 abcd	0.103 g	2.464 cde	0.407 e	11.342 abcd	1.014 de	21.732 de	0.111 f	2.725 cde
	C1T0(W)	Shiraz	0.761 de	14.568 cd	0.124 de	3.147 abcde	0.427 de	10.590 bcd	1.310 b	29.969 abcd	0.127 c	3.079 bcde
		Merlot	0.589 f	15.019 bcd	0.099 g	2.372 de	0.427 de	10.993 abcd	0.997 de	21.952 de	0.113 ef	2.786 cde
	C0T1(D)	Shiraz	0.918 a	22.100 a	0.175 a	3.914 ab	0.543 a	11.853 abc	1.249 bc	29.546 abcd	0.154 a	3.637 ab
		Merlot	0.732 e	16.191 bcd	0.109 fg	1.997 e	0.462 bcd	8.752 d	0.995 de	18.248 e	0.126 cd	2.367 e
	C0T1(W)	Shiraz	0.822 c	22.057 a	0.141 bc	3.973 a	0.448 bcd	12.813 ab	1.247 bc	35.635 a	0.142 ab	4.034 a
		Merlot	0.632 f	14.244 d	0.106 fg	2.480 cde	0.442 cde	10.609 bcd	0.984 de	22.836 cde	0.128 c	3.131 bcde
	C1T1(D)	Shiraz	0.833 c	20.486 ab	0.147 b	3.808 ab	0.445 cd	11.622 abcd	1.217 c	31.290 abc	0.124 cde	3.170 bcde
		Merlot	0.643 f	20.077 abc	0.116 ef	3.153 abcd	0.464 bc	13.702 a	0.969 e	26.667 bcde	0.115 def	3.280 abc
	C1T1(W)	Shiraz	0.799 cd	19.808 abcd	0.134 cd	3.734 ab	0.447 cd	11.481 abcd	1.178 c	34.789 ab	0.140 b	3.633 ab
		Merlot	0.603 f	17.553 abcd	0.115 ef	3.006 abcde	0.453 bcd	12.656 abc	0.964 e	25.479 cde	0.113 ef	3.243 abcd

			N (g.100 g^{-1})	N (mg.vine^{-1})	P (g.100 g^{-1})	P (mg.vine^{-1})	K (g.100 g^{-1})	K (mg.vine^{-1})	Ca (g.100 g^{-1})	Ca (mg.vine^{-1})	Mg (g.100 g^{-1})	Mg (mg.vine^{-1})
NMF	C0T0(D)	Shiraz	0.963 efg	26.852 efg	0.203 bcd	6.199 fgh	1.472 b	35.974 def	1.729 ab	40.061 def	0.241 bcd	5.671 de
		Merlot	1.153 bc	21.831 g	0.166 ef	3.319 i	1.218 cd	24.384 g	1.412 defg	25.225 g	0.238 bcd	4.787 e
	C0T0(W)	Shiraz	1.084 cd	34.734 bcd	0.217 ab	9.129 cd	1.345 bc	44.260 bcd	1.804 ab	56.879 c	0.246 bc	7.810 b
		Merlot	0.973 efg	26.703 efg	0.177 de	5.111 h	1.108 de	33.120 efg	1.428 cdefg	38.047 defg	0.232 bcd	6.549 bcde
	C1T0(D)	Shiraz	1.069 d	32.908 bcde	0.211 abc	8.055 de	1.199 cd	40.218 cde	1.625 bcde	53.239 c	0.226 cd	7.530 bc
		Merlot	0.904 gh	26.955 efg	0.171 e	4.666 hi	0.980 ef	30.499 efg	1.301 gh	34.284 efg	0.213 cd	5.973 cde
	C1T0(W)	Shiraz	0.901 gh	37.631 b	0.182 cde	10.540 bc	1.055 de	48.373 bc	1.577 bcdef	71.893 b	0.215 cd	9.746 a
		Merlot	0.842 h	34.607 bcd	0.136 f	5.537 gh	0.784 g	34.972 def	1.014 i	38.181 defg	0.159 e	6.358 bcde
	C0T1(D)	Shiraz	1.342 a	36.774 b	0.218 ab	7.651 def	1.715 a	47.506 bc	1.894 a	50.311 cd	0.286 a	7.655 bc
		Merlot	1.188 b	23.984 fg	0.177 de	3.316 i	1.357 bc	28.961 fg	1.574 bcdef	28.572 fg	0.267 ab	5.291 e
	C0T1(W)	Shiraz	1.084 cd	50.605 a	0.239 a	14.245 a	1.468 b	67.773 a	1.887 a	85.772 a	0.238 bcd	10.714 a
		Merlot	1.006 def	29.527 def	0.177 de	5.434 gh	1.129 de	36.346 def	1.364 fgh	36.164 efg	0.216 cd	6.344 bcde
	C1T1(D)	Shiraz	1.061 d	36.382 bc	0.203 bcd	8.794 cd	1.216 cd	43.896 bcd	1.647 abcd	54.385 c	0.223 cd	7.548 bc
		Merlot	1.035 de	30.151 cdef	0.179 de	4.691 hi	1.083 de	30.283 efg	1.377 efgh	34.693 efg	0.207 d	5.628 de
	C1T1(W)	Shiraz	0.953 fg	45.191 a	0.192 bcde	11.826 b	1.101 de	51.867 b	1.675 abc	76.428 ab	0.234 bcd	10.558 a
		Merlot	0.925 g	35.200 bcd	0.168 e	6.936 efg	0.872 fg	38.364 cdef	1.136 hi	46.351 cde	0.171 e	7.158 bcd

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			N ($g.100 g^{-1}$)	N ($mg.vine^{-1}$)	P ($g.100 g^{-1}$)	P ($mg.vine^{-1}$)	K ($g.100 g^{-1}$)	K ($mg.vine^{-1}$)	Ca ($g.100 g^{-1}$)	Ca ($mg.vine^{-1}$)	Mg ($g.100 g^{-1}$)	Mg ($mg.vine^{-1}$)
LEAVES	C0T0(D)	Shiraz	2.091 b	98.580 efg	0.160 bcd	7.533 g	2.042 abc	87.210 h	1.307 abc	64.636 f	0.361 a	15.927 g
		Merlot	2.452 a	146.750 a	0.182 a	9.926 cde	2.183 a	134.850 de	1.408 a	79.363 def	0.328 bc	20.257 cdef
	C0T0(W)	Shiraz	1.567 e	110.060 cdef	0.148 def	10.763 bc	1.974 cd	130.510 de	1.198 cdef	89.792 bcd	0.326 bc	21.258 abcde
		Merlot	1.905 c	135.840 ab	0.144 defg	9.248 cdefg	1.934 cde	136.520 de	1.239 bcd	81.006 cdef	0.278 efg	19.036 defg
	C1T0(D)	Shiraz	1.394 fg	90.660 fg	0.138 efghi	8.604 defg	1.653 ghi	94.320 h	1.032 ghi	66.899 f	0.305 cde	17.598 efg
		Merlot	1.344 g	129.400 abc	0.140 efgh	10.549 c	1.837 def	167.360 bc	1.109 efgh	94.680 bcd	0.240 hij	21.017 bcde
	C1T0(W)	Shiraz	1.072 h	110.630 cdef	0.125 hij	12.584 ab	1.766 efg	144.980 d	1.020 ghi	101.191 b	0.282 defg	24.487 abc
		Merlot	1.093 h	126.240 abcd	0.122 ij	11.082 abc	1.569 hi	147.860 cd	0.990 hi	96.620 bc	0.218 j	21.687 abcd
	C0T1(D)	Shiraz	2.387 a	105.970 defg	0.170 ab	7.836 fg	2.063 abc	88.110 h	1.334 ab	64.989 f	0.351 ab	16.493 fg
		Merlot	2.453 a	128.950 abc	0.166 abc	7.631 g	2.059 abc	99.860 fgh	1.400 a	66.257 f	0.330 abc	16.592 fg
	C0T1(W)	Shiraz	1.736 d	114.580 bcde	0.146 def	9.952 cd	1.907 cde	118.720 efg	1.222 bcde	90.562 bcd	0.313 cd	21.087 bcde
		Merlot	1.886 c	130.770 abc	0.152 cde	9.603 cdef	2.003 bcd	131.590 de	1.341 ab	86.645 bcde	0.303 cdef	19.877 cdefg
	C1T1(D)	Shiraz	1.451 f	85.670 g	0.129 ghi	8.009 efg	1.715 fgh	96.890 gh	1.073 fghi	70.322 ef	0.271 fgh	18.923 defg
		Merlot	1.751 d	137.550 a	0.135 fghi	9.567 cdef	2.169 ab	175.830 b	1.142 defg	85.584 bcde	0.256 ghi	19.883 cdefg
	C1T1(W)	Shiraz	1.126 h	97.450 efg	0.112 j	9.980 cd	1.513 i	120.700 ef	0.976 i	88.314 bcd	0.257 ghi	23.118 abc
		Merlot	1.342 g	145.800 a	0.132 fghi	12.741 a	2.022 abc	225.660 a	1.128 defg	118.524 a	0.237 ij	25.188 a

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APPENDIX 1b. Comparison of the micro-nutrient concentration and approximate content of Shiraz and Merlot vines at 12 weeks after planting.

			Na ($mg.kg^{-1}$)	Na ($mg.vine^{-1}$)	Mn ($mg.kg^{-1}$)	Mn ($mg.vine^{-1}$)	Fe ($mg.kg^{-1}$)	Fe ($mg.vine^{-1}$)	Cu ($mg.kg^{-1}$)	Cu ($mg.vine^{-1}$)	Zn ($mg.kg^{-1}$)	Zn ($mg.vine^{-1}$)	B ($mg.kg^{-1}$)	B ($mg.vine^{-1}$)
OT	C0T0(D)	Shiraz	444.79 ab	0.705 c	19.94 bc	0.054 bc	435.06 fg	1.274 defghi	9.57 defg	0.012 e	39.21 abc	0.038 c	15.85 ef	0.037 def
		Merlot	392.37 cdef	1.015 abc	14.19 fgh	0.041 cde	384.09 g	0.812 i	9.09 g	0.025 abcd	28.77 ef	0.074 ab	14.93 f	0.038 def
	C0T0(W)	Shiraz	427.53 abcd	0.834 bc	15.20 efg	0.044 cde	467.74 efg	1.573 cdefg	10.64 abcdef	0.023 bcd	37.09 abcd	0.049 bc	17.37 abcde	0.047 bcd
		Merlot	416.16 bcde	1.332 a	13.04 ghi	0.042 cde	526.82 abcdef	1.379 defghi	9.12 g	0.031 ab	25.14 fg	0.080 a	18.79 a	0.059 a
	C1T0(D)	Shiraz	391.62 cdef	0.778 c	19.45 bc	0.054 bc	543.64 abcdef	1.698 bcdef	11.57 ab	0.022 cd	36.23 bcd	0.050 abc	18.42 ab	0.045 bcde
		Merlot	350.61 fg	1.028 abc	12.21 ghi	0.034 de	514.09 bcdef	1.208 efghi	10.29 bcdefg	0.033 a	18.00 h	0.053 abc	16.17 bcdef	0.040 cdef
	C1T0(W)	Shiraz	383.11 def	1.0.906 bc	19.51 bc	0.067 ab	622.37 ab	2.577 a	11.16 abc	0.027 abc	32.11 de	0.047 bc	18.23 abc	0.055 ab
		Merlot	323.96 g	1.058 abc	10.80 i	0.029 e	480.15 defg	1.282 defghi	8.80 g	0.029 abc	16.50 h	0.055 abc	15.97 def	0.045 bcde
	C0T1(D)	Shiraz	469.52 a	1.002 abc	26.30 a	0.078 a	606.19 abc	2.143 abc	12.09 a	0.027 abc	43.75 a	0.057 abc	18.27 abc	0.051 abc
		Merlot	467.21 a	0.978 abc	16.04 def	0.032 e	634.25 a	1.153 fghi	9.13 fg	0.021 cd	21.88 gh	0.049 bc	17.37 abcde	0.031 f
	C0T1(W)	Shiraz	448.16 ab	0.997 abc	17.85 cde	0.056 bc	561.03 abcde	2.263 ab	11.71 ab	0.028 abc	37.83 abcd	0.057 abc	18.20 abcd	0.053 ab
		Merlot	374.77 efg	0.868 bc	13.25 fghi	0.031 e	620.59 ab	1.064 ghi	9.75 cdefg	0.026 abcd	23.16 fgh	0.055 abc	16.05 cdef	0.035 ef
	C1T1(D)	Shiraz	419.16 abcde	0.751 c	21.00 b	0.052 bcd	588.5 abcd	1.769 bcde	10.98 abcd	0.018 de	39.87 ab	0.048 bc	17.70 abcde	0.041 cdef
		Merlot	408.96 bcde	0.868 bc	13.33 fghi	0.033 e	477.9 defg	0.945 hi	9.46 efg	0.026 abcd	25.27 fg	0.060 abc	17.37 abcde	0.037 def
	C1T1(W)	Shiraz	436.26 abc	0.921 bc	18.51 bcd	0.052 bcd	534.59 abcdef	1.868 bcd	10.66 abcde	0.022 cd	33.03 cde	0.046 bc	19.25 a	0.050 abc
		Merlot	380.41 def	1.200 ab	11.84 hi	0.040 cde	499.11 cdefg	1.426 defgh	9.76 cdefg	0.033 a	22.51 fgh	0.075 ab	16.05 cdef	0.050 abc

			Na (mg.kg^{-1})	Na (mg.vine^{-1})	Mn (mg.kg^{-1})	Mn (mg.vine^{-1})	Fe (mg.kg^{-1})	Fe (mg.vine^{-1})	Cu (mg.kg^{-1})	Cu (mg.vine^{-1})	Zn (mg.kg^{-1})	Zn (mg.vine^{-1})	B (mg.kg^{-1})	B (mg.vine^{-1})
OMF	C0T0(D)	Shiraz	564.08 b	0.892 e	20.08 bcd	0.039 cd	959.39 ab	2.217 bcd	13.54 d	0.026 d	48.48 a	0.058 bc	21.95 de	0.038 c
		Merlot	469.61 de	1.721 a	16.74 efg	0.042 cd	666.93 de	1.669 cd	15.47 cd	0.037 bc	31.89 de	0.074 abc	20.67 def	0.056 a
	C0T0(W)	Shiraz	578.77 ab	1.040 cde	17.09 ef	0.045 cd	1023.05 ab	3.554 ab	17.36 abc	0.032 cd	34.62 cde	0.071 abc	24.49 ab	0.051 abc
		Merlot	436.00 efg	1.518 ab	17.01 ef	0.041 cd	649.17 e	1.569 cd	16.09 bcd	0.039 bc	24.61 hi	0.053 bc	21.21 def	0.053 abc
	C1T0(D)	Shiraz	453.04 def	0.959 de	18.46 bcde	0.048 bcd	983.88 ab	2.825 ab	17.24 abc	0.040 bc	29.44 efgh	0.065 abc	21.14 def	0.051 abc
		Merlot	386.48 h	1.377 abc	14.53 fg	0.037 d	563.50 e	1.439 d	15.31 cd	0.036 bc	23.68 i	0.050 c	19.69 f	0.051 abc
	C1T0(W)	Shiraz	469.86 de	0.928 de	17.87 cde	0.052 abcd	916.26 bc	3.594 a	18.04 abc	0.040 bc	31.66 de	0.072 abc	23.84 bc	0.054 abc
		Merlot	387.60 gh	1.264 bcde	14.83 fg	0.036 d	633.76 e	1.581 cd	15.80 bcd	0.038 bc	25.47 ghi	0.054 bc	21.11 def	0.055 abc
	C0T1(D)	Shiraz	615.18 a	1.291 bcd	23.73 a	0.068 a	1134.47 a	4.048 a	18.11 abc	0.038 bc	40.08 b	0.072 abc	23.98 abc	0.049 abc
		Merlot	542.3 bc	1.177 bcde	18.21 bcde	0.036 d	684.05 de	1.303 d	15.15 cd	0.026 d	34.42 cde	0.055 bc	22.10 d	0.039 bc
	C0T1(W)	Shiraz	540.64 bc	1.342 abc	20.97 ab	0.065 ab	994.40 ab	4.116 a	19.46 a	0.054 a	36.41 bcd	0.093 a	24.76 ab	0.062 a
		Merlot	418.07 fgh	1.243 bcde	17.31 def	0.041 cd	726.58 cde	1.741 cd	16.16 bcd	0.040 bc	30.89 ef	0.068 abc	22.32 cd	0.054 abc
	C1T1(D)	Shiraz	497.36 cd	1.233 bcde	20.18 bc	0.049 bcd	852.61 bcd	3.671 a	16.16 bcd	0.039 bc	38.76 bc	0.079 ab	22.18 d	0.058 a
		Merlot	429.58 efgh	1.490 ab	15.01 fg	0.042 cd	636.34 e	1.828 cd	14.99 cd	0.042 b	29.86 efg	0.077 abc	20.41 ef	0.055 ab
	C1T1(W)	Shiraz	533.48 bc	1.258 bcde	19.51 bcde	0.056 abc	933.91 ab	3.903 a	19.00 ab	0.043 b	30.48 efg	0.074 abc	25.51 a	0.066 a
		Merlot	405.76 fgh	1.387 abc	14.15 g	0.041 cd	560.44 e	1.696 cd	15.63 cd	0.045 ab	25.87 fghi	0.064 abc	20.36 ef	0.060 a

			Na (mg.kg^{-1})	Na (mg.vine^{-1})	Mn (mg.kg^{-1})	Mn (mg.vine^{-1})	Fe (mg.kg^{-1})	Fe (mg.vine^{-1})	Cu (mg.kg^{-1})	Cu (mg.vine^{-1})	Zn (mg.kg^{-1})	Zn (mg.vine^{-1})	B (mg.kg^{-1})	B (mg.vine^{-1})
NMF	C0T0(D)	Shiraz	788.94 c	1.807 cde	26.54 def	0.070 ef	1623.80 bcd	3.652 d	57.84 bcd	0.135 fghi	21.50 bcd	0.064 fgh	26.26 abcd	0.066 fg
		Merlot	821.16 bc	1.618 e	32.95 abc	0.065 f	1849.30 ab	3.641 d	50.20 d	0.102 hi	24.65 ab	0.040 h	27.29 abc	0.055 g
	C0T0(W)	Shiraz	671.9 d	2.018 cde	23.89 f	0.085 def	1742.80 abc	5.494 bcd	72.04 a	0.234 c	23.74 abc	0.097 cde	26.54 abcd	0.092 cde
		Merlot	664.70 d	1.905 cde	37.85 a	0.108 bcd	1530.00 bcd	3.783 d	56.88 cd	0.161 efgh	24.11 ab	0.057 fgh	26.78 abcd	0.079 ef
	C1T0(D)	Shiraz	666.14 d	2.176 bcd	28.92 cdef	0.085 def	1685.80 abc	5.484 bcd	58.18 bcd	0.185 cdefg	23.77 abc	0.108 bcd	28.06 abc	0.098 cd
		Merlot	566.70 def	1.817 cde	35.14 ab	0.072 ef	1621.10 bcd	4.073 d	50.69 d	0.133 fghi	34.32 ab	0.052 gh	22.83 d	0.066 fg
	C1T0(W)	Shiraz	509.42 efg	2.179 bcd	23.90 f	0.123 abc	1400.70 bcd	7.551 ab	70.02 ab	0.341 ab	18.23 d	0.134 ab	23.00 d	0.107 bc
		Merlot	420.31 g	1.736 de	29.92 bcde	0.096 cde	1220.00 d	4.606 cd	55.75 cd	0.221 cde	19.96 bcd	0.064 fgh	17.48 e	0.070 fg
	C0T1(D)	Shiraz	956.88 a	2.585 ab	30.66 bcd	0.093 def	1791.10 ab	5.499 bcd	59.06 bcd	0.170 defg	21.52 bcd	0.076 efg	28.55 ab	0.081 def
		Merlot	908.12 ab	1.771 de	28.57 cdef	0.066 f	2109.9 a	4.022 d	52.38 d	0.094 i	28.40 a	0.046 gh	29.31 a	0.057 g
	C0T1(W)	Shiraz	665.94 d	2.990 a	28.36 cdef	0.148 a	1453.10 bcd	8.577 a	75.29 a	0.391 a	20.00 bcd	0.147 a	26.90 abcd	0.127 a
		Merlot	608.54 de	1.593 e	25.09 ef	0.088 def	1744.40 abc	4.853 cd	56.77 cd	0.168 defg	24.16 ab	0.055 gh	24.40 cd	0.066 fg
	C1T1(D)	Shiraz	643.90 d	2.310 bc	24.71 ef	0.071 ef	1498.20 bcd	4.377 d	57.20 cd	0.195 cdef	20.75 bcd	0.088 def	25.21 abcd	0.099 cd
		Merlot	660.23 d	1.703 de	29.97 bcde	0.080 def	1719.60 abc	4.839 cd	49.37 d	0.130 ghi	23.58 abc	0.054 gh	24.47 bcd	0.067 fg
	C1T1(W)	Shiraz	579.71 de	2.655 ab	25.04 ef	0.124 abc	1448.30 bcd	7.069 abc	65.38 abc	0.325 b	18.81 cd	0.125 abc	24.23 cd	0.121 ab
		Merlot	456.08 fg	2.024 cde	31.20 bcd	0.126 ab	1288.70 cd	5.060 bcd	54.89 cd	0.229 cd	21.81 bcd	0.077 efg	18.68 e	0.082 def

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			Na ($mg.kg^{-1}$)	Na ($mg.vine^{-1}$)	Mn ($mg.kg^{-1}$)	Mn ($mg.vine^{-1}$)	Fe ($mg.kg^{-1}$)	Fe ($mg.vine^{-1}$)	Cu ($mg.kg^{-1}$)	Cu ($mg.vine^{-1}$)	Zn ($mg.kg^{-1}$)	Zn ($mg.vine^{-1}$)	B ($mg.kg^{-1}$)	B ($mg.vine^{-1}$)
LEAVES	C0T0(D)	Shiraz	133.65 c	0.614 fg	52.36 ab	0.261 abcd	367.37 a	1.080 bcde	8.05 a	0.032 def	51.99 de	0.280 cd	51.89 cd	0.298 hi
		Merlot	214.74 a	1.256 b	48.62 bcd	0.268 abcd	216.30 cde	1.016 bcdef	8.21 a	0.039 bcd	93.40 a	0.265 d	51.13 cd	0.273 i
	C0T0(W)	Shiraz	125.91 c	0.916 de	39.30 efg	0.296 abc	301.11 b	1.363 ab	6.99 b	0.042 abc	43.25 g	0.389 ab	49.57 d	0.393 def
		Merlot	177.86 b	1.183 bc	37.74 efg	0.239 cd	259.97 bc	1.315 b	6.81 b	0.034 cde	83.61 b	0.244 de	43.13 ef	0.278 i
	C1T0(D)	Shiraz	81.11 d	0.525 g	33.91 ghi	0.271 abcd	134.30 ghi	0.743 ef	3.03 g	0.022 g	33.34 h	0.273 cd	42.93 ef	0.305 ghi
		Merlot	181.88 b	1.333 b	35.98 fgh	0.253 bcd	168.54 efgh	1.133 bcd	4.52 cd	0.044 ab	31.77 h	0.224 def	40.77 fg	0.303 ghi
	C1T0(W)	Shiraz	87.35 d	0.798 def	26.99 i	0.315 ab	117.48 i	1.021 bcdef	2.85 g	0.031 def	25.26 i	0.332 bc	42.66 ef	0.434 bcd
		Merlot	187.45 b	1.589 a	29.05 hi	0.237 cd	199.25 def	1.713 a	3.98 def	0.049 a	27.80 hi	0.220 def	36.14 g	0.304 ghi
	C0T1(D)	Shiraz	127.41 c	0.505 g	57.83 a	0.291 abc	261.02 bc	0.672 f	6.39 b	0.028 efg	57.65 cd	0.194 ef	61.97 a	0.374 ef
		Merlot	217.33 a	0.960 cd	50.22 bc	0.212 d	287.34 b	1.287 bc	8.07 a	0.037 bcd	58.15 c	0.337 bc	59.76 ab	0.257 i
	C0T1(W)	Shiraz	119.71 c	0.695 efg	42.59 def	0.322 a	215.34 cde	0.926 cdef	4.76 c	0.031 def	46.11 fg	0.223 def	55.14 bc	0.476 ab
		Merlot	221.84 a	1.337 b	44.06 cde	0.256 bcd	226.77 cd	1.344 b	7.00 b	0.042 abc	50.35 ef	0.412 a	59.31 ab	0.359 efg
	C1T1(D)	Shiraz	93.17 d	0.501 g	38.95 efg	0.304 ab	183.10 defg	0.680 f	4.44 cde	0.025 fg	32.10 h	0.165 f	51.83 cd	0.405 cde
		Merlot	177.04 b	1.282 b	38.91 efg	0.268 abcd	152.39 fghi	0.918 cdef	4.74 c	0.028 efg	33.17 h	0.167 f	48.67 d	0.337 fgh
	C1T1(W)	Shiraz	90.9 d	0.757 def	27.33 i	0.286 abc	149.45 ghi	0.804 def	3.39 fg	0.028 efg	24.83 i	0.194 ef	48.74 d	0.527 a
		Merlot	174.32 b	1.735 a	32.49 ghi	0.305 ab	120.49 hi	0.918 cdef	3.87 ef	0.034 de	30.22 hi	0.189 ef	47.29 de	0.463 bc

Values in the same column followed by the same letter do not differ significantly ($p \leq 0.05$). (D): Dry; (W): Wet; OT: Old thick roots; OMF: Old medium and fine roots; NMF: New medium and fine roots

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CHAPTER 6: INTEGRATIVE EFFECTS OF CLIMATE CHANGE FACTORS (TEMPERATURE, CO_2 AND WATER) ON METABOLITE TRANSPORT AND ACCUMULATION IN YOUNG, GRAFTED GRAPEVINES

ABSTRACT:

The metabolite translocation and accumulation patterns were investigated in young, potted grapevines at 4 weeks and 12 weeks after planting. The effect of different combinations of ambient temperature (maximum ranges of 27-31 °C compared to 30-34 °C), ambient CO_2 (400 ppm *vs* 800 ppm) and soil water (irrigation to water-holding capacity and 50 % thereof), applied immediately after planting, on the synthesis, transport and accumulation of metabolites in young vines was investigated under glasshouse conditions. Two scion cultivars (Shiraz and Merlot), both grafted onto 101-14 Mgt, were used in this study. Absolute differences between the cultivars were found, but the direction of response to treatment combinations was similar. Elevated CO_2 levels and adequate water supply resulted in higher leaf hexose and organic acid contents in the vines. Decreased concentrations were ascribed to the attenuation effect of higher vegetative growth. Starch accumulation in roots and shoots commenced between 4 and 8 weeks after planting, after the rate of primary shoot growth decreased. Differences in reserve storage between root classes were found that indicated the respective importance of old thick, old medium-fine and new medium-fine roots in any root system. The level of water supply was the environmental factor that had the largest effect on the total phenolic index of roots, while CO_2 was the dominant factor in leaves. Grapevine metabolism and dynamics of translocation and assimilation (as referred to in this study) were not significantly affected by the treatment combinations and increased physiological activity and growth of young vines may be expected as long as water and nutrient supply remain adequate.

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6.1 INTRODUCTION

At the beginning of the growth season, the grapevine is dependent on nitrogen (N) and carbon (C) reserves accumulated in especially the roots and the rest of the perennial structure to support initial metabolism and growth. Between bud break and flowering, the growing shoots and leaves are the strongest sinks for C and N, resulting in remobilisation of reserves from mainly the roots, trunk (rootstock and scion part, as well as cordon/s) to the above-ground growth (Conradie, 1992; Mullins et al. 1992; Bates et al. 2002). This translocation of reserves to the shoots reaches a maximum at the 8-10 leaf stage (Yang & Hori, 1979) after which the rate decreases when the shoots become self-sufficient. All photosynthetic products of the young leaves are however allocated to the strong localised and adjacent sinks (Hunter et al. 1994), with the result that sucrose export to the roots is limited. The root system also experiences a growth flush around flowering (Van Zyl, 1984; Callejas et al. 2009), but growth of the new roots is mainly supported by root C (Bates et al. 2002) and N (Araujo & Williams, 1988) reserves. The demand for N by the new growth in the vine between budding and flowering is higher than the uptake ability of the roots, resulting in remobilisation of N reserves from the roots and perennial structure to make up for the difference (Conradie, 1986).

The replacement of reserves in the roots only occurs after flowering around the end of rapid shoot growth (Bates et al. 2002; Hunter et al. 1995), when canopies are at maximum activity and able to provide to all the various sinks in the vine. During this time, roots are also able to provide in the N requirement of the vine, avoiding further depletion of the N reserve pool (Conradie, 1986). From this stage onwards until leaf fall, roots cease to be a major source to the rest of the vine, with the exception of stress conditions when remobilisation of root reserves is essential for sustaining growth and maintenance to ensure the survival of the vine (Mullins et al. 1992; Keller et al. 1995).

Plants are known to accumulate phenolics in tissues in response to adverse conditions and shift from primary to secondary metabolism under conditions of both biotic and abiotic stress (Lattanzio, 2013). Secondary metabolites have important defensive, protective and signalling functions in plants (Griesser et al. 2015). Phenylalanine (PAL), produced during the shikimic-acid pathway, is the common precursor of proteins and phenolic acids (Hättenschwiler & Vitousek, 2000). This explains why the energy intensive shift to the production of phenols may be detrimental to vegetative growth (Lattanzio, 2013). The secondary metabolites, such as polyphenols, serve as anti-oxidants that scavenge the reactive oxygen species (ROS) produced during stress conditions (Lattanzio, 2013) and thereby protect the photosynthetic system against photo-damage. The difference in the combination of secondary metabolites in the plant is dependent on genetic expression (Lattanzio, 2013) as well as the type, intensity and duration of the stress (Król et al. 2014). Griesser et al. (2015) found that a few metabolites increased during a short period of drought stress, while the increase in all polyphenols tested were only

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induced in the prolonged drought stress treatment (8 days without water) in grapevine leaves. In contrast, Król et al. (2014) found a reduction in total phenolic compounds in grapevine leaves and roots in reaction to prolonged water stress (soil moisture at 30 % for two weeks).

After cessation of shoot growth, both the production of photosynthetates and the utilisation thereof generally decrease in the grapevine (Hunter & Visser, 1990). Grape ripening and shoot lignification occur simultaneously, with an increase in starch in all perennial structures and fine roots from véraison onwards (Bates et al. 2002). The starch concentration in leaves also increases from véraison, reaching maximum levels after harvest (Hunter et al. 1995). Even though starch accumulate in roots during the season from about berry set, the low levels at harvest indicate the importance of the post-harvest period for accumulation of reserves (Bates et al. 2002). According to Conradie (1980) N uptake by the grapevine decreases or even ceases around berry ripeness, while the post-harvest uptake of N occurs at a relatively high rate until leaf fall (Conradie, 1986). In general, the relative long period of time between harvest and leaf fall in warmer climates should be beneficial to reserve accumulation, especially since a linear relationship was found between leaf N content and the CO_2 assimilation rate at this stage (Hunter & Ruffner, 1997). The remobilisation and export of leaf nutrients such as C and N out of old leaves just before leaf fall may further contribute to the total reserve pool (Conradie, 1986; Hunter et al. 1995).

Atmospheric C is fixated in the plant during the carboxylation of RuBP in the Calvin cycle. One of the intermediates, glyceraldehyde-3-phosphate, may either be converted to sucrose in the cytoplasm; incorporated into starch in the chloroplast or kept in the Calvin cycle for the regeneration of RuBP (Wardlaw, 1990; Mulllins et al. 1992). Sucrose and starch are thus products of different metabolic pathways where the synthesis of one occurs at the expense of the other (Huber, 1983).

Especially during the active vegetative stage in the first part of the season, high levels of sucrose synthase are found in the leaves (Hunter et al. 1994). This enzyme catalyses the reversible splitting of sucrose into fructose and glucose forms, either uridine diphosphate glucose (UDP-G) or adenosine diphosphate glucose (ADP-G) and is mainly active in growing tissues (Stein & Granot, 2019). Sucrose is the main form of carbohydrate transport in the grapevine (Wardlaw, 1990) and is actively loaded into the phloem to be transported to sink tissues. High levels of invertase are present in actively growing parts (Hunter et al. 1994) that would irreversibly hydrolyse the sucrose into its hexose monomers, glucose and fructose (Stein & Granot, 2019). Leaf glucose and fructose concentrations increase sharply between the second and third weeks after budding (Kliewer & Nassar, 1966) and remain higher than sucrose until berry set. After that, sucrose increases relative to the hexose sugars and becomes the main sugar in the leaves (Kliewer & Nassar, 1966; Hunter et al. 1994). It remains at a constant level until senescence (Hunter & Ruffner, 2001). The degree of sucrose loading and translocation is strongly related to the sink strength (Hunter et al. 1995) and should the latter decrease, sucrose would accumulate in the leaves (Hunter et

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al. 1994). This would cause the leaf to switch to the production of starch (Huber; 1983; Mullins et al. 1992). This storage of C may serve as a temporary buffer against short term changes/inhibition in photosynthetic output, to be remobilised when needed (Wardlaw, 1990).

In any growing tissue, the utilisation of sucrose is dependent on the provision of amino acids for growth (Stitt & Krapp, 1999). Nitrogen is mainly absorbed as nitrate by the grapevine (Keller et al. 1995), which is an active process. The energy and C skeletons required for N assimilation are mainly obtained from the sugars that migrate from the chloroplast to the cytoplasm (Klepper et al. 1971). Thus C is removed from mainly the sucrose synthesis pathway for the production of nucleic acids, amino acids, protein and organic acids, such as malate (Stitt & Krapp, 1999). The first step of N assimilation is the reduction of nitrate to nitrite by nitrate reductase (NR). The activity of NR may be used to evaluate the nutrient status of the vine (Hunter & Ruffner, 1997) and is stimulated by the presence of a nitrate substrate.

Since nitrate reductase, sucrose synthase and invertase are regulated by products of photosynthesis (amino acids and sucrose), it may be expected that their activity is coordinated with each other as well as with the rate of photosynthesis (Hunter & Ruffner, 1997). In growing tissues, the utilisation of sucrose depends on the concurrent provision of amino acid (Stitt & Krapp, 1999). Should the N be limited, the sucrose might accumulate and revert to starch synthesis (Wardlaw, 1990), or starch synthesis may directly be enhanced by the absence of nitrate (Stitt & Krapp, 1999). In photosynthesizing leaves, limited N supply is associated with a general decrease in leaf protein (including RubisCO) (Stitt & Krapp, 1999), while the N in the older, less active leaves may be remobilised and allocated to young roots and leaves to support their growth (Mullins et al. 1992; Keller, 2005). When carbohydrates are in short supply, N assimilation is limited, which will result in decreased synthesis of amino and organic acids as well as protein (Stitt & Krapp (1999). The relationships between the rate of photosynthesis and the various respective concentrations of the non-structural carbohydrates (NSC) and N in the vine may be used to make possible deductions regarding the source:sink balances in the vine and help better understand the effect of the environment on the translocation and assimilation of metabolites.

Based on climatic modelling (Webb et al. 2007; 2013; Hall & Jones, 2009; Hunter & Bonnardot, 2011; Fraga et al. 2016) as well as real-time climatic data (Jones et al. 2005; Jones, 2007; Koch & Oehl, 2018), the current climatic conditions will be different in future. The general consensus is that vineyards will be exposed to elevated CO_2, increased temperature and limited water availability in future (Morales et al. 2016), combined with an increased frequency in extreme temperature and rainfall conditions (Jones, 2007).

Water deficit decreases stomatal gaseous exchange and therefore photosynthesis (Flexas & Medrano, 2002; Martínez & Chacon, 2010; Dayer et al. 2013; Zufferey, 2013) with an associated decrease in vegetative growth (Palliotti et al. 2008). It may also limit the uptake of minerals from the soil, which

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could have a direct impact on plant growth and functioning, e.g. P plays an important role in exchanging triose-P for inorganic phosphate during the dark phase of photosynthesis (Thuynsma et al. 2016); K is involved in the osmoregulation of stomatal guard cells and together with Mg are involved in protein (and thus RubisCO) synthesis, whereas Mg is functional in chlorophyll synthesis and RubisCO activation (Tränkner et al. 2018); N forms part of proteins and nucleic acids and thus plays and integral role in plant structures, photosynthesis, metabolic pathways and the storage and translation of genetic information (Novoa & Loomis, 1981).

Elevation of ambient CO_2 levels increases the rate of photosynthesis (Long et al. 2004; Moutinho-Pereira et al. 2009; Pan et al. 2018) and enhances both vegetative and reproductive growth (Norby et al. 1986; Bindi et al. 2001; Long et al. 2004). A temperature increase up to 30 ^oC enhanced photosynthesis (Greer & Weedon, 2012), whereas at higher temperatures it started to decrease again. When high CO_2 and temperature were combined, the higher photosynthetic rate with an increase in temperature was further enhanced (Alonso et al. 2008; Edwards et al. 2017), while the optimal temperature range for photosynthesis increased with a simultaneous increase in ambient CO_2 (Kriedemann et al. 1976; Long, 1991; Alonso et al. 2008; Greer & Weedon, 2012). However, acclimation of photosynthesis is often mentioned with longer term plant exposure to high CO_2 levels (Leibar et al. 2015; Salazar-Parra et al. 2012, 2015; Martínez-Lüscher et al. 2015). Acclimation occurs when the photosynthetic properties of leaves that developed at elevated CO_2 are different in comparison to those that developed at the current ambient CO_2 (Drake et al. 1997). When acclimatised plants are put in an ambient (400 ppm) CO_2 environment, the photosynthetic rate would be lower than control plants that grew in the lower CO_2 from the start. Acclimation in plants is often indicated by high levels of leaf starch content (provided that N is not limiting), a decrease in leaf protein and RubisCO content, as well as lower carboxylation capacity and activity (Drake et al. 1997; Moore et al. 1999; Long et al. 2004; Aranjuelo et al. 2008). Photosynthetic acclimation is commonly associated with the inability of plants to acquire sufficient nitrogen (Alonso et al. 2009) and decreased leaf N concentration is generally found where plants are grown under elevated CO_2 (Norby et al. 1986; Moutinho-Pereira et al. 2009; Morales et al. 2016). This may either indicate that the rate of uptake by the roots was not sufficient to meet the higher demand for N by the increased growth, or that the growth medium used in the studies contained insufficient nutrients to support enhanced growth. Geiger et al. (1999) stated that many of the effects on photosynthate allocation, photosynthetic acclimation and growth ascribed to elevated CO_2 are rather due to a shift in the nitrogen status of the plant. This is supported in the review of Stitt & Krapp (1999) who claimed that when N supply is adequate, photosynthetic rates do not decline over the longer term in high CO_2 conditions, with no decrease in the internal N concentration or levels of N metabolites in the plants and the stimulating effect on growth is sustained.

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It is thus important to distinguish between the direct effects of elevated CO_2 and the concomitant increase in photosynthates on the plant, and the indirect effects of a possible N limitation that may occur due to the increased plant growth. This will *inter alia* entail a detailed analysis of both the organic and inorganic fractions of N in the plant over time, which falls outside the scope of this study.

In this part of the study the combined effect of projected climate change conditions (high ambient temperature, elevated CO_2 and water deficit) on the transport and accumulation of metabolites in young, grafted grapevines during the first 12 weeks after planting was measured under controlled conditions in glasshouse compartments. This is a novel approach to gain a better understanding of how young vines would function and grow (at leaf, root and whole-plant level) under future climates during the very important young vineyard establishment stage.

6.2 MATERIALS AND METHODS

6.2.1 Study location and glasshouse compartments

Four glasshouse rooms situated at ARC Infruitec-Nietvoorbij, Stellenbosch, were used to accommodate the different treatments. The rooms were 2.4 m X 6.0 m each and prepared according to the treatment criteria explained in detail in Chapter 3 and summarised in Table 6.1. The experiment comprised of five consecutive growth cycles (planting times during the first week of February and the first week of September), using Shiraz (SH 470) as scion cultivar for the first three, and Merlot noir (MO 348) for the other two. Both scions were grafted onto rootstock 101-14 Mgt. The potted vines were randomly allocated per glasshouse compartment in a randomised complete block design. There were 108 vines per room (54 per irrigation treatment) and thus 432 vines were used for each growth cycle.

Table 6.1 Treatment combinations randomly allocated in four glasshouse compartments for five growth cycles.

PARAMETER	TREATMENTS											
	C0T0			C1T0			C0T1			C1T1		
Vine age (weeks)	4	8	12	4	8	12	4	8	12	4	8	12
CO_2 levels (ppm)	400	400	400	800	800	800	400	400	400	800	800	800
Temperature (°C)	15/27	15.5/29	16/31	15/27	15.5/29	16/31	18/30	18.5/32	19/34	18/30	18.5/32	19/34
Water treatments	Wet: WC Dry: $\frac{1}{2}$WC	Wet: WC Dry: $\frac{1}{2}$WC	Wet: WC Dry: $\frac{1}{2}$WC	Wet: WC Dry: $\frac{1}{2}$WC	Wet: WC Dry: $\frac{1}{2}$WC	Wet: WC Dry: $\frac{1}{2}$WC	Wet: WC Dry: $\frac{1}{2}$WC	Wet: WC Dry: $\frac{1}{2}$WC	Wet: WC Dry: $\frac{1}{2}$WC	Wet: WC Dry: $\frac{1}{2}$WC	Wet: WC Dry: $\frac{1}{2}$WC	Wet: WC Dry: $\frac{1}{2}$WC

C0: Lower CO_2 (400 ppm); C1: Higher CO_2 (800 ppm); T0: Lower temperature; T1: Higher temperature (T0 max + 3°C); WC: Water-holding capacity

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6.2.1.1 Potting soil

The soil used for each growth cycle was obtained from the same fallow vineyard site in Robertson, South Africa, and transported to the experiment location. Each plastic planting pot (25 cm diameter; roughly 7.2 L) was provided with a Bidim-layer at the bottom before the vine was planted in 6.50 kg of soil. The soil was a sandy clay loam with a high pH (Chapter 3). Soils were analysed before each planting date by a SANAS Accredited Testing Laboratory, in accordance with ISO 17025:2005 (more details were provided in Chapter 5). No additional nutrients were provided to the young, growing vines for the duration of the study.

6.2.1.2 Grafted vines

Vines were obtained from a SAPO (South African Plant Improvement Organisation) accredited nursery in the Wellington/Paarl region. Shiraz (SH 470), grafted onto rootstock 101-14 Mgt, was used for the first three growth cycles and Merlot noir (MO 348), grafted onto the same rootstock, for the last two cycles.

Before planting, vines were pruned back to two buds and roots (only those originating from the basal node were kept) cut to a length of 10 cm. Shoot removal and weed control were continuously done during the growth cycles to ensure optimal growth of the vines under the respective growth conditions. Primary shoot tips were not removed and all developing secondary shoots were allowed to grow.

6.2.2 Measurements

The plant material of six vines was combined for each of the three replications of each treatment combination at the respective times of sampling. All the primary leaves on a shoot (from the basal to the apical part) were combined to obtain the leaf samples. Roots were separated into old (dark in colour and suberized) and new (soft and light brown to white in colour) roots. Each group was further separated into thick (> 2.0 mm in diameter), medium (0.5 - 2.0 mm) and fine (< 0.5 mm) roots before analysis. Three representative samples (2 g fresh mass) of leaves were sent to an accredited analytical laboratory for nitrogen analysis. The rest of the plant material in the leaf and root samples was freeze-dried for a week in a CHRIST BETA 1-8 freeze-dryer [Wirsam Scientific & Precision Equipment Pty (Ltd), Cape Town, South Africa]. Leaf samples were analysed after 4 and 12 weeks of growth for their sugar (glucose and fructose) and acid (tartaric and malic acid) contents. Starch analysis of the rootstock trunk, shoots and roots (in their separate classes) was done at 4, 8 and 12 weeks after planting. The respective total nutrient contents per Old Thick (OT), Old Medium-Fine (OMF) and New Medium-Fine (NMF) root classes and leaves were also calculated by using the average dry mass per fraction for each.

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Photosynthesis

Photosynthetic rate of basal leaves on primary shoots (nodes 2-4; one leaf per vine) was measured at midday at each of the three sampling dates using a LI-6400 portable photosynthesis system (LI-COR Biosciences, Lincoln, Nebraska, United States). The PAR of the LED light source (6400-02B) was set at 350 µmol.m^{-2}.s^{-1}. During the measurements, CO_2 levels in the sample chamber were controlled by means of a CO_2 injector system (6400-01) according to the treatment criteria and a constant flow rate of 300 µmol.s^{-1} was applied.

6.2.2.1 Laboratory analysis

Leaf N

Each leaf sample was washed with a Teepol solution, rinsed with de-ionised water and dried overnight at 70 °C in an oven. The dried leaves were then milled and ashed at 480 °C, shaken up in a 50:50 HCl (32 %) solution for extraction through filter paper (Campbell & Plank, 1998; Miller, 1998). Total N content of the ground leaves was determined through total combustion in a Leco Truspec® CN N-analyser (Leco Africa, Kempton Park, South Africa).

Starch

An adapted method of Hunter et al. (1995) was used for determination of the starch concentration of plant tissues. Since the free glucose in the sample was not required for further analyses, soluble sugars were not first extracted from the sample, as described in the reference method. A volume of 550 mm^3 of water was added to 50 mg of the freeze-dried sample, vortexed for 10 s and sonicated for 10 min. Samples were then centrifuged (10 min at 12 000 rpm) and a 50 mm^3 aliquot was removed as control. Gelatinisation of starch was induced by heating samples in a water bath with boiling water, where after 500 mm^3 of an enzyme mix [10 000 U α-amylase and 4000 U amylo-glucosidase added to a 0.1 M Na-acetate buffer (9.57 g $NaAc.3H_2O$ and 1.776 cm^3 CH_3COOH made up to 1 000 cm^3 with water and pH adjusted to 5.0)] was added to each sample. Samples were vortexed (10 s) and incubated at 40 °C (with shaking at 35 rpm) for 3 hours to hydrolyse the starch. They were then removed and shaken every 30 min. After this, samples were centrifuged (10 min at 12 000 rpm) and diluted. Controls were not incubated. The amount of glucose generated from starch was determined by using an ABTS [2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)] reagent, containing 13.8 g $Na_2HPO_4.2H_2O$, 6.4 g $NaH_2PO_4.H_2O$, 9 400 U glucose oxidase, 1 500 U peroxidase and 0.5 g ABTS reagent per 1 000 cm^3 water. A 50 mm^3 aliquot of the diluted sample was mixed with 950 mm^3 of the reagent. Absorbance was read at 420 nm, using a Cary 60 UV-Vis spectrophotometer (Agilent Technologies, South Africa). A standard curve was drawn, using 0, 0.625, 1.25, 2.5, 5 and 10 mg glucose.100 cm^{-3} standards.

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The equations used for the determination of starch are:

Control value:

$$\frac{A420}{x\ quotient\ of\ linear\ regression\ line\ through\ (0;0)} \times dilution\ factor\ of\ control \times \left(\frac{0.55}{100}\right) \times \frac{1}{sample\ mass}$$

Sample value:

$$\frac{A420}{x\ quotient\ of\ linear\ regression\ line\ through\ (0;0)} \times dilution\ factor\ of\ sample \times \frac{1}{100} \times \frac{1}{sample\ mass}$$

Starch (mg.g^{-1} dry mass) = (Sample value – Control value) x 0.9 (due to reduced molecular weight of glucose in the polymer)

Total phenolic index (TPI)

The method according to Ribéreau-Gayon et al. (2006) was used. A representative sample of 2 g fresh plant material was mixed with 40 cm^3 of buffer solution [5 g tartaric acid, 22 cm^3 1 N sodium hydroxide (NaOH), 2 g sodium metabisulfite ($Na_2S_2O_5$) and 120 cm^3 ethanol made up with deionized water to 1000 cm^3 and pH adjusted to 3.2] and then incubated at 30 °C for 7 days. After mixing, 2 cm^3 of the aliquot was centrifuged (10 min at 12 000 rpm) and diluted as necessary. Absorbancy at 280 nm was determined with a LKB Biochrom Ultrospec spectrophotometer (II E) (Biochrom, Cambridge, England; Model 4057) using 10 mm quartz cells. Values obtained were multiplied by the applicable dilution factor.

Sucrose, glucose and fructose

Sample preparation: approximately 250 mg of freeze-dried sample was weighed into a 15 cm^3 plastic centrifuge tube and 3 cm^3 of water added. Samples were subsequently vortexed for 60 s, placed in an inversion mixer for 15 min and sonicated for 20 min. They were then centrifuged for 10 min at 2 500 rpm before 0.9 cm^3 of the supernatant was transferred to a 2 cm^3 Eppendorf and 0.9 cm^3 acetonitrile (MeCN) added, followed by a quick vortex to mix. Samples were then centrifuged for 10 min at 3 000 rpm where after 1.4 cm^3 of the supernatant was transferred to 2 cm^3 HPLC vials. A 0.2 cm^3 xylose solution (20 mg.cm^{-3} in 50 % MeCN) was added to each vial as internal standard before being placed in the HPLC autosampler.

HPLC: The HPLC instrument (Waters 600 pump and Waters 717 Plus Autosampler) was equipped with a Phenomenex AMINE column with the following dimensions: 250 x 4.6 mm, 5 µm particle size. The injection volume was set to 10 µL. An isocratic mobile phase of 75 % MeCN was used with a flow rate of 1 cm^3.min^{-1} at column temperature of 30 °C. The run time was set at 15 min. Detection of the column eluents was done by a Polymer Lab PL-ELS 1000 evaporative light scattering detector with nebuliser

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temperature set at 80 °C, evaporator temperature at 120 °C and the flow-rate of the inert gas (nitrogen) at 1.5 SLM (standard litres per minute).

Malic and tartaric acid

Sample preparation: approximately 200 mg of a freeze-dried sample was weighed into a 15 cm^3 plastic centrifuge tube and 3 cm^3 of 0.05 M HCl added. Samples were subsequently vortexed for 60 s, placed in an inversion mixer for 15 min and sonicated for 20 min. They were then centrifuged for 10 min at 3 000 rpm before aliquots of 1.0 cm^3 of the supernatant were put into 2 cm^3 Eppendorf vials. Samples were then centrifuged for 10 min at 3 000 rpm where after 0.5 cm^3 of the supernatant was transferred to HPLC sample vials.

HPLC: The HPLC instrument (Agilent 1100) was equipped with an YMC Triart C18 plus column with the following dimensions: 250 x 4.6 mm, 5 µm particle size. The injection volume was set at 10 µL. This analysis made use of a binary gradient elution – Eluent A was 25 mM KH_2PO_4 at pH 2.5 (adjusted with H_3PO_4) and Eluent B 50 % MeCN and 0.05 % H_3PO_4. The mobile phase was set to a flow rate of 1 $cm^3.min^{-1}$ at column oven temperature of 30 °C. The run time was set at 30 min for the column. For the first 10 min 100 % Eluent A was used, for the following 10 min a 50:50 mix of A and B, followed by 10 min of pure Eluent A. Detection of the column eluents were done by UV spectroscopy at 216 nm.

6.2.3 Statistical layout of project

The data was subjected to analysis of variance (ANOVA) using the General Linear Models Procedure (PROC GLM) of SAS software (Version 9.2; SAS Institute Inc., Cary, USA). The ANOVA was performed in order to evaluate the main influences/effects of cultivar, CO_2, temperature and H_2O, as well as to detect interaction effects among these factors. Measurements over sampling times were included in a split-plot analysis of variance with sampling times as sub-plot factor (Little & Hills, 1978) where applicable. The Shapiro-Wilk test was performed on the standardised residuals from the model to verify normality (Shapiro & Wilk, 1965). Levene's test showed dissimilarity of cultivar variances (Levene, 1960). To correct for variance differences between cultivars, a weight was included in the ANOVA. The weight was the inverse of the experimental error of each cultivar (John & Quenouille, 1977). Fisher's least significant difference was calculated at the 5 % level to compare means of the factors (main effects) and factor interaction means (Ott & Longnecker, 2001). A probability level of 5 % was considered significant for all significance tests. The Pearson product moment (Pearson) correlation tests were performed using XLSTAT (Version 2015.1.03.15485, Addinsoft, Paris).

6.3 RESULTS AND DISCUSSION

At 12 weeks after planting, the rate of photosynthesis was still higher in the C1 than C0 plants in both cultivars (Fig. 6.1). This occurred despite the lower N (Fig. 6.2) and chlorophyll (Chapter 3) content under elevated CO_2 conditions. However, internal CO_2 levels were higher in C1 plants (Fig. 6.3). The results indicate higher chloroplast efficiency (as demonstrated by Girardin et al. 1985) and more effective use of the available N.

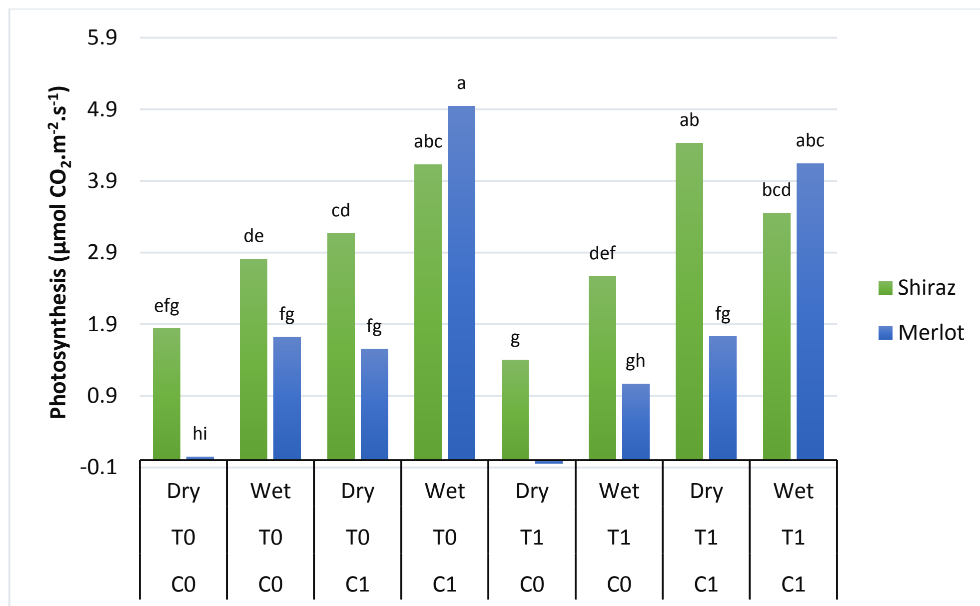

Fig. 6.1 Photosynthetic rate of basal primary leaves of Shiraz and Merlot at 12 weeks after planting. Bars with the same letters do not differ significantly ($p \leq 0.05$).

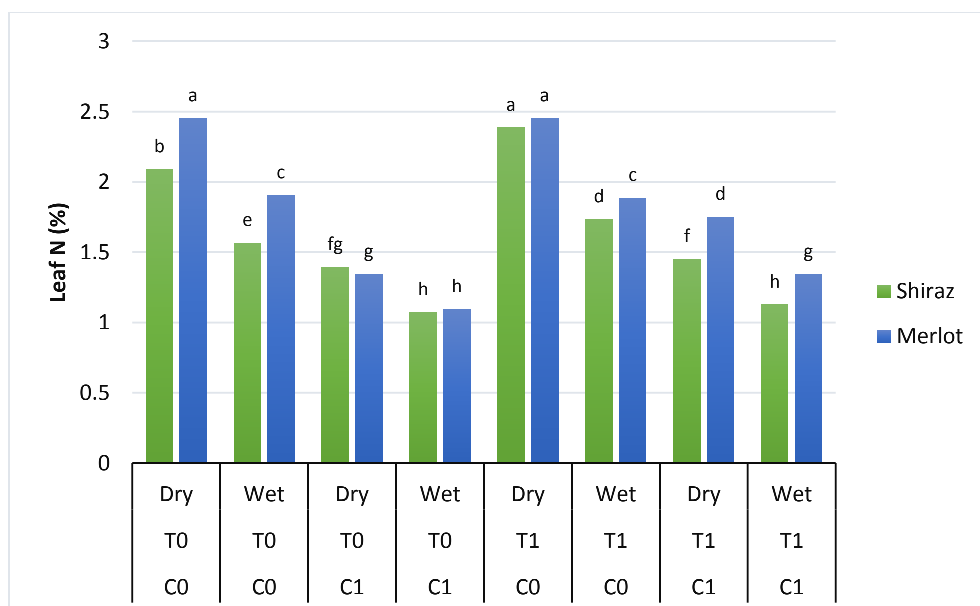

Fig. 6.2 Nitrogen concentration of primary leaves of Shiraz and Merlot at 12 weeks after planting. Bars with the same letters do not differ significantly ($p \leq 0.05$).

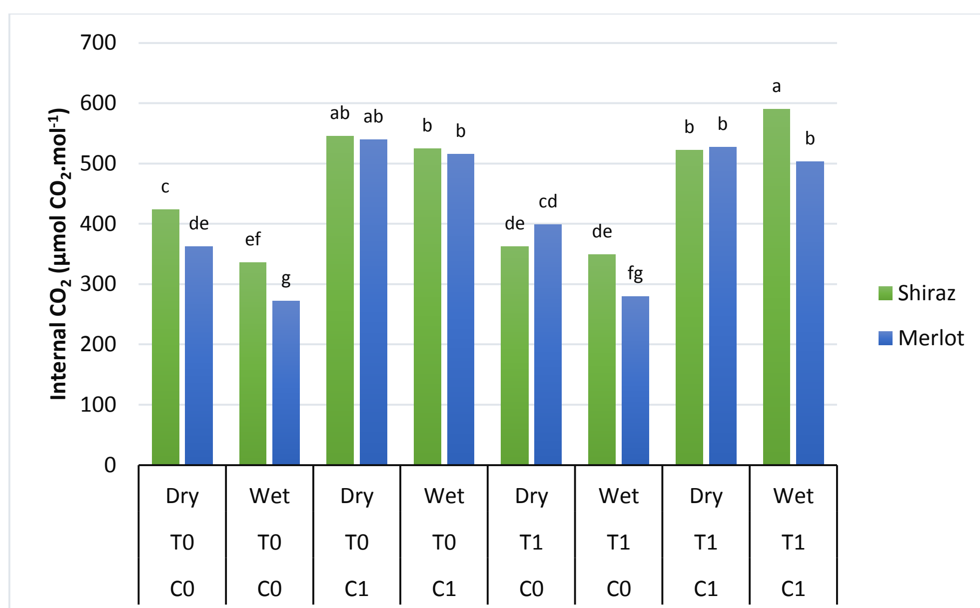

Fig. 6.3 Internal CO_2 of basal primary leaves of Shiraz and Merlot at 12 weeks after planting. Bars with the same letters do not differ significantly ($p \leq 0.05$).

A certain degree of photosynthetic acclimation was therefore observed in this study that may be related to increased N remobilisation out of the leaves (Aranjuelo et al. 2015) to other vine parts to improve the N use efficiency (Drake et al. 1997; Leakey et al. 2009) and maintain vegetative growth of non-photosynthesising plant parts. This may have been illustrated by the continued increase in shoot and root biomass between 8 and 12 weeks after planting (Chapter 4). Photosynthetic rates in future may be higher than the current rates due to the elevated ambient CO_2 (Long et al. 2004; Moutinho-Pereira et al. 2009; Morales et al. 2016; Edwards et al. 2017). Such acclimation may not necessarily have a negative impact on crop production, although the chlorophyll content (Chapter 3) and the leaf N may decrease (Fig. 6.2). The patterns of N allocation in plants may also change under elevated CO_2 with accelerated leaf N remobilisation (Aranjuelo et al. 2015) and increased translocation to and assimilation in other, growing plant parts (Stitt & Krapp, 1999). This may be beneficial to the vine, since N supply, uptake and assimilation are often found to be limiting in C1 treatments (Coleman et al. 1993; Stitt & Krapp, 1999; Alonso et al. 2009; Morales et al. 2016), possibly due to the higher N demand as a result of increased vegetative growth (Aranjuelo et al. 2015). Biomass accumulation (calculated as the sum of new roots, primary shoots and primary leaves dry mass per vine) in both cultivars was the highest in the C1(wet) treatments in this study. It was also found (Chapter 5) that the N concentration in the leaves and roots (especially NMF roots) decreased in C1 treatments, although the total N content in the canopies did not differ significantly between treatments within the same cultivar, while the NMF N content was enhanced by both water supply and elevated CO_2. Thus, although the leaf N concentrations in C1 treatments were very low, (if compared with industry guidelines used for commercial vineyards), N deficiency was not reflected in the vegetative growth or in the overall N content of the leaves and roots.

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The sucrose synthesised in mature leaves may be translocated to carbohydrate sinks in the rest of the vine; used during N assimilation for the production of nucleic acids and protein; or split by invertase to glucose and fructose for metabolic maintenance (Hunter et al. 1994). Under circumstances where sucrose translocation and/or respiration decrease (limited N supply and/or decrease in sink strength), the metabolic pathway is switched to starch synthesis (Huber; 1983; Mullins et al. 1992). It may therefore be expected that a decrease in vegetative growth or N levels in plants would result in a relative increase in starch accumulation.

The starch concentrations and content of the various root fractions and shoots were determined at 4, 8 and 12 weeks after planting (Table 6.2; Figs 6.4 & 6.5). At 4 weeks, when active shoot and root growth occurred (Chapter 4), starch was present at either extremely low levels or totally absent. Starch reserves present in the root fractions during planting were probably remobilised to support the budding and initial growth, in accordance with Hunter et al. (1995). Starch started to accumulate in root material and shoots at 8 weeks after planting (Table 6.2; Figs 6.4 & 6.5). This coincided with the sharp decrease in shoot growth rate (Chapter 4) and the start of lignification of the green shoots and is in accordance with the findings of Hunter et al. (1995) and Bates et al. (2002). The levels of starch continued to increase in roots and shoots between 8 and 12 weeks after planting and this increase is expected to continue until leaf fall as a result of continued photosynthetic activity as well as recycling of C and N resources from senescent leaves just before leaf fall (Hunter et al. 1995).

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Table 6.2 Comparison of the starch concentration in vine tissue fractions of Shiraz and Merlot vines at 4, 8 and 12 weeks after planting.

STARCH		C0T0(D)	C0T0(W)	C1T0(D)	C1T0(W)	C0T1(D)	C0T1(W)	C1T1(D)	C1T1(W)
RS ($mg.g^{-1}$)	Shiraz Week 4	3.926 a	1.489 bcde	1.62 bcde	0.716 de	1.716 bcde	1.064 cde	2.962 ab	1.391 bcde
	Merlot Week 4	2.094 bcde	2.425 bcd	1.471 cde	2.599 abc	0.716 de	0.705 de	0.65 de	0.416 e
	Shiraz Week 8	22.09 g	23.57 efg	29.113 bcd	30.676 abcd	20.909 g	27.079 cdef	34.856 a	32.099 abc
	Merlot Week 8	21.333 g	25.834 defg	35.128 ab	32.675 abc	23.816 fg	28.761 cde	34.448 ab	35.999 a
	Shiraz Week 12	44.91 de	41.98 e	47.131 cde	46.925 cde	45.909 cde	42.983 de	51.389 c	48.307 cd
	Merlot Week 12	69.275 a	62.274 b	64.458 ab	65.51 ab	61.333 b	61.584 b	67.045 a	68.981 a
OT ($mg.g^{-1}$)	Shiraz Week 4	1.245 a	0.547 b	0.315 bcd	0.095 d	0.432 bc	0.128 cd	0.1 d	0.112 d
	Merlot Week 4	0.27 bcd	0.177 cd	0.074 d	0.244 bde	0.201 cd	0.209 cd	0.155 cd	0.22 cd
	Shiraz Week 8	2.37 bcde	2.242 cdef	3.134 abc	2.881 abc	2.567 abcd	3.638 abc	3.925 a	3.853 ab
	Merlot Week 8	0.539 g	0.802 fg	1.009 efg	1.085 defg	0.428 g	0.526 g	0.619 g	1.1 defg
	Shiraz Week 12	14.448 bcde	19.005 abcd	19.429 abc	19.851 ab	24.083 a	22.258 a	24.031 a	23.602 a
	Merlot Week 12	14.806 bcde	14.646 bcde	12.799 e	14.654 bcde	14.372 cde	13.521 e	13.866 de	15.17 bcde
OMF ($mg.g^{-1}$)	Shiraz Week 4	6.127 a	4.838 ab	1.016 e	1.038 e	2.500 bcde	2.018 cde	2.226 cde	1.926 cde
	Merlot Week 4	5.324 a	4.313 abc	3.758 abcd	3.751 abcd	1.747 de	1.922 cde	1.043 e	1.711 de
	Shiraz Week 8	13.806 cd	13.956 cd	18.032 bc	11.412 de	16.594 bcd	18.535 bc	26.803 a	20.187 b
	Merlot Week 8	0.283 g	0.269 g	4.249 fg	4.621 fg	0.910 g	0.488 g	8.111 ef	1.864 g
	Shiraz Week 12	57.463 ab	54.780 ab	55.992 ab	56.983 ab	60.166 ab	64.815 a	58.738 ab	54.244 b
	Merlot Week 12	31.353 cd	30.105 cd	23.814 cd	33.465 c	21.486 d	23.824 cd	28.863 cd	30.36 cd

Values in two rows representing the same week (Shiraz and Merlot combined) followed by the same letter do not differ significantly ($p \leq 0.05$).
RS: Rootstock trunk; OT: Old thick roots; OMF: Old medium and fine roots.

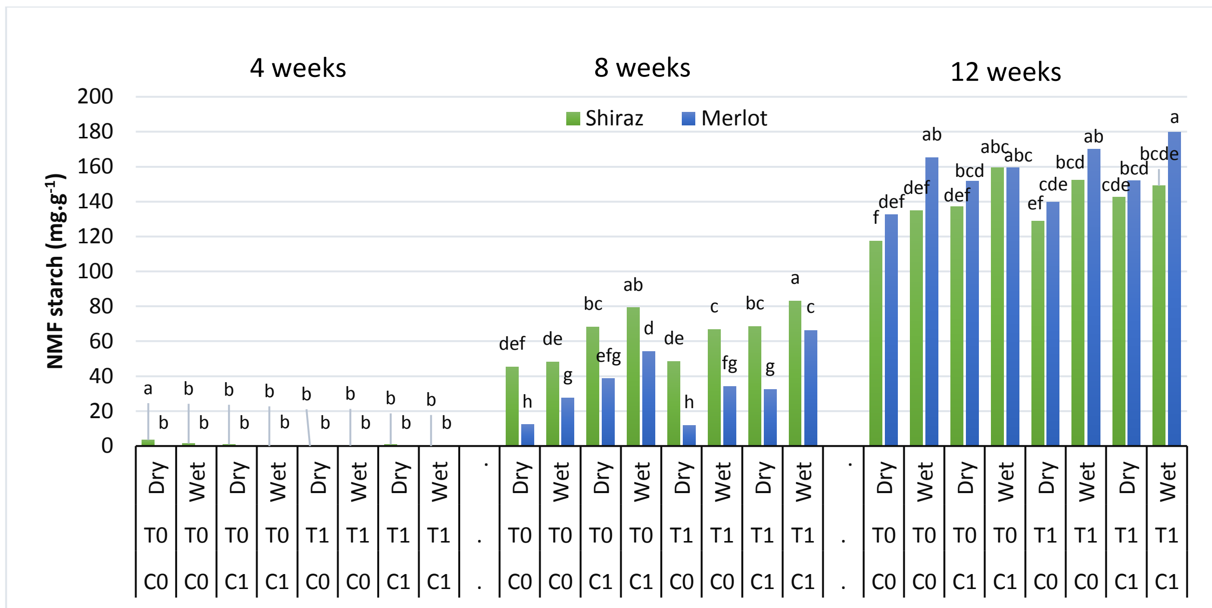

Fig. 6.4 Starch concentration in new medium-fine roots of Shiraz and Merlot at 4, 8 and 12 weeks after planting. Bars with the same letters within the same week do not differ significantly ($p \leq 0.05$).

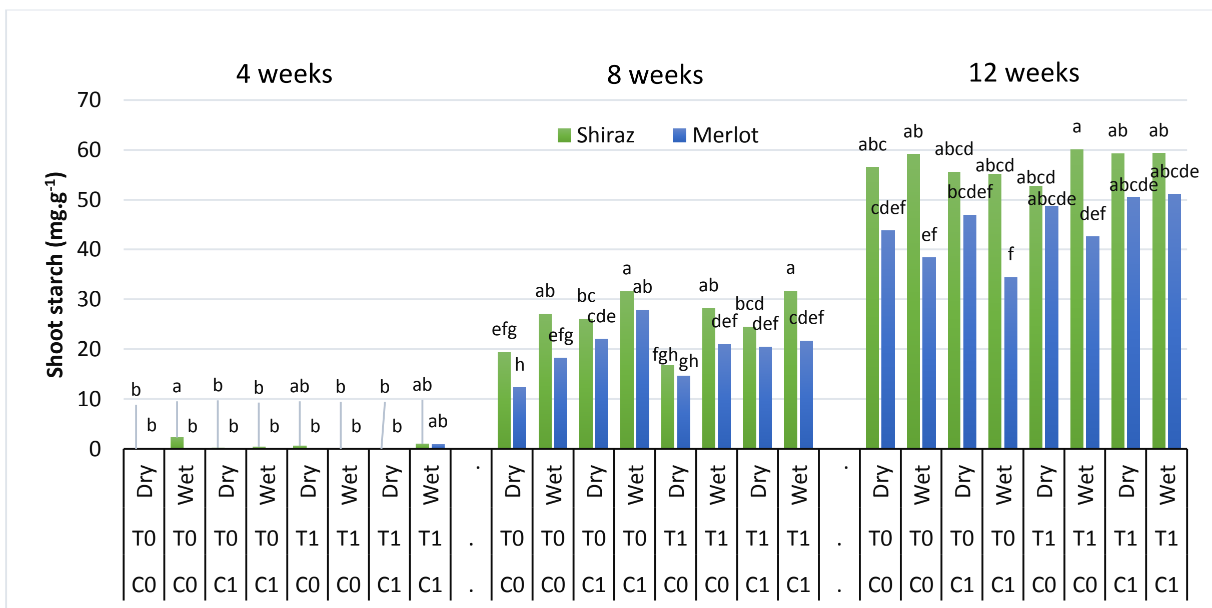

Fig. 6.5 Starch concentration in primary shoots of Shiraz and Merlot at 4, 8 and 12 weeks after planting. Bars with the same letters within the same week do not differ significantly ($p \leq 0.05$).

Scion cultivars seem to affect the patterns of starch accumulation in the roots, since Shiraz accumulated higher levels of starch in OT, OMF and shoots than Merlot, and had similar or lower concentrations in the NMF roots and less in the rootstock trunks (Table. 6.2).

Starch accumulation in the rootstock trunk seems to be affected by the treatments in week 8 with higher concentrations in the C1 and the (wet) treatments with no apparent difference between the cultivars (Table 6.2). At 12 weeks after planting the effect of the cultivar was stronger than any treatment effect, with rootstocks with Merlot as scion cultivar containing higher starch concentrations than Shiraz. No

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difference in starch level was found within the cultivars among the various treatments. Starch seemed to accumulate very slowly in the OT roots, with concentration less than 50 % of that in the rootstock material at 12 weeks after planting. No difference between the treatments was found for Merlot, while starch accumulation in the OT roots seems to be enhanced in T1 temperatures in Shiraz (although not always statistically significant). Regarding the OMF roots, the cultivar effect was already apparent at 8 weeks after planting with higher concentrations in Shiraz than in Merlot. This difference became more apparent at 12 weeks, but as was found for the OT roots, no differences in the OMF starch concentration occurred among the treatment combinations within cultivars, with the exceptions of C0T1(wet) containing higher starch levels than C1T1(wet) in Shiraz and C1T0(wet) with higher levels in Merlot than C0T1(dry).

Environmental factors did not affect the starch concentrations in the rootstock trunk, OT or OMF roots (Table 6.3), with the exception of the CO_2 level that seemed to increase the starch in rootstock trunks. It was mainly the number of weeks after planting, the cultivar and the interaction between them that affected the starch levels of these fractions. It is expected that starch would have further accumulated in the older roots and perennial parts should the study have been extended, since the highest starch concentration during dormancy was found in the 0.5 – 2.0 mm and 2 – 5 mm roots (Hunter, 1998), which is comparable to the OMF and OT root fractions in this study.

Therefore, regarding the perennial fractions analysed, starch accumulation commenced when the sink strength of growing shoots decreased. At the end of the growth period, the highest starch concentrations were found in the rootstock trunk, followed by the OMF roots and then the OT roots containing the lowest starch concentrations. Cultivars differed in their starch accumulation patterns, once again displaying the interactive effect between scion and rootstock, with Shiraz seemingly accumulating more starch in the old roots and Merlot more in the rootstock trunk.

An interesting aspect to consider is the concept that the vine ontogeny is hastened under C1 conditions (Miller et al. 1997). This is supported by findings of earlier N remobilisation (Aranjuelo et al. 2015) and earlier natural decrease in the photosynthetic rate as a result of leaf senescence (Miller et al. 1997). In a study such as this one, it could therefore be possible that vines planted at the same time might be at different phenological "ages" at the same number of weeks after planting. It is also quite possible that various cultivars would be differently affected, making interpretation of the results more difficult, since measurements were taken with four week intervals. Based on this reasoning, it might even be that starch accumulation in Shiraz shoots, OT and OMF roots is not higher than in Merlot, but only commenced earlier during the growth period.

Table 6.3 Indication of significance level of main treatment factors and interaction effects on the starch concentration of tissue fractions of Shiraz and Merlot.

STARCH	Cv	Weeks after plant	CO_2	Temp	H_2O	Cv x Weeks	Cv x CO_2	Cv x Temp	Cv x H_2O	Weeks x CO_2	Weeks x Temp	Weeks x H_2O	CO_2 x Temp	CO_2 x H_2O	Temp x H_2O
RS	*** 2 %	*** 83 %	***	NS	NS	*** 3 %	NS	NS	NS	***	*	**	*	NS	NS
OT	*** 2 %	*** 60 %	NS	NS	NS	*** 2 %	NS	**	NS	NS	NS	NS	NS	NS	NS
OMF	*** 12 %	*** 60 %	NS	NS	NS	*** 8 %	NS	*	NS	**	*	*	**	NS	NS
NMF	**	*** 84 %	***	**	***	*** 2 %	NS	NS	NS	***	NS	***	NS	NS	NS
1°shoots	*** 1 %	*** 57 %	**	NS	*	***	NS	NS	*	**	*	***	NS	NS	NS

	Cv x Weeks x CO_2	Cv x Weeks x Temp	Cv x Weeks x H_2O	Cv x CO_2 x Temp	Cv x CO_2 x H_2O	Cv x Temp x H_2O	Weeks x CO_2 x Temp	Weeks x CO_2 x H_2O	Weeks x Temp x H_2O	CO_2 x Temp x H_2O	Cv x CO_2 x Temp x H_2O	Cv x Weeks x Temp x H_2O	Cv x Weeks x CO_2 x H_2O	Cv x Weeks x CO_2 x Temp	Weeks x CO_2 x Temp x H_2O	Cv x Weeks x CO_2 x Temp x H_2O
RS	NS	NS	NS	NS	NS	NS	NS	**	NS	NS	NS	NS	NS	NS	NS	NS
OT	NS	**	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
OMF	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	**	NS	NS
NMF	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
1° shoots	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

(*, ** and *** indicate significance at $p \leq 0.05$, 0.01 and 0.001, respectively. NS indicates no significant difference ($p > 0.05$).
The percentage values included in the table indicate the contribution by the specific factor or interaction to the total variance of each parameter measured (values only included if contribution is > 1 %).
RS: Rootstock trunk; OT: Old thick roots; OMF: Old medium-fine roots; NMF: New medium-fine roots; 1°: Primary; Cv: Cultivar; Temp: Temperature

The starch concentration in young roots that developed during the growth period was affected by all three environmental factors, as well as the cultivar and the number of weeks after planting when samples were taken. Similarly to the old roots, starch accumulation in the NMF roots also commenced around 8 weeks after planting, with higher concentration in Shiraz than in Merlot (Table 6.3). After that, starch levels increased sharply between 8 and 12 weeks with the concentration in Merlot roots equal to or higher than that of Shiraz. The storage of carbohydrate reserves seemed to be enhanced by water supply and elevated CO_2 in both cultivars, which is consistent with the higher photosynthetic rate found in these treatments.

Since the concentration does not indicate the size of the reserve pool in the roots, the starch content of the root fractions was also calculated. It was found that the NMF starch content of Shiraz and Merlot in the respective treatments was the same, except for C0T1(wet) that was higher in Shiraz than in Merlot (Figs 6.6 & 6.7). The total starch accumulation in the root system per vine was also similar between the cultivars, which may indicate an inherent regulatory role by the rootstock genotype regarding reserve accumulation.

At the end of the growth period, the root systems that developed in C0(dry) conditions contained the least amount of starch. These treatments were also associated with the lowest photosynthetic rate (Fig. 6.1) and total vegetative growth (Chapter 4). The opposite was found to be true for C1(wet) vines, with the highest photosynthetic activity and strongest growth, accumulating the most starch in their root systems.

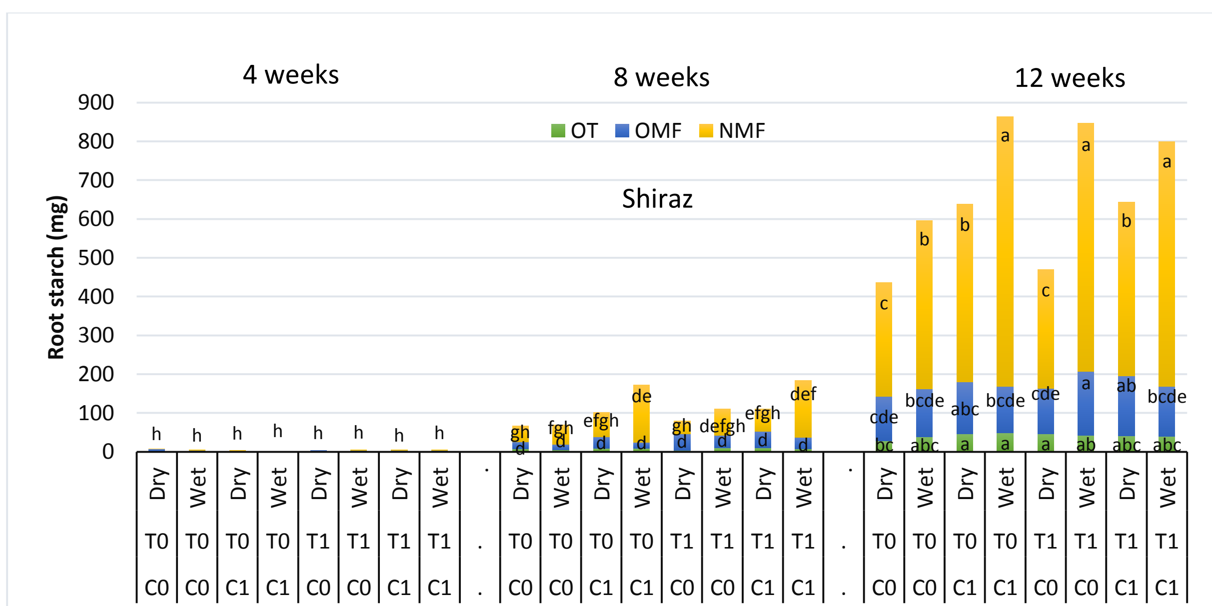

Fig. 6.6 Starch content in root fractions of Shiraz at 4, 8 and 12 weeks after planting. Bars with the same letters within the same series do not differ significantly ($p \leq 0.05$). Significance of only NMF roots is indicated at week 4; OMF and NMF at week 8; and NMR, OMF and OT at week 12.

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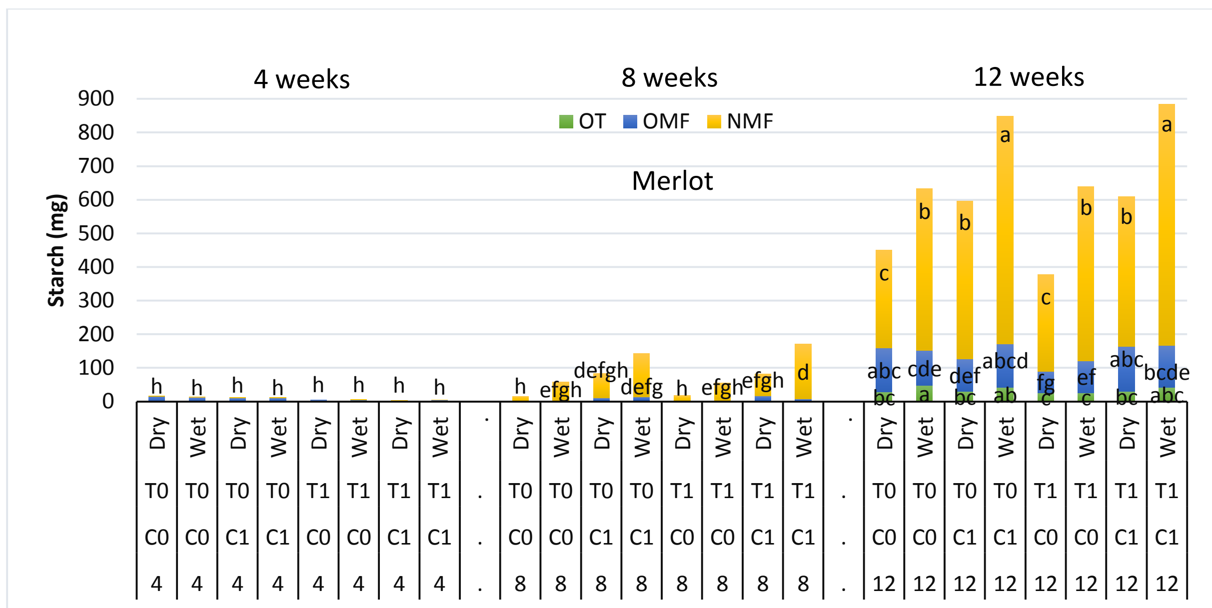

Fig. 6.7 Starch content in root fractions of Merlot at 4, 8 and 12 weeks after planting. Bars with the same letters within the same series do not differ significantly ($p \leq 0.05$). Significance of only NMF roots is indicated at weeks 4 and 8.

Although the leaf N concentrations were lower in C1 and (wet) treatments (Fig. 6.2), no negative effects normally related to N deficiency were found. Comparable N content (Chapter 5) and starch concentration (Fig. 6.5) were found between C1 and C0 treatments, while stimulation of vegetative growth seemed to be sustained for the duration of the growth periods (Chapter 3). The total N in this study was measured at 12 weeks after planting and it is therefore not possible to make any deductions regarding the N dynamics during the growth season or even the ratio between organic and inorganic N levels in the vines.

Another indication of nutrient (especially N) deficiency is the ratio between new shoot and new root growth. In N deficit plants, root growth is stimulated in relation with shoot growth (Keller, 2005) in order for the plant to reach new, unutilised soil regions for the uptake of the necessary nutrients (Volder et al. 2005). It was found that the level of CO_2 and water supply had no effect on the dry mass ratio between new shoot and new root growth (Fig. 6.8), while the cultivar and temperature variables affected the dry mass ratio significantly with respective p-values of 0.007 and 0.004. It therefore did not seem as if any vines were suffering from nutrient deficiency compared to the others, and that the differences in growth patterns and physiological activity may be ascribed to the treatment effects.

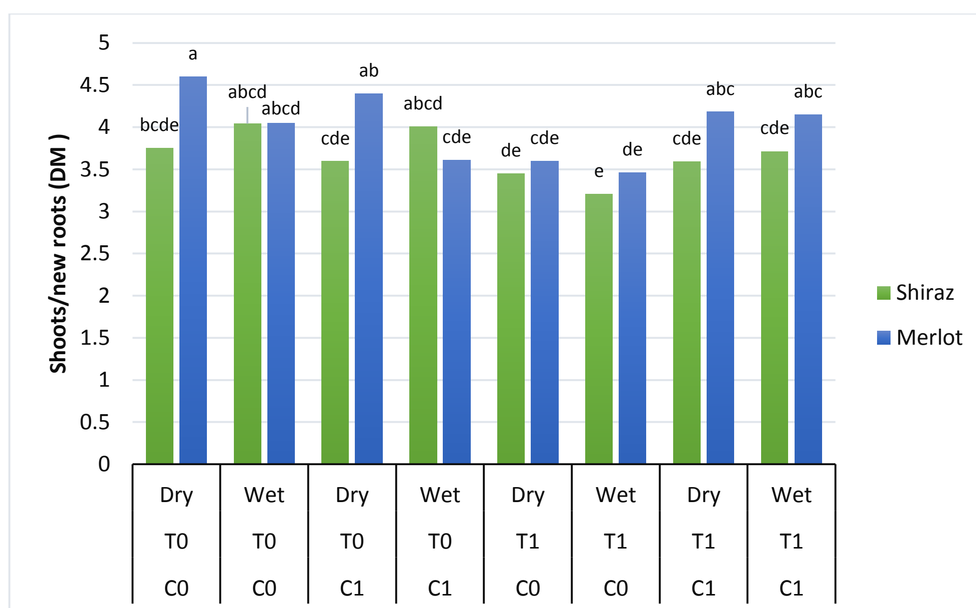

Fig. 6.8 Dry mass ratio of shoots (including leaves) and new root growth of Shiraz and Merlot at 12 weeks after planting. Bars with the same letters do not differ significantly ($p \leq 0.05$).

It was found that leaf glucose and fructose concentrations were significantly higher than sucrose throughout the growth period for both cultivars (Figs 6.9 & 6.10; Table 6.4) with an increase in both hexose levels between 4 and 12 weeks. Sucrose concentrations were extremely low and were only detected in Shiraz. This is an indication of very active vegetative tissue in which high activities of sucrose synthase and invertase are splitting sucrose into hexoses for metabolic processes (Hunter et al. 1994). Sucrose is utilised in the leaves as soon as it is produced, while the leaf demand for sucrose increased between 4 and 12 weeks after planting to support maintenance of the plant with a much larger canopy compared to 4 weeks after planting. It is assumed that towards the end of the growth period in particular, any sucrose not hydrolysed in the leaves would have been immediately transported to the shoots and roots for accumulation as starch reserves. Under field conditions, leaf glucose and fructose concentrations increase sharply within the first few weeks after bud burst and remain high until berry set (Kliewer & Nassar, 1966). These patterns are similar to those that were found in this study. However, the leaf sucrose did not increase and actually surpassed the hexose concentrations as was found by Hunter et al. (1994) and Hunter & Ruffner (2001) from berry set to leaf senescence. This might be explained by the different conditions in the glasshouse and/or the fact that newly planted vines without reproductive growth and perennial structures were used.

At 4 weeks after planting both glucose and fructose concentrations were higher in the C1 than C0 treatments for both Shiraz and Merlot (Figs 6.9 & 6.10), which might be linked to enhanced leaf growth under these conditions (Chapter 4). Ambient temperature and level of water supply did not seem to greatly affect leaf hexose sugar concentrations at this stage in comparison with CO_2 (Table 6.5). At 12

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weeks the various hexose concentrations were still comparable between Shiraz and Merlot, while differences between treatments became less pronounced and patterns not as consistent.

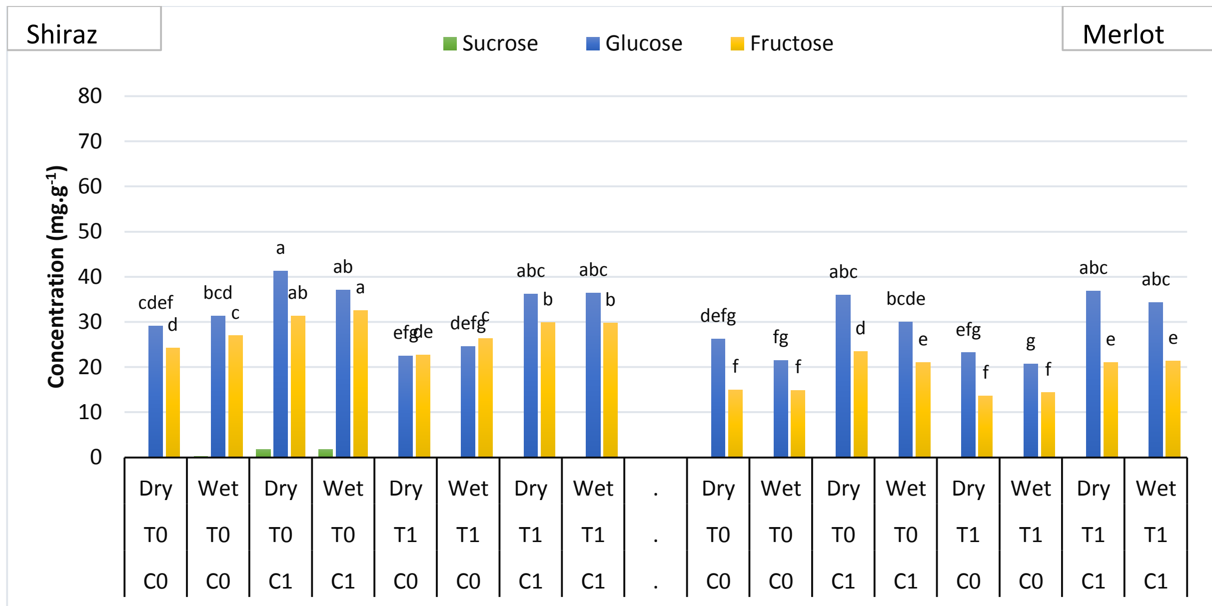

Fig. 6.9 Leaf sugar concentrations of Shiraz and Merlot at 4 weeks after planting. Bars with the same letters within the same series do not differ significantly ($p \leq 0.05$).

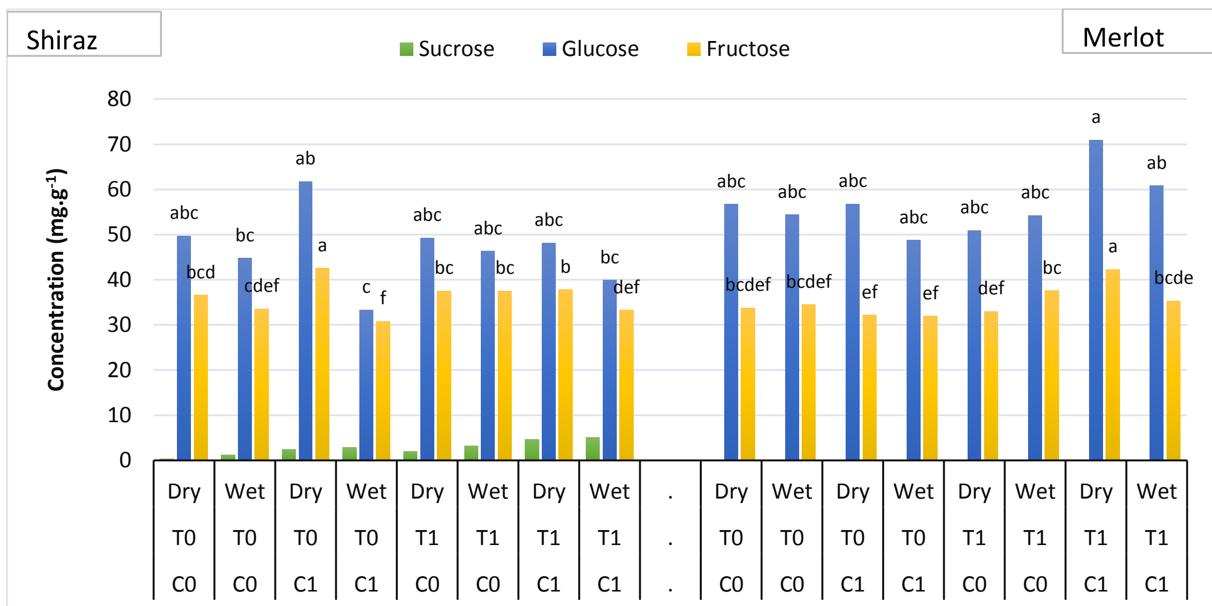

Fig. 6.10 Leaf sugar concentrations of Shiraz and Merlot at 12 weeks after planting. Bars with the same letters within the same series do not differ significantly ($p \leq 0.05$).

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Table 6.4 Comparison of the leaf glucose, fructose, tartaric acid and malic acid concentrations of Shiraz and Merlot vines at 4 and 12 weeks after planting.

		Glucose ($mg.g^{-1}$) 4 Weeks	Glucose ($mg.g^{-1}$) 12 Weeks	Fructose ($mg.g^{-1}$) 4 Weeks	Fructose ($mg.g^{-1}$) 12 Weeks	Tartaric acid ($mg.g^{-1}$) 4 Weeks	Tartaric acid ($mg.g^{-1}$) 12 Weeks	Malic acid ($mg.g^{-1}$) 4 Weeks	Malic acid ($mg.g^{-1}$) 12 Weeks
C0T0(D)	Shiraz	29.172 cdef	49.710 abc	24.23 d	36.58 bcd	107.845 c	82.921 a	27.237 de	38.857 a
	Merlot	26.252 defg	56.720 abc	14.997 f	33.771 bcdef	99.846 de	76.381 c	19.531 hi	34.848 bc
C0T0(W)	Shiraz	31.321 bcd	44.740 bc	26.939 c	33.546 cdef	105.207 cd	71.432 de	23.9 fg	34.345 bcd
	Merlot	21.498 fg	54.360 abc	14.839 f	34.496 bcdef	99.497 de	72.057 de	15.554 j	33.749 bcde
C1T0(D)	Shiraz	41.333 a	61.740 ab	31.301 ab	42.56 a	94.028 ef	58.288 fg	29.392 cd	31.249 efgh
	Merlot	36.047 abc	56.780 abc	23.467 d	32.155 ef	84.397 gh	56.102 fgh	22.498 g	29.772 fgh
C1T0(W)	Shiraz	37.072 ab	33.360 c	32.568 a	30.818 f	82.995 gh	52.078 h	25.748 ef	29.287 gh
	Merlot	30.006 bcde	48.760 abc	21.085 e	31.906 ef	87.397 fg	54.708 gh	19.243 hi	28.473 h
C0T1(D)	Shiraz	22.436 efg	49.290 abc	22.688 de	37.524 bc	130.711 a	80.719 ab	32.956 b	39.189 a
	Merlot	23.212 efg	50.930 abc	13.666 f	33.003 def	94.147 ef	77.357 bc	21.862 gh	33.1 bcde
C0T1(W)	Shiraz	24.591 defg	46.310 abc	26.395 c	37.512 bc	123.62 b	69.318 e	26.89 de	32.233 cdef
	Merlot	20.732 g	54.230 abc	14.343 f	37.574 bc	95.469 e	73.701 cd	17.308 ij	31.729 defg
C1T1(D)	Shiraz	36.220 abc	48.160 abc	29.949 b	37.779 b	109.707 c	59.981 f	36.75 a	32.803 bcde
	Merlot	36.897 abc	70.970 a	21.005 e	42.296 a	78.721 h	55.567 gh	29.19 cd	41.444 a
C1T1(W)	Shiraz	36.446 abc	39.910 bc	29.773 b	33.261 def	105.898 cd	54.425 gh	30.541 bc	25.652 i
	Merlot	34.352 abc	60.800 ab	21.343 e	35.341 bcde	80.661 gh	53.91 h	23.354 fg	35.126 b

Values in the same column followed by the same letter do not differ significantly ($p \leq 0.05$).
(D): Dry; (W): Wet.

Table 6.5 Indication of significance level of main treatment factors and interaction effects on leaf parameters of Shiraz and Merlot.

Parameter	Cv	Weeks after plant	CO_2	Temp	H_2O	Cv x Weeks	Cv x CO_2	Cv x Temp	Cv x H_2O	Weeks x CO_2	Weeks x Temp	Weeks x H_2O	CO_2 x Temp	CO_2 x H_2O	Temp x H_2O
N (%)	*** 3 %	-	*** 52 %	*** 2 %	*** 20 %	-	**	NS	NS	-	-	-	NS	*** 1 %	*
Glucose (mg.g^{-1})	***	*** 57 %	*** 6 %	*	*** 2 %	*** 6 %	***	**	NS	*** 1 %	NS	NS	*** 1 %	***	*
Fructose (mg.g^{-1})	*** 6 %	*** 44 %	*** 3 %	*	*	*** 4 %	NS	*	*	*** 2 %	***	*	NS	***	NS
Sucrose (mg.g^{-1}	-	*** 18 %	*** 7 %	NS	NS	-	-	-	-	NS	*** 9 %	NS	NS	NS	NS
MA (mg.g^{-1})	*** 3 %	*** 14 %	NS	*** 2 %	*** 4 %	*** 5 %	*** 1 %	*	*	*** 3 %	***	NS	*** 1 %	NS	**
TTA (mg.g^{-1})	*** 3 %	*** 48 %	*** 18 %	NS	**	*** 3 %	NS	*** 2 %	**	***	NS	**	NS	NS	NS

Parameter	Cv x Weeks x CO_2	Cv x Weeks x Temp	Cv x Weeks x H_2O	Cv x CO_2 x Temp	Cv x CO_2 x H_2O	Cv x Temp x H_2O	Weeks x CO_2 x Temp	Weeks x CO_2 x H_2O	Weeks x Temp x H_2O	CO_2 x Temp x H_2O	Cv x CO_2 x Temp x H_2O	Cv x Weeks x Temp x H_2O	Cv x Weeks x CO_2 x H_2O	Cv x Weeks x CO_2 x Temp	Weeks x CO_2 x Temp x H_2O	Cv x Weeks x CO_2 x Temp x H_2O
N (%)	-	-	-	*** 2 %	NS	NS	-	-	-	NS	NS	-	-	-	-	-
Glucose (mg.g^{-1})	NS	NS	*	*** 1 %	NS	NS	*	*	NS	NS	NS	NS	NS	*	NS	NS
Fructose (mg.g^{-1})	NS	NS	**	**	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Sucrose (mg.g^{-1}	-	-	-	-	-	-	NS	NS	NS	NS	-	-	-	-	NS	-
MA (mg.g^{-1})	**	**	NS	***	NS	NS	NS	NS	NS	NS	NS	NS	NS	**	NS	NS
TTA (mg.g^{-1})	NS	*** 2 %	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

(*, ** and *** indicate significance at $p \leq 0.05$, 0.01 and 0.001, respectively. NS indicates no significant difference ($p > 0.05$). – indicates missing data
The percentage values included in the table indicate the contribution by the specific factor or interaction to the total variance of each parameter measured (values only included if contribution is > 1 %). N: Nitrogen; MA: Malic acid; TTA: Tartaric acid; Cv: Cultivar; Temp: Temperature

Tartaric acid in *Vitis* is synthesised mainly via ascorbic acid (Saito & Loewus, 1989), while malic acid synthesis is associated with N assimilation (Stitt & Krapp, 1999). This means that these two acids are not metabolically linked and have independent accumulation patterns (Ruffner, 1982). Tartaric acid develops very early during the season and reaches maximum concentration in the leaf between 4 weeks after budding (Kliewer & Nassar, 1966) and the pea-size berry stage (Hunter & Ruffner, 2001). The concentration after that remains constant (Ruffner, 1982). The findings of this study are in agreement with these patterns and show a decrease in leaf tartaric acid concentration between 4 and 12 weeks after planting (Figs 6.11 & 6.12). At 4 weeks, tartaric acid concentrations in Shiraz leaves seemed to depend on all three environmental factors with higher levels associated with higher temperature, lower ambient CO_2 and adequate water supply. Concentrations in Merlot leaves were also lower in well-watered vines and elevated CO_2, but with the T0 temperatures seemingly more conducive to tartaric acid synthesis. At 12 weeks, the temperature did not affect leaf tartaric acid (also Table 6.5), while the effect of ambient CO_2 and water supply seem to continue during the whole season. Lower tartaric acid concentrations were therefore found in the stronger growing vines, namely those in the C1(wet) treatments (Chapter 4). However, when the total tartaric acid content in the leaves of the various treatments was calculated, it was found that the content was linked with the plant size (data not shown). Thus it is very possible that tartaric acid synthesis continued in the leaves, albeit at a slower rate than the vegetative growth which resulted in the observed decrease in concentration.

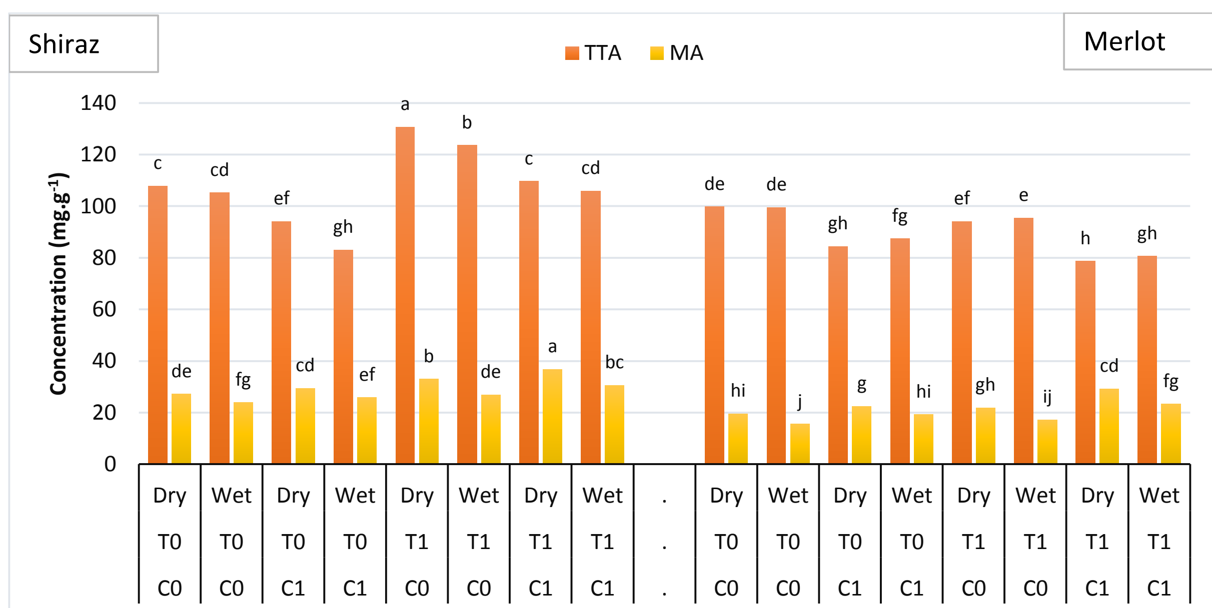

Fig. 6.11 Leaf tartaric and malic acid concentrations of Shiraz and Merlot at 4 weeks after planting. Bars with the same letters within the same series do not differ significantly ($p \leq 0.05$).

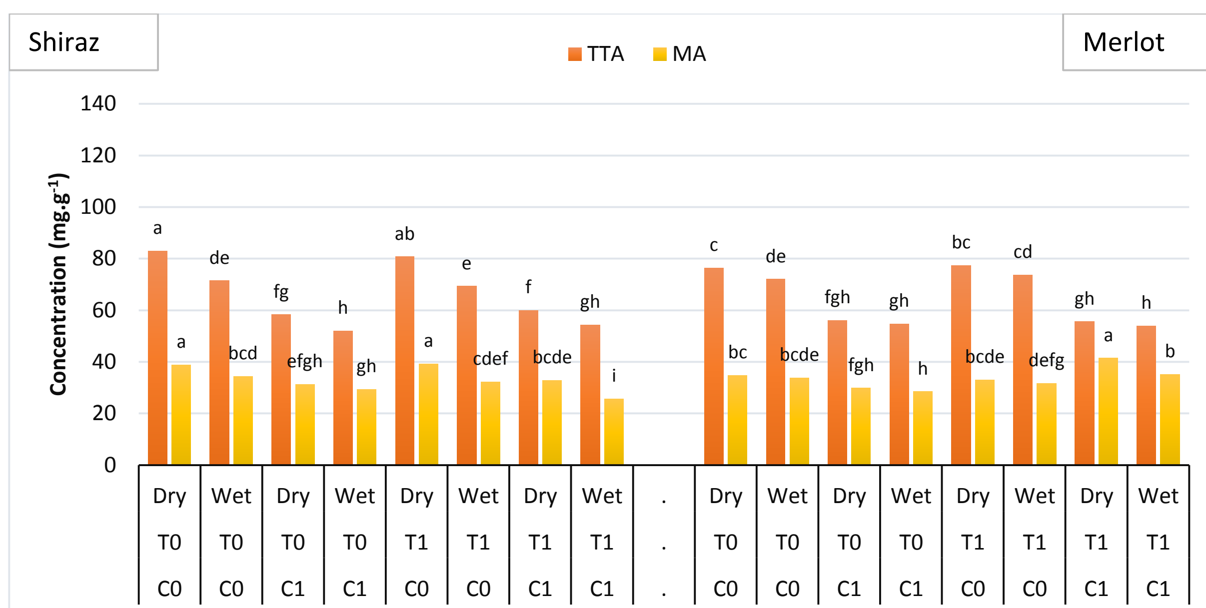

Fig. 6.12 Leaf tartaric and malic acid concentrations of Shiraz and Merlot at 12 weeks after planting. Bars with the same letters within the same series do not differ significantly ($p \leq 0.05$).

Malic acid concentration is very low in young leaves (Hunter & Ruffner, 2001) and starts to increase rapidly when leaves reach about 25 % of their mature size (Ruffner, 1982). Malic acid levels in leaves continue to increase until senescence (Kliewer & Nassar, 1966). The increase in leaf malic acid concentration between 4 and 12 weeks after planting was therefore expected (Figs 6.11 & 6.12). Leaf malic acid concentrations generally seem to decrease in the (wet) treatments for both cultivars, while CO_2 had no effect on leaf malic acid levels (also Table 6.5). The synthesis of malic acid did not seem to be drastically impaired by any treatment combination, which also provide support to the assumption that N deficiency was not experienced by the vines in any of the treatment combinations.

Although the effect of temperature treatment on the total phenolic index (TPI) in the case of the rootstock trunk, CO_2 level for OMF and water supply for both OT and OMF was found to be statistically significant (Table 6.6), the strength of these effects was very small and contributed less than 1 % to the total variance of the respective parameters. The TPI of these perennial fractions seemed to increase a little during the course of the growth period in the case of Shiraz (Table 6.7), while values for Merlot seemingly increased between 4 and 8 weeks and decreased again thereafter. These fluctuations and trends between weeks after planting and treatments applied were much smaller than the cultivar effect (Table 6.6), which is in accordance with Lattanzio (2013) who mentioned the dependency of secondary metabolite production on the genetic expression of the plant. Throughout the growth periods monitored, TPI values in perennial tissues of Shiraz were significantly higher than in those of Merlot.

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Table 6.6 Comparison of the total phenolic index (TPI) concentration in vine tissue fractions of Shiraz and Merlot vines at 4, 8 and 12 weeks after planting.

TPI		C0T0(D)	C0T0(W)	C1T0(D)	C1T0(W)	C0T1(D)	C0T1(W)	C1T1(D)	C1T1(W)
RS	Shiraz Week 4	13.924 ab	13.532 abc	13.951 ab	13.280 bc	14.688 a	13.550 abc	13.843 ab	14.266 ab
	Merlot Week 4	11.528 de	10.788 e	10.991 e	11.399 de	11.758 de	10.774 e	11.522 de	12.539 cd
	Shiraz Week 8	14.374 a	13.295 abcd	14.025 abc	13.267 abcd	14.360 a	14.709 a	14.309 ab	14.820 a
	Merlot Week 8	12.001 de	11.916 de	12.064 de	11.466 e	12.626 bcde	12.402 cde	12.364 cde	11.708 de
	Shiraz Week 12	15.648 c	16.605 bc	16.921 b	16.062 bc	15.717 c	16.384 bc	16.779 bc	18.113 a
	Merlot Week 12	12.127 e	12.653 de	12.504 de	13.445 d	12.663 de	13.240 de	13.246 de	13.545 d
OT	Shiraz Week 4	27.658 bc	26.061 c	29.393 ab	26.389 bc	29.388 ab	25.017 cd	31.182 a	27.757 bc
	Merlot Week 4	19.551 e	19.36 e	19.17 e	19.434 e	22.213 de	19.571 e	20.217 e	20.159 e
	Shiraz Week 8	30.91 ab	28.338 bcd	31.05 a	27.687 cde	29.085 abc	28.275 bcd	30.866 ab	27.332 cde
	Merlot Week 8	25.052 ef	24.215 f	25.862 def	23.679 f	26.298 def	26.303 def	27.31 cde	25.328 ef
	Shiraz Week 12	33.558 ab	33.073 abc	34.295 a	32.528 abc	30.993 cd	29.973 d	31.601 bcd	30.302 d
	Merlot Week 12	22.869 ef	23.028 ef	24.652 e	24.711 e	21.29 f	22.924 ef	22.449 f	23.245 ef
OMF	Shiraz Week 4	23.945 e	23.941 e	27.000 bd	24.951 de	27.783 bc	26.604 cd	30.145 a	28.694 ab
	Merlot Week 4	16.106 f	15.807 f	16.636 f	17.352 f	16.89 f	15.603 f	16.038 f	16.142 f
	Shiraz Week 8	31.397 ab	29.347 bcd	32.763 a	31.261 abc	27.654 d	28.308 d	31.274 abd	28.793 cd
	Merlot Week 8	21.094 efg	20.623 fg	23.234 e	19.738 g	22.437 ef	22.72 ef	23.076 ef	20.998 efg
	Shiraz Week 12	32.392 abc	30.051 cd	34.142 a	29.171 d	30.386 bcd	29.744 cd	32.847 ab	30.07 cd
	Merlot Week 12	17.318 f	17.089 f	20.381 e	18.653 ef	16.371 f	17.02 f	17.875 ef	17.907 ef

Values in two rows representing the same week (Shiraz and Merlot combined) followed by the same letter do not differ significantly ($p \leq 0.05$).
(D): Dry; (W): Wet; RS: Rootstock trunk; OT: Old thick roots; OMF: Old medium and fine roots

Table 6.7. Indication of significance level of main treatment factors and interaction effects on the total phenolic index (TPI) of tissue fractions of Shiraz and Merlot

TPI	Cv	Weeks after plant	CO_2	Temp	H_2O	Cv x Weeks	Cv x CO_2	Cv x Temp	Cv x H_2O	Weeks x CO_2	Weeks x Temp	Weeks x H_2O	CO_2 x Temp	CO_2 x H_2O	Temp x H_2O
RS	*** 16 %	*** 10 %	NS	***	NS	***	NS	NS	NS	*	NS	**	NS	NS	*
OT	*** 36 %	*** 11 %	*	NS	***	*** 4 %	NS	NS	**	NS	*** 1 %	*	NS	NS	NS
OMF	*** 37 %	*** 8 %	***	NS	***	*** 1 %	NS	NS	*	NS	*	NS	*	*	NS
NMF	*** 4 %	*** 56 %	NS	NS	*** 2 %	*** 1 %	*	NS	NS	NS	NS	NS	NS	NS	NS
Leaves	***	*** 47 %	*** 12 %	***	*** 2 %	*** 1 %	***	*	NS	*** 2 %	*	*** 2 %	*	NS	NS

	Cv x Weeks x CO_2	Cv x Weeks x Temp	Cv x Weeks x H_2O	Cv x CO_2 x Temp	Cv x CO_2 x H_2O	Cv x Temp x H_2O	Weeks x CO_2 x Temp	Weeks x CO_2 x H_2O	Weeks x Temp x H_2O	CO_2 x Temp x H_2O	Cv x CO_2 x Temp x H_2O	Cv x Weeks x Temp x H_2O	Cv x Weeks x CO_2 x H_2O	Cv x Weeks x CO_2 x Temp	Weeks x CO_2 x Temp x H_2O	Cv x Weeks x CO_2 x Temp x H_2O
Rootstock	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
OT	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
OMF	NS	***	NS	NS	NS	NS	NS	*	NS	NS	NS	NS	NS	NS	NS	NS
NMF	NS	*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Leaves	***	NS	NS	*	NS	NS	NS	*	NS	NS	NS	NS	NS	***	NS	NS

(*, ** and *** indicate significance at $p \leq 0.05$, 0.01 and 0.001, respectively. NS indicates no significant difference ($p > 0.05$).
The percentage values included in the table indicate the contribution by the specific factor or interaction to the total variance of each parameter measured (values only included if contribution is > 1 %).
RS: Rootstock trunk; OT: Old thick roots; OMF: Old medium-fine roots; NMF: New medium-fine roots; Cv: Cultivar; Temp: Temperature

Roots that developed during the growth period (NMF) were more sensitive to the level of water supply than the suberized roots (Table 6.6), while the CO_2 and temperature treatments had no effect on their TPI values. Conditions of water deficit increased the TPI in the NMF roots (Fig. 6.13), which is in accordance with the findings of Griesser et al. (2015) who found higher levels of polyphenols in leaves where grapevines were exposed to prolonged drought conditions. Since it is known that plants accumulate phenols in their tissues under stress conditions (Lattanzio, 2013), it would seem as if both cultivars experienced increased stress levels as the growth period progressed with consistently increasing NMF TPI values between 4 and 12 weeks after planting.

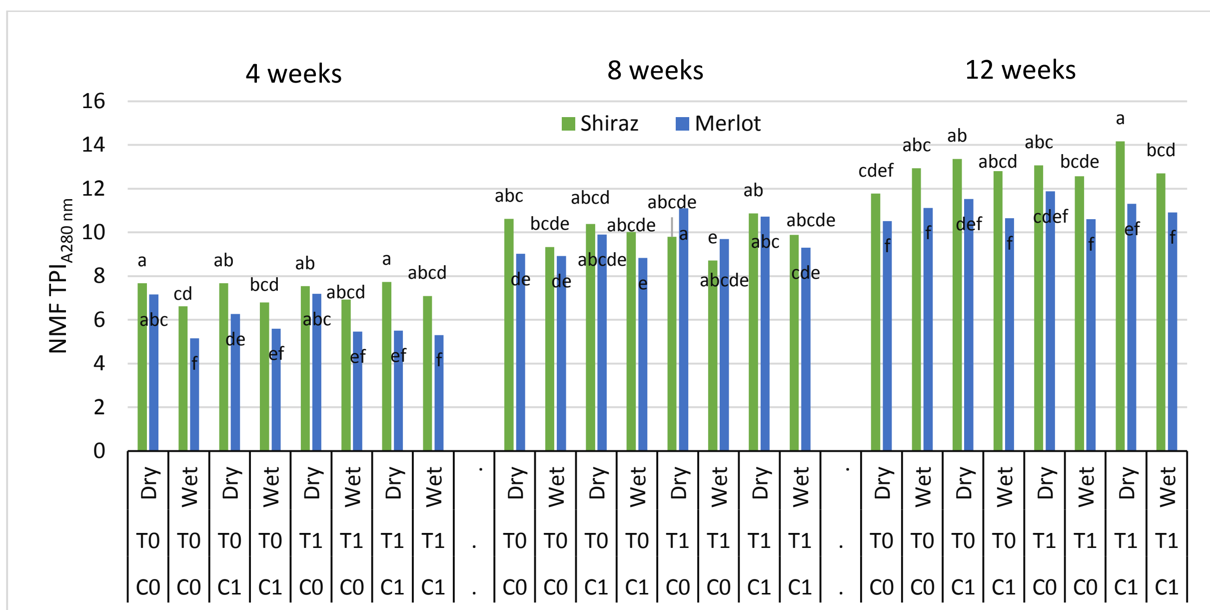

Fig. 6.13 Total phenolic index (TPI) in new medium-fine roots of Shiraz and Merlot at 4, 8 and 12 weeks after planting. Bars with the same letters within the same week do not differ significantly ($p \leq 0.05$).

At 4 weeks, the most significant difference between (wet) and (dry) conditions was found for the C0 treatments, which might indicate a higher resistance against drought stress under elevated CO_2 conditions. At 8 and 12 weeks after planting, the strength of the irrigation effect decreased and there was no significant difference ($p \leq 0.05$) between (wet) and (dry) treatments for both cultivars, although the trend of higher TPI values in water-stressed roots continued.

Grapevine roots are directly exposed to soil water conditions and soil borne pests and diseases. Unsuberized NMF roots are more water permeable than suberized roots (Kramer & Bullock, 1966) and they are more susceptible to root drying under drought conditions and pathogen invasion (Enstone et al. 2003). This may also explain why soil water conditions affected phenol synthesis in the NMF roots more strongly than in the older roots.

The TPI in the leaves was clearly determined by environmental factors, and less strongly by the cultivar effect compared to the (especially old) roots (Table 6.6). The trend of increasing TPI during the growth period in roots was repeated in leaves (Fig. 6.14). At 4 weeks after planting, the response of leaves to water deficit seemed similar to that of roots, with higher TPI values found in the (dry) treatments. Shiraz leaves also contained more phenols than Merlot leaves. The exception to these findings was the C1T1 treatments, with no difference between cultivars or the level of water supply.

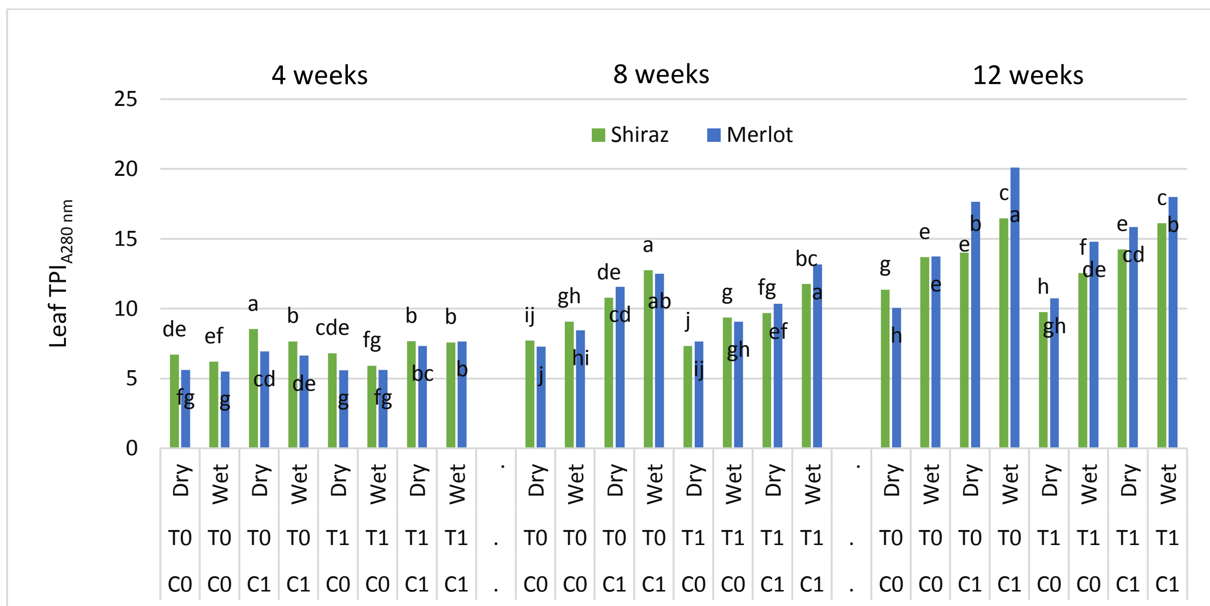

Fig. 6.14 Total phenolic index (TPI) in primary leaves of Shiraz and Merlot at 4, 8 and 12 weeks after planting. Bars with the same letters within the same week do not differ significantly ($p \leq 0.05$).

It is expected that leaves would react differently to environmental conditions than roots. As already discussed, roots are more directly exposed to plant water provision than the leaves, which in turn depend on the roots for their water supply *via* the xylem. Leaves, on the other hand, are directly in contact with ambient air conditions, which may explain the larger effect of CO_2 to secondary metabolism (as indicated by the TPI) in leaves compared to roots (Table 6.6).

According to Close & McArthur (2002) patterns in the development of phenolics in plants are often used to support the idea of a trade-off between defence against pathogens and vegetative growth. However, after strong primary shoot growth ceased around 8 weeks, leaf TPI was higher in the (wet) than in the dry treatments; higher in T0 than in T1, and also higher in the C1 than in the C0 conditions (Fig. 6.14). This seems to be in direct contrast with the concept of increased phenol synthesis under plant stress conditions, since photosynthetic activity (Chapter 3; Fig. 6.1) and vegetative growth (Chapter 4) were the highest in the C1T0(wet) treatment combination. It should be kept in mind that only an index of the total phenolics in the various fractions was determined in this study. Plant phenols is a very wide

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classification comprising a diverse group of compounds with various functions in the plant, such as the stabilisation of structures, signal transduction and protection against photo-damage (Edreva et al. 2008). It may therefore be possible that the synthesis of some of these phenolic compounds is not enhanced by stress conditions *per se*, but rather increases as a result of active photosynthesis and stimulated growth.

Aranjuelo et al. (2008) found that plants in elevated CO_2 conditions did not suffer from photo-oxidative damage. They ascribed it to the enhanced de-epoxidation of violaxanthin to zeaxanthin and antheraxanthin that facilitated thermal dissipation and thus protected the photosystem from the excess photo-energy. Zinta et al. (2014, 2018) also noticed that elevated CO_2 had a stress mitigating effect, but in turn ascribed it partially to the upregulation of lipophilic anti-oxidant synthesis in the plants in high CO_2 conditions. Close & McArthur (2002) linked lower phenolic levels with environmental conditions with low risk of photo-damage. The higher TPI found in the C1 treatments may therefore be partially explained by the higher risk of photo-oxidative damage under these conditions with the enhanced production of phenols as a preventative measure.

6.4 CONCLUSIONS

The higher physiological activity (Chapter 3) and vegetative growth (Chapter 4) found for treatments with adequate water supply and those in elevated CO_2 were also reflected in the metabolic parameters. Higher amounts of starch accumulated in the root systems of these vines, indicating continued and active photosynthetic activity and sucrose export. The relative starch concentration and content accumulated differed between the various root fractions. This may indicate the importance of the composition of any root system and the balance required between OT, OMF and NMF roots. The timing and degree of shoot lignification were similar among the various treatments, which could indicate a balance between growth and reserve accumulation within each respective treatment combination. This balance is also possibly reflected in the comparable shoot:new root ratio among the treatments. It seems as if the water deficit treatment was effective (as confirmed by the stem water potential levels discussed in Chapter 3) in decreasing general physiological rates and vegetative vigour, but not sufficiently extreme to change the allocation patterns to enhance root growth relative to shoot growth.

The very low leaf sucrose relative to hexose levels found at 12 weeks after planting indicate active leaf metabolism as well as immediate splitting of sucrose for export and utilisation in other departments and for reserve storage, since no accumulation of sucrose occurred in the leaf tissues. The dynamics of TTA and MA synthesis and accumulation in the leaves were similar to those found under field conditions. Hexose and organic acid concentrations were lower in C1 treatments and when vines were well-supplied

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with water. This could be explained by an attenuation effect on the sugars and acids due to the larger vines (stronger growth), since the leaf hexose and organic acid contents per vine were higher in the C1 and (wet) than in the C0 and (dry) treatments.

The most important factors determining the TPI in the roots were the number of weeks after planting and the scion cultivar. An increasing effect of water deficiency on root phenol content was observed. The phenol index in leaves was dependent on the CO_2 level as well as the stage during the growth period when samples were collected. Higher phenol content in C1 treatments might have been functional in protecting the photosynthetic system in those plants, while higher TPI seemed to be linked with photosynthetic rate and growth.

It is expected that grapevine physiology and growth would be enhanced by future climatic conditions. The elevated CO_2 should (within limits) be able to negate the negative effect of limited water on growth and functioning. There was no indication of nutrient deficiency in any vines. Stimulated photosynthetic and growth rates of vines in elevated CO_2 conditions were sustained during the first 12 weeks after planting young vines. Grapevine metabolism and dynamics of translocation and assimilation were not greatly affected by the treatment combinations.

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CHAPTER 7: GENERAL DISCUSSION AND CONCLUSIONS

7.1 INTRODUCTION

The grapevine is indigenous to the Mediterranean region and was traditionally cultivated over a relatively narrow range of climatic and geographical regions (Jones & Webb, 2010). Over recent decades, this range has increased substantially across a wide climate range and grapes are currently cultivated on six of the seven continents (Schultz, 2016). It is clear that the grapevine has a natural ability to adapt to the environmental conditions in which it is grown; this is referred to as its ecophysiological adaptation capacity (plasticity). In addition, more than 4000 wine grape cultivars were listed (OIV, 2013), which indicate the large genetic variability and plasticity of the grapevine genome (Medrano et al. 2015; Bota et al. 2016). This array of physiological and morphological traits allow successful wine grape production over a wide range of climates (Anderson et al. 2008; Keller, 2010).

The Western Cape province in South Africa is characterised by a complex topography with large variation in elevation, slope gradients and aspects as well as a long coastline with various degrees of exposure to the sea breeze effects from both the Indian and Atlantic oceans, but which is intensified by the cold Benguela current of the latter (Carey, 2001; Hunter & Myburgh, 2001; Bonnardot et al. 2002). There is also significant variation in soil type regarding texture, depth and water and nutrient holding capacity (Hunter & Myburgh, 2001). All of these factors result in a variety of meso climates within very short distances (Hunter & Bonnardot, 2011; Midgley et al. 2016) that often require small scale (spatial) adaptation of agricultural practices to accommodate the respective local growth conditions. Local producers have adopted cultivars and cultivation practices best suited to the diverse local environment (Jones & Alves, 2013).

However, based on climatic modelling (Webb et al. 2007, 2013; Hall & Jones, 2009; Hunter & Bonnardot, 2011; Fraga et al. 2016) as well as real-time climate data (Jones et al. 2005; Jones, 2007; Koch & Oehl, 2018), current climatic conditions will in future change to a larger extent than the current natural variability range (UNFCCC, 2011) and possibly exceed the effective limit of short-term adaptation of cultivation practices (Howden et al. 2007). More drastic changes in the regional climate would require an integrated approach to mitigation and adaptation (IPCC, 2014, 2019) that would necessitate well-aligned policies on land and climate and collaboration among stakeholders on different levels (e.g. individuals; local, regional and national government; private sector; research and training facilities).

Multi-disciplinary research is therefore required to address the various aspects involved in successful adaptation of the agricultural industry to climate change (Howden et al. 2007) and this should include contributions from both natural and social sciences.

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7.2 GENERAL MITIGATION AND ADAPTATION STRATEGIES ON GRAPEVINE PRODUCTION LEVEL

According to the IPCC Report of 2019, Agriculture, Forestry and Other Land Use (AFOLU) activities contributed 23 % to the net global greenhouse gas (GHG) emissions between 2007 and 2016. Global data for only agricultural CO_2 emissions is not available (IPCC, 2019.) In the Confronting Climate Change report of 2019 for South African Wine Grapes, it was reported (based on combined data from 2011-2018) that wine grape production emits 3-4 tCO_2-eq/ha into the atmosphere, which is relatively low compared to table grapes (9-10 tCO_2-eq/ha), stone and pome fruit (9 tCO_2-eq/ha) and citrus (7 tCO_2-eq/ha) (CCC, 2019). These values were obtained from normalised, graded data from the current database (representing less than 10 % of the wine industry (in hectares) (Blignaut, 2019). The main contributors to GHG emissions on wine grape farms are electricity (46-48 %), fuel consumption (28 %) and the use of fertilisers (20 %). Emission values would most probably differ between regions, cultivars used and cultivation practices, but due to the small representing sample it is too early to make any deductions at this time. However, it is clear that these three aspects should receive special attention when developing strategies to decrease GHG emissions on a farm.

Mitigation practices enforced by the producer should be aimed at decreasing or limiting GHG emissions as far as possible, while investigating options to increase the extraction and sequestration of CO_2 from the atmosphere by enhancing carbon sinks on farm level (IPCC, 2014; Tubiello et al. 2014). Carbon fuels and electricity could possibly be replaced by cleaner energy sources, such as solar radiation and wind, while traffic in the vineyards should be limited to the minimum. Less traffic would prevent soil compaction, which in turn would prevent the necessity of soil tillage to improve water infiltration and root distribution. Excessive tillage would further cause soil degradation (Keller, 2010; IPCC, 2019) and increase evaporation from the soil (Schultz, 2000). Open soil cultivation would increase CO_2 release from the enhanced breakdown of organic matter and erosion where increased precipitation intensities are expected (Schultz & Stoll, 2010). All of these may be prevented by using a cover crop, which has the additional advantages of extracting CO_2 from the atmosphere (Tezza et al. 2019), decrease water run-off, and improve soil structure and fertility (Medrano et al. 2015).

It was found that woody, perennial crops (such as the grapevine) may serve as moderately strong carbon sinks on a farm, which is not the case for annual crops (Vendrame et al. 2019). According to these authors, the sink strength of a vineyard depends on the terroir (soil type, climate and potential vigour) and vineyard management practices such as the choice of a trellising system, canopy management and the usage of inter-row cover crops. In arid and semi-arid regions, care should be taken to avoid excessive vine stress due to competition with cover crops for water and nutrients (Schultz & Stoll, 2010) and it is

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therefore imperative that summer-dormant cover crops are used in these regions to maximise carbon (C) extraction without compromising water availability to the grapevines (Tezza et al. 2019).

Since the impact of climate change is highly heterogeneous across grape varieties and regions (Jones et al. 2005; Fraga et al. 2016), its effects on viticulture will depend on the cultivar and the cultivation strategies followed within a specific region or vineyard. In order to protect the grapevine against detrimental effects caused by climate change and to improve its resilience, a total cultivation strategy should be adopted regarding both long term practices (starting at site selection, soil preparation, and matching the terroir and scion/rootstock) and short term practices performed seasonally (Hunter et al. 2010).

7.2.1 Long term practices

7.2.1.1 Site selection

Climate models may be used to determine the suitability of a region for a specific purpose and are often used in combination with crop models to generate future projections of yield, phenology and possible stress indicators for grapevines (Chapter 2). To increase the accuracy and the application value of these models, climatic suitability of a region should be assessed on a fine scale (regarding time and space) to better understand physiological activity at a specific terroir, especially in regions with a complex terrain (Hunter & Bonnardot, 2011; Quénol et al. 2017; Sturman et al. 2017). It is possible to create high-resolution projections of future yield, phenology and possible stress indicators for vineyards within a specific region by combining dynamic crop models with high-resolution climate model simulations (Fraga et al. 2016), but even with sophisticated methods like these certain assumptions and generalisations are made in the programming.

Neethling et al. (2013) conducted interviews with producers in the Loire Valley regarding their adaptive response to climate variability. They found that the physical characteristics of a vineyard site are one of the most important reasons for producers changing their standard cultivation practices and therefore there is a need for real-time, accurate information about local conditions. In South Africa, a study was done to investigate thermal remote sensing as alternative technology to supplement weather station data (Southey, 2017). An integrated platform was developed that provides information to the agricultural sector on climate, terrain and soils to better understand the topographical and climatic complexity of the Western Cape and to help producers with long and short term decision making.

In future it may even become necessary to relocate existing vineyards to higher latitudes and higher elevations where projected climates will be more conducive to high quality production (Jones et al. 2005; Duchêne et al. 2010; Keller, 2010; Hannah et al. 2013; Fraga et al. 2016; 2017).

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7.2.1.2 Soil preparation

Due to the balance between canopy and root growth (Hunter & Volschenk, 2001; Archer & Hunter, 2005, 2010), canopy growth depends on the root system for the provision of water, minerals and hormones, while strong shoot and canopy development would enhance root growth through the supply of carbohydrates, amino acids and hormones.

Root growth and distribution are largely affected by physical (Van Huyssteen, 1988) and chemical (Conradie, 1988) soil properties and it is therefore critical that any potential limitations to root penetration and distribution are eliminated during soil preparation. Grapevine physiology and growth were improved through deeper soil preparation (Van Huyssteen, 1988; Myburgh et al. 1996). Since these effects were more pronounced in dryland vineyards, it was deduced that the deeper penetrating roots were able to exploit the available soil volume better for the uptake of water and nutrients (Van Huyssteen, 1988).

7.2.1.3 Choice of scion and rootstock cultivars

In regions where climate change is occurring at a relatively fast rate, the new climatic conditions might necessitate the establishment of cultivars that are unkown to the region, but which are better suited to the current and predicted local environment (Keller, 2010). Potential wine grape cultivars should be evaluated under regional conditions and selected based on traits such as adaptability to variable climatic conditions (Clingeleffer 2010); high fruit: leaf area ratio (Clingeleffer et al. 2013) to delay véraison and ripening (Van Leeuwen et al. 2019); late seasonal ripening to extend the harvest (Van Leeuwen et al. 2008; Duchêne et al. 2010; Schwab & Maass, 2010) and high water use efficiency (WUE), particularly under conditions of water stress (Clingeleffer et al. 2013; Bota et al. 2016)

The sensitivity of the scion cultivar to climate change may be reduced by the rootstock choice. Under such circumstances, the most important characteristics for the rootstock seem to be moderate vigour (Clingeleffer, 2010); tolerance to soil salinity (Keller, 2010) and tolerance to low soil water conditions and drought (Serra et al. 2014; Hunter et al. 2016; Simonneau et al. 2017; Peccoux et al. 2018). The rootstock may also affect the phenology of the scion and could possibly be used to manage and control the time of ripening (Van Leeuwen et al. 2019).

In the long term, genetic improvement of cultivars (scion and rootstock) is a strategy to support sustainable wine production systems (Torregrosa et al. 2017) and it could be advantageous for Wine Industries to invest in breeding programmes (Jones, 2010), despite them being slow and expensive (Bota et al. 2016). Although modification of the grapevine genotype to incorporate desirable traits is possible, its practical application is currently prevented by policies and legislation (Anderson et al. 2008).

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7.2.1.4 Planting new vines, fertilisation and irrigation

During the first season after planting, strict weed and pest control should be applied and water stress of the young vines avoided (Creasy & Creasy, 2009; Jackson, 2014). Young grapevines are generally well-supplied with water and nutrients (especially nitrogen) to maximise vegetative growth (Myburgh et al. 1996) in an attempt to reap a harvest already in the second season (Keller, 2005). The promotion of shoot growth in the young vines will ensure strong root growth due to the established relationship between aerial and subterranean growth. A well-developed root system, in combination with judicious management practices, would buffer the vine against adverse climate conditions or environmental stress for the rest of its productive life (Archer & Hunter, 2010).

Conradie et al. (1996) found (on a granitic Glenrosa soil in the Stellenbosch area) that soil-derived N is sufficient to supply the required N of the vine for the first three years after soil preparation and that additional N fertilisation would only be required from the fourth year onwards. On more sandy soils, N fertilisation would probably have to commence sooner. According to Myburgh et al. (1996) the water requirement of grapevines (on the same site) during their first two years of growth is 55.4 % and 34.5 %, respectively, lower than the amount of irrigation generally required by an established vineyard in full production. Keller (2005) argued that over-supply of water and nitrogen to newly planted vines might cause NO_3 to accumulate in the leaf tissue resulting from the faster uptake of N by the roots. High NO_3 levels in leaves would favour local N assimilation and growth to the detriment of sucrose export (Stitt & Krapp, 1999). Sucrose import by the roots would then decrease, forcing them to metabolise their own reserves for the energy requirement of nutrient uptake and assimilation, growth and maintenance (Keller, 2005). Starch reserves in the roots may therefore be depleted unnecessarily, which could have a negative impact on vine longevity and performance.

7.2.1.5 Vine training and trellising

The higher expected vigour in future climatic conditions would necessitate adjustments in vine training and trellising systems. Higher trellis systems (bunch zone further away from the soil surface) improve the micro climate inside canopies in warm regions by decreasing the average leaf and bunch temperature (Zeeman, 1981). These findings were confirmed in Van Leeuwen et al. (2019), who added that this knowledge may be used to delay ripening so that it may occur under more optimal temperature conditions after mid-summer.

A good understanding and application of the inherent balance between shoot and root growth and between vegetative and reproductive growth (Hunter & Volschenk, 2001; Archer & Hunter, 2005, 2010) will increasingly be required to manage vineyard vigour and to translate that into increased yield. The choice of vine spacing (Archer et al. 1988; Hunter 1998a 1998b), type and size of trellising system and

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thus the perennial structure of the vine (Hunter & Volschenk, 2001) as well as vineyard row orientation (Hunter et al. 2017) are all tools for the producer to optimise the canopy micro climate (sunlight and wind exposure and thus temperature) in which the leaves photosynthesise and the bunches ripen.

7.2.2 Short term practices

7.2.2.1 Soil surface cultivation

Minimum tillage and the cultivation of a non-competing cover crop between vineyard rows have advantages. According to Blanco-Canqui et al. (2015), cover crops improve soil structure, water infiltration and retention, moderate soil temperature (more optimum conditions for grapevine root growth), increase biodiversity and microbial properties, recycle nutrients and suppress the growth of weeds. Cover crops also contribute to CO_2 extraction from the atmosphere and the C sequestration potential of the agrosystem (Tezza et al. 2019).

7.2.2.2 Pruning and canopy management

According to Van Leeuwen et al. (2019) adaptations in the vineyard to delay grape ripening have received much attention. Although the importance of delayed ripening *per se* is relative to the context of the region, these practices may be considered to manage or extend the harvest period and prevent logistical problems at the cellar. Late pruning in warm areas delayed the harvest by postponing the onset and suppress the rate of ripening (Moran et al. 2019), while light or minimal pruning may also serve to delay véraison and harvest (Clingeleffer, 2010) by increasing the reproductive:vegetative growth balance.

7.3 OVERVIEW, RESULTS, LIMITATIONS AND APPLICATION OF THIS STUDY

A lot of research has been (and is still currently being) done on the relationship between environmental factors and the physiological and growth response (vegetative and reproductive) of the grapevine. Meta-analysis could be used to combine and interpret the findings of various research projects, but cannot replace well-designed, multifactorial experiments (Curtis & Wang, 1998). However, results of these experiments are often contradictory, which may partly be due to a difference in experiment design and/or methodology used, rather than a direct effect of a particular treatment. Interpretation is thus often difficult, and the inherent uncertainty further complicates the critical aspect of knowledge and technology transfer between researchers and end-users (Howden et al. 2007).

A better understanding of how plants would react morphologically and physiologically (at leaf, root and whole-plant level) to climatic stress factors would facilitate the challenge of translating scientific knowledge to recommendations for practical application to ensure sustainable/profitable production of good quality grapes under future climatic conditions.

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7.3.1 Overview of the study

The literature available on the possible effects of future climatic conditions on grapevines and winegrape production evoked a few questions:

- How would newly-planted commercial "vineyards of the future" function under projected climate conditions?
- How will such vineyards cope in wine regions that are currently already considered as warm?
- Would current cultivation practices still apply, or would significant adaptations be required in future?

The establishment of a new vineyard is an expensive endeavour and a high survival rate of the young vines is very important for the producer to prevent re-planting and ensure that the vines come into full production as soon as possible. It is expected that future climatic scenarios will put additional strain on the first year of vine growth and therefore the physiological functioning and growth of the vine during its first few months after planting were studied. Initial growth of the young vines is very sensitive to the environment and has a direct effect on the performance and longevity of the mature vine. Strong vegetative growth during the first few years is required to establish a root system that is as deep, wide and dense as conditions would allow to maximise the buffer capacity of the vine, especially in the context of an increasingly variable climate expected in the future.

The study comprised five growth periods of 12 weeks each. The same rootstock (101-14 Mgt) was used throughout with Shiraz (SH 470) as scion cultivar for the first three plantings and Merlot noir (MO 348) for the remaining two. The effects of different combinations of ambient temperatures [maximum ranges 27 – 31 °C (T0), compared to 30 – 34 °C (T1)]; ambient CO_2 [400 ppm (C0) *vs* 800 ppm (C1)]; soil water [irrigation to water holding capacity (wet) and 50 % thereof (dry)]; phenological stage (4, 8 and 12 weeks after planting); and scion cultivar (Shiraz and Merlot) on the physiological activity, vegetative growth response, mineral uptake and translocation as well as the synthesis and allocation of metabolites to the various vine parts were investigated, using newly potted vines during their first 12 weeks of growth in glasshouses.

The study was laid out as a completely randomised block design, with 108 vines per each of the four CO_2/temperature combinations. Within each combination, water supply treatments were allocated in pairs, resulting in a sample population of 54 vines per CO_2/temperature/water treatment combination. Measurements and analyses (three replicates comprising 6 vines each) were done at 4, 8 and 12 weeks after planting.

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7.3.2 Main results of the study

It was found that higher CO_2 levels (irrespective of the temperature ranges applied in this study) increased the photosynthetic activity of the young vines and improved the efficiency of water and nitrogen use, provided that water stress did not increase to severe levels. Higher CO_2 levels mitigated the negative effect of water deficit on physiological activity to a certain extent. Inherent shoot and root growth patterns of the vine remained unaltered under simulated future climatic conditions. A strong relationship between new aerial and root growth was maintained in all treatment combinations. Adequate water supply was the most important environmental factor that determined the degree of vegetative growth, followed by the enhancing effect of higher CO_2. Cultivars differ in their physiological and growth response to environmental variables. Merlot seemed more sensitive to water deficit than Shiraz, but was at the same time more stimulated by elevated CO_2 levels. The effect of the treatment factors on macro- and micro-nutrient levels in vine tissues depended on the particular nutrient, the tissue type, as well as the scion/rootstock genotype. Stronger vegetative growth was associated with lower nutrient concentrations in the tissues, but resulted in similar (or higher) content. Established metabolite synthesis, translocation and accumulation patterns linked to grapevine phenology were the same in the various treatments. Higher CO_2 levels increased (and sustained) physiological activity and metabolism and induced stronger vegetative growth. These effects were further enhanced by higher water supply.

7.3.3 Possible limitations to the study

This study was done in a four-room glasshouse situated outside with specific light requirements and provided with infrastructure to obtain different levels of temperature and CO_2. It is a challenge to set up a study of this nature with regards to the preparation of the rooms, the installation of the various measuring and controlling equipment as well as the irrigation system. The successful maintenance of various treatment levels required meticulous monitoring that may be a technical limitation, depending on the available resources in the environment where such a study is executed.

A relevant limitation to the study from an industry point of view is the fact that it was executed in glasshouse rooms with vines planted in pots. Glasshouse conditions are very different to field conditions regarding environmental aspects, such as soil, light intensity, air movement and wind, relative humidity of the air and thus the water vapour pressure deficit of the leaf-air interface as well as the level of vine exposure to pests and diseases. However, information obtained under controlled conditions can be used effectively in understanding and extrapolation of grapevine behaviour in different scenarios.

It stands to reason that the absolute values of levels obtained cannot be directly extrapolated to mature vineyards grown under field conditions. In this dissertation an effort was made to avoid this and to focus

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on grapevine response to treatment combinations. However, since scientific literature on the effects of environmental parameters on young vine development is scarce, and even more so under predicted climate change scenarios, information obtained under controlled as well as field conditions was accommodated in the discussion of results in order to find value points that would be of interest to both scientists and producers.

7.3.4 Value of the study and possible applications

It is expected that producers will have to cultivate their grapevines in higher atmospheric CO_2 levels and in warmer, mostly drier conditions. Climatic variability is also projected to increase, which necessitates the need to improve the resilience of the vines against adverse and fluctuating growth conditions. Producers will be required to access and apply available climatic, environmental and technical information to ensure sustainable production.

Within the context of a changing climate and the expected decrease in available irrigation water, it would be advantageous to maximise the depth of soil preparation within the limits of economic feasibility. This would enhance depth penetration of roots and improve access to available water in the subsoil regions and possibly reduce the irrigation water requirement over the long term. Based on the results of this study, overall vine performance and growth would increase in future under elevated CO_2 conditions. These positive effects could possibly be exploited by increasing the time interval between irrigations, as well as the volume of water applied per irrigation, to facilitate wider, deeper and denser distribution of the root system.

Cover crops are used to prevent soil compaction and improve the soil structure. They may also compete with the vines for water and nutrients. Under limiting growth conditions, it would be advisable to start cultivating a cover crop after the young vineyard has been successfully established to ensure optimal growth of the vines.

The two scion cultivars used in this study reacted differently to the treatment combinations, even though they were grafted onto the same rootstock. It was interesting that the functioning and growth of Merlot seemed to be more stimulated by the higher CO_2 levels than Shiraz. This shows that the reaction of scion cultivars to changing climatic conditions will not be identical. These differences may even be enlarged when different rootstock genotypes are used. Improved and expanded knowledge of various cultivars (scion and rootstock as well as various combinations) and their expected performance under future climatic conditions would help the producer to make better informed decisions regarding this increasingly important aspect. It is very possible that cultivar profiles of estates and regions could change as well as the current cultivar wine styles, which could be a challenge for wine marketing and tourism.

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Elevated CO_2 mitigated the negative effects of water stress on growth and functioning to a certain extent. Nitrogen was more effectively utilised in the plants and photosynthesis was more efficient since high rates were maintained with significantly lower chlorophyll and leaf N concentrations. It seems prudent to revise and re-evaluate irrigation and fertilisation requirements and programmes, especially for young vines in the context of a changing climate since there will be less water available and vegetative growth will be enhanced as a result of the higher atmospheric CO_2. Excessive N fertilisation would only be wasted, since N uptake, shoot growth and yields are only enhanced up to a certain point in response to increased N availability in the soil. The unutilised soil NO_3 would be leached out of the soil profile and may also result in the eutrophication of nearby water sources and rivers.

This study is a further proof of the extremely efficient adaptability of the grapevine to changes in its environment. Physiological activity and vegetative growth are expected to increase in the future, although closer monitoring of water and nutrient application would be required. Information obtained in this study fulfils a gap in our knowledge regarding young vine behaviour. The study allows better understanding as well as extrapolation possibilities to other controlled as well as field conditions where young vines are established and monitored.

Most of the advised adaptation practices that are recommended to address the different climatic conditions in future are based on research already done over the last four decades in various regions of the world. Improved collaboration and knowledge transfer between wine producing countries on international level may show that one region’s climatic future is already another one’s present.

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