Effect of post-harvest summer pruning on carbohydrate reserve status, bud break and fertility of Sultanina H5 in the Lower Orange River region.

by

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Declaration

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Summary

Carbohydrate reserves produced from photosynthesis are stored in perennial tissues of the grapevine in the form of starch and free-sugar fractions or soluble sugars, mostly sucrose, glucose, and fructose. These reserves are highly affected by viticultural practices altering the source-sink relationship in the grapevine. Post-harvest pruning is a practice widely applied by several table grape producers in South Africa aiming to channel carbohydrate reserve accumulation to the remaining shoots. Due to the high input costs of table grape production, any manipulation, including post-harvest summer pruning, should be applied only if it is scientifically proven to have practical and economic benefits. This study, comprising of two trials, aimed to determine whether post-harvest pruning results in increased carbohydrate reserve status, improved bud break and fertility, as well as to establish a base for quantifying and practically assessing the carbohydrate reserve status of grapevines.

The first trial focused on establishing the seasonal dynamics of non-structural carbohydrate (NSC) reserves of *Vitis vinifera* L. cv. Sultanina H5 in the semi-arid Lower Orange River (LOR) and the Mediterranean Hex River Valley (HRV). Root, trunk, cane and/or shoot tissues were sampled monthly and analysed for NSC. The Anthrone method was used to analyse soluble sugars and starch, while enzymatic analysis was used to quantify specific sugars (sucrose, d-fructose and d-glucose). Starch and sucrose were the most abundant forms of NSC in all tissues in both regions. In both regions, soluble sugars in permanent tissues (roots, trunks, canes) reached their highest concentration during dormancy (June-July). The starch concentration was low in all tissues in winter (July), during grapevine dormancy, whereafter it increased to a peak occurring in August (before bud break). A steep decrease in starch concentration was recorded from dormancy to flowering in both regions, indicating a dependency of the vine on carbohydrate reserves during that period. Accumulation of NSC reserves began after flowering to the post-harvest period, reaching their second peaks in autumn. The overall higher soluble sugars and starch (roots and canes) concentrations in the tissues of the Mediterranean region is ascribed to the earlier accumulation of reserves, lower crop load and a shorter post-harvest period characteristic of this region.

A basis was established for sampling grapevine tissues for qualitative assessment of grapevine NSC reserve status, linking sampling time to occurrence of peaks in soluble sugars and starch concentrations. It is recommended that sampling for qualitative assessment of soluble sugars should be done after leaf fall, during dormancy (June-July under the conditions of this study). Starch concentrations should be assessed before bud break (August under the conditions of this study). Based on significant positive correlations between NSC concentrations of different tissue types, tissue types that could be sampled for indication of the overall NSC status of the grapevine

were identified. Canes and shoots could be sampled and analysed to indicate the overall NSC reserve status of the vine. These tissues are considered the most practical tissue types to sample for determination of grapevine NSC reserve status.

The second trial investigated the effect of post-harvest summer pruning applied in the semi-arid Lower Orange River region on NSC reserve status, bud break and fertility of Sultanina H5 grapevines. Five post-harvest pruning treatments were applied, namely an early 33% and a 66% shoot removal pruning treatment one day after harvest (33_1dAH and 66_1dAH respectively), a late 33% and a 66% shoot removal pruning treatment 45 days after harvest (33_45dAH and 66_45dAH respectively) and a control (Ctr), in which no post-harvest summer pruning was applied. To quantify pruning severity, the number and length of removed shoots, as well as the number of leaves and leaf area removed were determined at the time of the post-harvest summer pruning treatment application. After winter pruning was applied, the removed canes and shoots were measured to calculate the overall shoot length and leaf area removed per vine. The day after pruning treatments were applied, photosynthetically active radiation (PAR), as well as photosynthetic activity and related physiological parameters were measured. Cane and/or shoot, stem and root tissue, were sampled on 4 dates for assessing the effect of pruning treatments on NSC reserve status. Bud break and fertility were assessed through forced bud break and bud dissection for potential bud break and fertility, while actual bud break and fertility were assessed in the vineyard.

Post-harvest pruning proved to be beneficial for light penetration, but it did not improve the photosynthetic rate of the leaves. A few significant differences were recorded on the impact of the treatments on TNC. These however, do not show a clear trend. Post-harvest summer pruning did not have a significant effect on final bud break and potential fertility of grapevines in the season following the treatment. Based on this one season’s results, post-harvest pruning did not have overall practical benefits. Repeating the treatments for two more seasons on the same data vines, would indicate whether there is a carry-over effect of the practice on NSC, bud break and fertility. It is recommended that in a further phase of this project, available rapid and accurate methods to quantify carbohydrate reserves should be used and/or evaluated for use in grapevine studies, including Near-Infrared spectroscopy, as well as the starch iodine test (already commercially used in the apple and forestry industries).

Opsomming

Koolhidraatreserwes geproduseer deur fotosintese, word gestoor in permanente strukture van die wingerdstok, in die vorm van stysel en ongebonde suikerfraksies, of oplosbare suikers, meestal sukrose, glucose en fruktose. Hierdie reserwes word grootliks beïmvloed deur wingerdboukundige praktyke wat die bron-vraagpuntbalans in die wingerdtsok verander. Na-oes snoei is ‘n praktyk wat algemeen toegepas word deur verskeie tafeldruiprodusente, met die doel om koolhidraatreserwe-akumulasie te kanaliseer na die oorblywende lote. Weens hoë insetkostes van tafeldruifproduksie, moet enige manipulaise, insluitend na-oes somersnoei, slegs toegepas word, indien daar wetenskaplik bewys is dat dit praktiese en ekonomiese voordele het. Die doel van hierdie studie, bestaande uit twee proewe, was om (i) te bepaal of na-oes snoei lei tot verhoogde koolhidraatreserwestatus, verbeterde bot en vrugbaarheid; en (ii) ‘n basis te vestig vir kwantifisering en praktiese assessering van die koolhidraareserwestatus van wingerdstokke.

Die eerste proef het gefokus op die vasstelling van die seisoenale patroon van nie-strukturele koolhidraat (NSK) reserwes van *Vitis vinifera* L. cv. Sultanina H5 in die semi-ariede Benede Oranjriviergebied (BOR) en die Mediterreënse Hex River Vallei (HRV). Wortel-, stam-. winterloot- en/of groenlootweefsels is maandeliks gemonster en ontleed vir NSK. Die Anthrone metode is gebruik om oplosbare suikers en stysel te ontleed, terwyl ensiematiese ontleding gebruik is om spesifieke suikers te kwantifiseer (sukrose, d-fruktose en d-glukose). Stysel was die vorm van NSK wat die meeste voorgekom het in alle weefsels in beide gebiede. In beide gebiede, het die oplosbare suikers in die permanente weefsel (wortels, stamme, winterlote) hul hoogste konsentrasie tydens dormansie bereik (Junie-Julie). Die styselkonsentrasie was laag in alle weefsels in die winter (Julie), tydens dormansie, waarna dit toegeneem het tot ‘n piek in Augustus (voor bot) in alle permanente weefsels in die Semi-ariede gebied (wortels, stamme en winterlote) en in winterlote en wortels van die Mediterreënse gebied. ‘n Skerp afname in styselkonsentrasie het voorgekom vanaf dormansie tot blom in beide gebiede, wat dui op die afhanklikheid van die wingerdstsok van koolhidraatreserwes gedurende daardie periode. Akkumulasie van NSK reserwes het begin vanaf na blom en gestrek tot in die na-oesperiode, met bereiking van ‘n tweede piek in die herfs. Die algehele hoër styselkonsentrasies in die weefsels van die Semi-ariede gebied gedurende na-oes periode, word toegeskryf aan die langer na-oes periode waartydens gunstige toestande vir fotosintese heers, naamlik ‘n langer fotoperiode, hoër temperature en stralingsvlakke, in vergelyking met die koeler, Mediterreënse gebied.

‘n Grondslag is gelê vir monsterneming van wingerdweefsel vir kwalitatiewe assessering van wingerd NSK reserwestatus, deur die monsternemingtyd te koppel aan die voorkoms van pieke in oplosbare suiker- en styselkonsentrasies. Dit word aanbeveel dat monsterneming vir kwalitatiewe assessering van oplosbare suikers gedoen moet word na blaarval, tydens dormansie

(Junie-Julie onder die toestande van hierdie proef). Monsterneming vir assessering van styselkonsentrasie moet voor bot (Augustus onder die toestande van hierdie proef) gedoen word. Op grond van die betekenisvolle interaksies tussen NSK konsentrasies van verskillende weefseltipes, is weefseltipes wat gemonster kan word vir bepaling van die algehele NSK status van die wingerdstok geïdentifiseer. In die Semi-ariede gebied, kan enige van die bogrondse weefseltipes gemonster en ontleed word vir bepaling van die algehele NSK status van die stok. In die Mediterreënse gebied kan enige van die weefseltipes ontleed word vir stysel, terwyl groenlote en winterlote ontleed kan word vir bepaling van oplosbare suikers. Groenlote en/of winterlote word beskou as die mees praktiese weefseltipes om te monster vir bepaling van die wingerstok se NSK reserwestatus.

In die tweede proef is die effek van na-oes somersnoei op NSK reserwestatus, bot en vrugbaarheid van Sultanina H5 in die semi-ariede Benede-Oranjeriviergebeid ondersoek. Vyf na-oes snoeibehandelings is toegepas, naamlik ‘n vroeë 33% en ‘n 66% lootverwydering snoeibehandeling een dag na oes (33_1dAH en 66_1dAH onderskeidelik), ‘n laat 33% en ‘n 66% lootverwydering snoeibehandeling 45 dae na oes (33_45dAH en 66_45dAH onderskeidelik) en ‘n kontrole (Ctr), waar geen na-oes somersnoei toegepas is nie. Ten einde die strafheid van snoei te kwantifiseer, is die aantal en die lengte van die lote, asook die aantal blare en die blaaroppervlak wat verwyder is, bepaal direk nadat die na-oes somersnoeibehandeling toegepas is. Nadat wintersnoei toegepas is, is die lote wat verwyder is ook gemeet, om totale lootlengte wat per stok verwyder is, te bepaal. Die dag na toepassing van die na-oes somersnoeibehandelings, is fotosintetiese aktiewe radiasie (FAR) en fotosintese gemeet. Winter- en/of groenloot-, stam- en wortelmonsters is op vier datums gemonster om die effek van snoeibehandlings op NSK reserwestatus te bepaal. Bot en vrugbaarheid is geassesseer deur middel van geforseerde bot en oogontledings vir potensiële bot en vrugbaarheid, terwyl werklike bot en vrugbaarheid in die wingerd geëvalueer is.

Na-oes somersnoei het ligindringing deur die lower bevorder, maar dit het nie fotosintese bevorder nie. Na-oes somersnoei het geen betekenisvolle effek gehad op NSK reserwes, bot en vrugbaarheid in die seisoen wat gevolg het op die seisoen waartydens behandelings toegepas is nie. Op grond van hierdie een seisoen se resultate, het na-oes somersnoei nie enige praktiese voordele nie, behalwe verbeterde ligindringing. Herhaling van die behandelings vir twee verdere seisoene op dieselfde datastokke, behoort aan te dui of daar ‘n oordragingsefeek van hierdie praktyk op NSK, bot en vurgbaarheid is. Dit word aanbeveel dat, in ‘n verdere fase van hierdie projek, beskikbare vinnige en akkurate metodes vir kwantifisering van koolhidraatreserwes gebruik en/of geëvalueer moet word vir gebruik in wingerstudies, insluitend Naby-Infra-Rooi spektroskopie, asook die jodiumtoets vir stysel (wat reeds kommersieel in die appel- en bosboubedrywe gebruik word).

Biographical sketch

Keboneilwe Boitumelo Toolo was born in Thaba Nchu, South Africa. She matriculated from Unicom Agricultural Secondary School in Tweespruit in 2012. Keboneilwe obtained her BSc Agric degree in Viticulture and Oenology from Stellenbosch University in 2017. Upon completion, she enrolled for an MSc Agric degree in Viticulture (table grapes) at Stellenbosch University. During her MSc study period, she worked as an intern and research project team member at the ARC Infruitec-Nietvoorbij and in 2021 she started working for the cultivar evaluation company, Provar, where she still works to date.

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Preface

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

Chapter 1 **General Introduction and project aims**

Chapter 2 **Review of literature**
Seasonal dynamics of carbohydrate reserves of grapevines and factors contributing to it.

Chapter 3 **Research results**
Comparing the seasonal dynamics of total non-structural carbohydrate reserves of *Vitis vinifera* L. cv. 'Sultanina H5' grapevines in a winter rainfall and a semi-arid summer rainfall region of South Africa.

Chapter 4 **Research results**
Effect of post-harvest summer pruning on carbohydrate reserve status, bud break and fertility of *Vitis vinifera* L. cv. Sultanina H5 in the Lower Orange River region.

Chapter 5 **General conclusions and recommendations**

List of abbreviations:

LOR – Lower Orange River
GAP – Growth Arrest Phenomenon
RSG – Restricted Spring Growth
TNC – Total Non-structural Carbohydrates
NSC – Non-structural Carbohydrates
SA – South Africa
SATI – South African Table-Grape Industry
GDP - Gross Domestic Product
ABA – Abscisic Acid
R:FR – Red: Far-Red

ATP - Adenosine Triphosphate

NADPH - Nicotinamide Adenine Dinucleotide Phosphate Hydrogen

CO_2 – Carbon Dioxide

PGAL – Phosphoglyceraldehyde

MPCT - Minimal Pruning Converted

HRV - Hex River Valley

EtOH - Aqueous Ethanol

dH_2O – Distilled Water

UV – Ultraviolet

SAS – Statistical Analysis System

GLM – General Linear Models

LSD – Least Significant Difference

ANOVA – Analysis Of Variance

GDD - Growing Degree Days

PAR - Photosynthetically Active Radiation

PPFD – Photosynthetic Photon Flux Density

L.A. – Leaf Area

LAI – Leaf Area Index

TAI – Tree Area Index

LiDAR – Light Detection And Ranging

Ctr – Control

33_1dAH – 33% pruning one day after harvest

66_1dAH - 66% pruning one day after harvest

33_45dAH - 33% pruning 45 days after harvest

66_45dAH - 66% pruning 45 days after harvest

E-L - Eichhorn and Lorenz

P - Phosphorus

K - Potassium

Na – Sodium

Ca – Calcium

Mg – Magnesium

HPLC – High-Performance Liquid Chromatography

ELSD – Evaporative Light-Scattering Detector

PAD – Pulsed Amperometric Detector

NIR – Near-Infrared Reflectance

NIRS – Near-Infrared Reflectance Spectroscopy

ATR-FT-IR – Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy

MicroCT – Micro Computed Tomography

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Chapter 1: General introduction and project objectives

1.1 INTRODUCTION

Post-harvest pruning is a practice widely applied by several table grape producers in South Africa. This practice was recommended by Van der Merwe (2017) for less fertile cultivars (Sultanina, Sugraone, Crimson Seedless and Autumn Royal), to channel carbohydrate reserve accumulation to the remaining shoots. These are shoots that are selected to be the main canes in winter pruning (Van der Merwe, 2017). This manipulation of carbohydrate reserves is believed to improve the accumulation thereof as well as bud break and fertility. Due to the high input costs of table grape production, it is important to evaluate the return on investment (ROI) of each management practice carried out in the entire table grape production system. Any manipulation should be applied only if it is scientifically necessary and economically viable.

Vine nutrition plays a key role in vine morphology and phenology (Conradie 1980, 1981, 1990, 1992), and is vital for both vegetative and reproductive development (Bates *et al*., 2002; Bennett, 2002; Smith & Holzapfel, 2012). In a study by Pradubsuk (2008), grapevine seasonal nutrient contents were reported to follow a pattern that included translocation from perennial tissues to actively growing tissues at the start of the season. Carbohydrates are used by the grapevine for metabolism and growth (Iland *et al*., 2011; Rustioni *et al*., 2017). Produced *via* photosynthesis during the growing season, carbohydrate reserves are stored in perennial parts of the vine in the form of starch and free-sugar fractions or soluble sugars, mostly sucrose, glucose, and fructose (Candolfi-Vasconcelos *et al*., 1994; Jones *et al*., 1999; Bates *et al*., 2002; Lebon *et al*., 2008; Holzapfel, 2009; Rogiers *et al*., 2011; Smith & Holzapfel, 2012). These reserves are used for both vegetative and reproductive growth, development and respiration, indicating the dependence of the grapevine on stored reserves (Bennett, 2002; Lebon *et al*., 2008; Rustioni *et al*., 2017).

Zapata *et al*. (2004) reported that significant amounts of starch as well as nitrogen are taken up from roots (E-L 1-7) (Figure 1.1). In early spring, when leaves unfold and expand (from E-L 7), they are considered to be sinks, as they are either non-photosynthetic or do not produce sufficient photosynthates to sustain their nutrient and energy requirements (Keller, 2010). A sink is a non-photosynthesising part of a plant or a part that does not produce enough photosynthates to sustain itself. A source on the other hand, is a part of the plant that produce and export photosynthates (Vivin *et al*., 2002; Keller, 2010). The mobilisation of carbohydrate reserves in early spring period is therefore important (Holzapfel *et al*., 2006). From E-L 7-19, a strong starch mobilisation was recorded and reached a minimum at early flowering (Figure 1.1) (Zapata *et al*., 2004)

According to Keller (2010), immature leaves need to build up their photosynthetic "machinery" before they can start to photosynthesise. Once leaves have attained about one-third of their mature size, they cease to be sinks and begin to produce and export photosynthates to the new sinks *via* the phloem (Holzapfel, 2009; Keller, 2010). Zapata *et al*. (2004) further reported an accumulation of starch in roots and canes from flowering to pea size (E-L 19-31) (Figure 1.1). At this stage, the vine is no longer solely dependent on carbohydrate reserves as its source. As the season progresses, there is a fluctuation in the level of carbohydrate reserves in the vine as source-sink relations are involved (Rustioni *et al*., 2017). From flowering to maturation of bunches, large quantities of carbohydrates are used mainly during the development and growth of fruit. Root development and growth have also been shown to be one of the competitive sinks for carbohydrate reserves, especially in the month before bud break (Holzapfel, 2009).

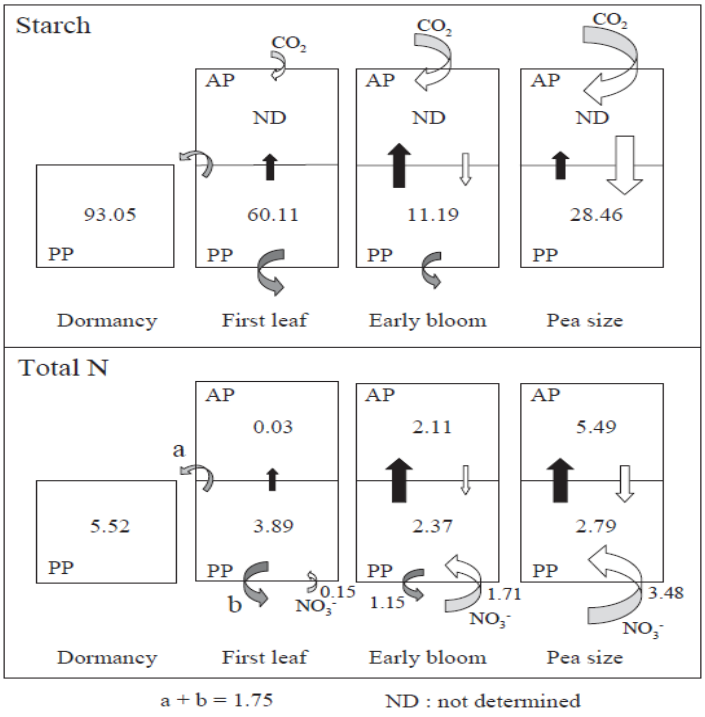

Figure 1.1: Contribution of the main nutrient flux during the third growing season to the partitioning of starch and total N in perennial tissues of grapevine cv. Pinot noir. Loss through root necrosis; N uptake; reserve mobilization; storage; CO_2 assimilation; loss through bleeding sap (Zapata *et al*., 2004). PP- Perennial parts, AN- Annual parts.

Accumulation of photosynthetic products in the perennial parts of the vine, viz., roots, trunks, canes and shoots is important in that it can affect as well as determine the carbohydrate reserve levels in the vine (Lebon *et al*., 2008; Smith & Holzapfel, 2012). The post-harvest period (E-L 41-74) has been reported to be vital for carbohydrate reserve replenishment as the only sinks are young leaves (Smith & Holzapfel, 2012). The level of carbohydrate reserves accumulated will be determined by the vigour of the vine, nutrition, growth activity, water as well as frost damage

(Holzapfel *et al*., 2010; Hunter *et al*. 1995; Rodrigues *et al*., 1993, Sommer *et al*., 2000; Smith & Holzapfel, 2012; Bennett *et al*., 2005).

In winter, during dormancy (E-L 1), carbohydrate reserves have been reported to contribute to cold hardiness and are used in the form of sugars, which have been converted from the breakdown of starch (Williams, 1996; Jones *et al*., 1999; Bennett, 2002; Kaplan & Guy, 2005; Smith & Holzapfel, 2012). The conversion of starch to soluble sugars and accumulation of sugars in the vacuole results in an increased negative osmotic potential and decreased freezing point of cellular sap (crypto protection) (Holzapfel *et al*., 2010).

Post-harvest summer pruning is applied, in addition to winter pruning, by several producers in warm arid, semi-arid, as well as subtropical grapevine production regions, in contrast to the general practice in Mediterranean regions, where only winter pruning is applied (Van der Merwe, 2017). These warm regions have been shown to possess a long, continuous period of vegetative growth activity post-harvest, during which summer pruning is applied (Conradie, 1990; Smith & Holzapfel, 2012). This practice has been reported to contribute to increasing bud break and fertility for the next season, as well as controlling vegetative growth in the following spring (Smith & Holzapfel, 2012; Van der Merwe, 2017). Contrary to this, Smith and Holzapfel (2012) and Bennett *et al*. (2005) reported that summer defoliation controls the vigour of the vine in the following spring by slowing down shoot elongation and a reduction in carbohydrate reserves is associated with decreases in inflorescence number per shoot and flower number per inflorescence.

Accumulation of reserves will be negatively affected by injudicious and severe removal of shoots, as well as continuous vegetative growth in the post-harvest period, which is characteristic of warmer production regions such as the Lower Orange River (LOR) region. In this region, post-harvest vegetative growth often continues into late autumn due to high prevailing temperatures (Volschenk & Hunter, 2009). Furthermore, sudden frost during April or May causes defoliation, forcing vines into dormancy and cutting short the natural process of reserve accumulation (van der Westhuizen, *et al.*, 2001).

The general seasonal pattern of carbohydrate reserves can be summarised as follows: Starch concentration reaches a peak in late autumn, declines as winter approaches and reaches a lower peak shortly before or at the beginning of bud break. In winter, sugar concentration increases to a peak as starch concentration decreases. This is associated with cold hardiness developed by the vine during the coldest part of winter, during which starch is converted to sugars, inducing freezing tolerance (Winkler & Williams, 1945; Kaplan & Guy, 2005; Mills *et al*., 2006; Ruelland *et al*., 2009; Janská *et al*., 2010; Keller, 2010; Ferguson *et al*., 2011). This conversion is reversed as temperatures begin to increase and dormancy is lifted (Williams, 1996). From bud break to

flowering, the carbohydrate reserve concentration declines to a minimum. From then, it accumulates again through to the post-harvest period (Holzapfel, 2009).

Studies on grapevine carbohydrate reserves have not yet been extensively carried out in South Africa, especially in the warm climate regions. Conradie (1980; 1981; 1990; 1992) and Uys (1981) conducted studies on seasonal nutrient and reserve status in a cooler climate (Mediterranean-Western Cape) which has a different seasonal pattern and on wine grape cultivars. The table grape industry, however, comprises of five regions, namely the Olifants River, Berg River, the Hex River (these three fall under the Western Cape with the Mediterranean climate), the Orange River and the Northern Provinces (which comprise of arid and semi-arid climates) (SATI, 2021). Of all these regions, the Orange River as well as the Hex River are the major production areas in South Africa (producing 19 207 361 and 18 649 830 cartons, respectively) (SATI, 2019).

The work conducted by Saayman (1983), focused on the link between the occurrence of the growth arrest phenomenon (GAP), and grapevine carbohydrate and nitrogen status in the LOR region. However, this study only focused on four stages within a growing season, namely dormancy, before and after harvest, as well as the growth arrest stage in October. There was an unbalanced carbohydrate and nitrogen status of GAP vines, which was correlated to vigour, fertile soil and high temperature during the growing season, contributing to vigorous and continuous shoot growth after harvest.

The longer period of post-harvest vegetative growth, as well as the effect of high temperatures on respiration rate (including carbohydrate depletion) in the warm climate of the LOR are expected to affect reserve accumulation negatively. It is also expected that the seasonal carbohydrate reserve pattern of the grapevine in the LOR will differ from that of the Hex River Valley (HRV) which possesses a Mediterranean climate like the few reported by previous studies, especially concerning when the starch and sugar peaks will occur. Accurate establishment of when these peaks take place will be crucial to identifying the optimal time for sampling to assess grapevine carbohydrate status. This is also required for informed decision-making when it comes to table grape cultivation practices such as post-harvest pruning.

Regarding post-harvest pruning, Van der Merwe (2017) recommended that all non-lignified (green) shoots, as well as strong shoots (lignified), should be removed (not more than one-third of the shoots) during the last two weeks of February in the early regions and in the first week of March in the late regions. This practice is recommended and applied in the industry for Sultanina, Sugraone, Crimson Seedless and Autumn Royal. These cultivars respectively comprise 11.4 %, 7.5 %, 2.3 % and 0.5 % of the 5 857 ha under table grape production in the LOR region (SATI, 2021).

The LOR was selected as the region in which the post-harvest summer pruning study was conducted as it was the second-largest table grape-producing region in South Africa at the initial stages of this study (SATI, 2019). Sultanina was selected for this study, because it is one of the major cultivars produced both in South Africa and globally and was reported to be the third-largest produced cultivar in South Africa, the second largest produced in the LOR, (SATI, 2021) and is prone to GAP, which is known to often occur in warm areas like the LOR region. Sultanina H5 was included, because this clone is considered slightly superior to H4 as a table grape (Evans & Smit, 1985; Habili *et al*., 1997).

This study was aimed at providing scientific results towards answering the following questions asked by the industry:

1. Does post-harvest summer pruning result in practical and economic benefits for the producer, *i.e*., increased carbohydrate reserve status and improved bud break and fertility?
2. How can “good reserve status” of a grapevine be quantified and practically assessed?

The following was therefore investigated:

1. The seasonal dynamics of total non-structural carbohydrates (TNC) in grapevines.
2. The effect of post-harvest summer pruning on carbohydrate reserve accumulation; and
3. The link between carbohydrate reserve accumulation, bud break and fertility.

1.2 PROJECT AIMS AND OBJECTIVES

1.2.1 Aims

This study aimed to determine whether post-harvest pruning results in increased carbohydrate reserve status, contributing to improved bud break and fertility, as well as to establish a base for quantifying and practically assessing the reserve status of grapevines.

Main aim: Determine the effect of post-harvest pruning on carbohydrate reserve status, bud break and fertility of table grapevines.

Sub aim: Establish the link between reserve status, vigour, bud break and fertility.

1.2.2 Objectives

Objective 1: Establish the seasonal pattern of TNC of table grapevines (Sultanina H5), in two South African production regions, namely the LOR region (warm, semi-arid climate) and the HRV (Mediterranean climate)

Objective 2: Determine the effect of post-harvest pruning treatments on carbohydrate reserve status, bud break and fertility of table grapevines (Sultanina H5) in the LOR region (warm, semi-arid climate).

Objective 3: Establish a base for quantifying and practically assessing the reserve status of grapevines.

The expected benefits of this study for the table grape industry:

1. Establish whether post-harvest pruning has scientifically proven benefits and is economically justified.
2. If post-harvest pruning has scientifically proven benefits, establish guidelines regarding how and when it should be done.
3. Establish a base for quantifying and practically assessing "good reserve status" of grapevines, linked to bud break, vigour and fertility.

1.3 LITERATURE CITED

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Chapter 2: Seasonal dynamics of carbohydrate reserves of grapevines and factors contributing to it

2.1 INTRODUCTION

The grapevine is a deciduous, woody, creeping plant that is perennial, polycarpic and adapted to warm temperate climates (Mullins *et al*., 1992; Holzapfel *et al*., 2010; Keller, 2010). The deciduous nature and associated dormancy rely on the accumulation of carbohydrates during favourable seasonal conditions to sustain plants through dormancy and provide for the resumption of vegetative and reproductive development and re-establishment of photosynthetic capacity in the following season (Scholefield *et al*., 1987; Bates *et al*., 2002; Bennett, 2002; Holzapfel *et al*., 2010; Smith & Holzapfel, 2012).

Carbohydrates are produced *via* photosynthesis, provide the building blocks for plant structure (Iland *et al*., 2011); and are either used in metabolism and growth or stored as reserves in the woody permanent structures (cordons, trunks and roots) of the vine (Candolfi-Vasconcelos *et al*., 1993; Bouard, 1996; Lebon *et al*., 2008; Wojnarowiez *et al*., 2008; Iland *et al*., 2011; Rogiers *et al*., 2011; Hartmann & Trumbore, 2016). Structural carbohydrates are molecules that provide building blocks for biomass, utilised for building structures, whereas non-structural carbohydrates (NSC) are critical substrates for plant growth and metabolism (Hartmann & Trumbore, 2016; Han *et al*., 2020). Structural carbohydrates, including hemicellulose and cellulose, represent about 80% of the total seasonally assimilated carbon in grapevines, while the balance of the assimilated carbon is accumulated as non-structural carbohydrate reserves (Holzapfel *et al*., 2010), comprising of starch and sugars (Candolfi-Vasconcelos *et al*., 1993, Bouard, 1996, Bates *et al*., 2002, Wojnarowiez *et al*., 2008, Holzapfel *et al*., 2009, Rogiers *et al*., 2011, Smith & Holzapfel, 2012). Non-structural carbohydrate reserves are available for use in respiration, or translocation to areas where they are required (Uys, 1981; Cheng *et al*., 2004).

Carbohydrate reserves are stored in the form of starch and sugars (Bates *et al*., 2002; Zapata *et al.*, 2004; Holzapfel, 2009). These sugars are soluble and consist primarily of sucrose, d-glucose and d-fructose (Candolfi-Vasconcelos *et al*., 1994; Jones *et al*., 1999; Bates *et al*., 2002; Lebon *et al*., 2008). Starch is the most important and main reserve compound in plants storage tissues (Winkler & Williams, 1945; Vaillant-Gaveau *et al*., 2014; Dayer *et al*., 2020).

In this chapter, the currently available published research results regarding the following are reviewed: Production and accumulation of carbohydrate reserves, seasonal dynamics of carbohydrate reserves of grapevines, factors affecting production and accumulation of

carbohydrate reserves, grapevine abnormalities associated with carbohydrate status, as well as methods for quantifying carbohydrate reserve content of grapevine tissues.

2.2 PRODUCTION AND ACCUMULATION OF CARBOHYDRATE RESERVES

Grapevines use light as an energy source for physiological processes, including photosynthesis, bunch primordia induction, chlorophyll degradation, formation of carotenoids and anthocyanins (Uys, 1991; Keller, 2010). The amount of light intercepted by the grapevine canopy differs with latitude, season, time of day and cloud coverage (Keller, 2015).

Photosynthesis is a vital process in which the plant produces organic material required for both vegetative and reproductive growth (Filimon *et al*., 2016). In this process, light in the visible wavelength range of 400-700 nm is used by the plant. This light penetrates the chloroplast and is converted from light into chemical energy in the form of adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide phosphate (NADPH), providing energy for assimilation of carbon and other nutrients as well as providing energy for other metabolic processes taking place in the chloroplast (Baker, 2008; Strever, 2012). The end products of carbon dioxide fixation are carbohydrates and oxygen (Rabinowitch & Govindjee, 1969). Sucrose is formed outside the chloroplast with energy provided by ATP, whereas starch is found in plastids, formed in most leaf chloroplasts. Starch is the primary carbohydrate reserve in grapevines and is stored as water-insoluble granules in amyloplasts (Hunter *et al*., 1995; Strever, 2012).

For the vine to produce the maximum quantity of photosynthetic products, the maximum surface of leaves per hectare should be exposed to PAR (Uys, 1991). In a study on Carbenet Sauvignon trained onto a two-wire vertical system, Dokoozlian and Kliewer (1995) reported that in canopies of low leaf area density (< 4 m^2 leaf area m^{-1} canopy of length), sun flecks illuminated 20% to 40% of the surface area. This illumination was reported to have contributed to the photosynthesis in the canopy. In the same study, in a dense canopy (> 8 m^2 leaf area.m^{-1} canopy length), however, the sun flecks were reported to be almost absent and their contribution to the photosynthesis in the canopy was minimal. When a leaf receives direct radiation, 10% is reflected, 9% transmitted, and 81% absorbed (Dokoozlian & Kliewer, 1995; Poni & Intrieri, 2001; Strever, 2012). From the absorbed light, 60% is used for transpiration and convection, 20% is emitted as infrared, and about 1% for photosynthesis (Champagnol, 1984; Strever, 2012).

The penetration of light into the canopy is affected greatly by the number of leaf layers in that canopy (Strever, 2012). The level of photosynthesis differs between sun-exposed and shaded leaves. Due to the longer palisade parenchyma cells or an additional cell layer in sun-exposed leaves, they are characteristically thicker and more absorption efficient (Strever, 2012). Leaves

developed in shade exhibit maximum photosynthesis of 30% to 50% less than those developed in the sun (Poni & Intrieri, 2001). The age of a leaf determines how much light is transmitted as the chlorophyll content and overall photosynthetic efficiency are affected by leaf age (Poni & Intrieri, 2001; Strever, 2012). Poni and Intrieri (2001) and Keller (2015) reported that the photosynthesis activity of a vine leaf rapidly increases until up to 40 to 45 days and thereafter, exhibits a decline.

Carbohydrates are produced *via* photosynthesis in the leaf; however, the process can also occur in green shoots due to the presence of chlorophyll (Keller, 2010). This is the process whereby carbon from the atmosphere is fixed to produce photosynthates. In this process, the light reaction products (Figure 2.1A) are consumed by the dark reaction (Figure 2.1B) to produce sugars and starch (Huglin & Schneider, 1998; Meyer, 2008; Strever, 2012; Keller, 2015). A schematic diagram of the light and dark reactions is presented in Figure 2.1A-B.

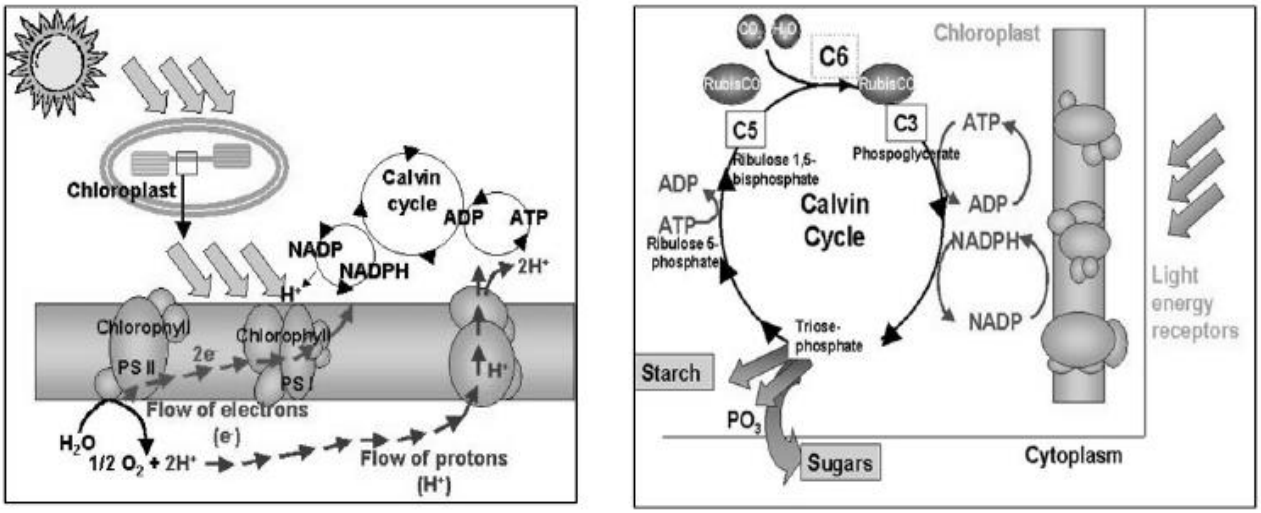

Figure 2.1: Simplified diagram of the light (left- A) and dark (right- B) reactions of grapevine photosynthesis (Huglin & Schneider, 1998 adapted from Strever, 2012).

The carbon dioxide from the atmosphere is taken up by the leaf through the stomata by diffusion. In the stroma, the CO_2 reacts with ribulose-1,5-bisphosphate to form phosphoglyceraldehyde (PGAL). This process requires a source of energy, which is provided by ATP and NADPH. Some of the PGAL molecules produced from this cycle generate carbohydrates while the rest are reused in the cycle (Figure. 2.1B) (Strever, 2012; Keller, 2015).

2.3 SEASONAL DYNAMICS OF CARBOHYDRATE RESERVES OF GRAPEVINES

The carbohydrate dynamics of grapevine carbohydrate reserves have been studied for various cultivars (Cabernet franc, Cabernet Sauvignon, Carignane, Chardonnay, Chasselas, Merlot, Pinot noir, Riesling, Sangiovese, Semillon, Shiraz, Tempranillo) (Winkler, 1929; Picket & Cowart 1941;

Winkler & Williams 1938 and 1945; Bernstein & Klein, 1957; Eifert *et al*., 1961; Marutyan, 1962; Eifert & Eifert 1963 and 1966; Kliewer 1965 and 1996; Scholefield *et al*., 1978; Baines *et al*., 1981; Loescher *et al*., 1990; Bates *et al*., 2002; Zapata *et al*., 2004; Bennett *et al*., 2005; Holzapfel, 2009; Smith & Holzapfel, 2012; Zufferey *et al*., 2012; Philips *et al*., 2015) as well as regions/climate (in Australia) (Holzapfel, 2009; Hall *et al*., 2016).

The seasonal change in starch concentration in grapevines can be summarised as follows (Eifert *et al.*, 1961 cited by Uys, 1981; Bates *et al*., 2002; Zapata *et al*., 2004; Holzapfel, 2009; Smith & Holzapfel, 2012; Köse & Ateş, 2017): Starch concentration peaks in autumn, decreases as temperatures and day length are decreased in winter and reaches a second lower peak shortly before or at the beginning of bud break. From bud break, there is a steep decrease in starch concentration to bloom, when it reaches a minimum level. The starch concentration increases after flowering, where after the increase is slowed down at véraison, with a continued decrease to harvest. In the post-harvest period, the starch concentration increases to reach a peak in autumn. Soluble sugar concentration reaches a peak in late summer, decreases as starch increases up to its peak in autumn and increases rapidly as winter approaches (Köse & Ateş, 2017). The reverse fluctuation in starch and sugar concentration is caused by sugar-starch interconversion (Winkler & Williams, 1945; Eifert *et al*., 1961; Williams, 1996; Rossouw *et al*., 2017).

The period between the two starch peaks in the grapevine seasonal cycle differs according to the temperature and the length of the winter period. With the work conducted in cool climate regions, the duration of this period was reported to vary from 5 months in Hungary (Eifert *et al*., 1961; Uys, 1981) to 4 months in California (Winkler & Williams, 1945) and the Western Cape of South Africa (Van der Westhuizen, 1980). Under warmer conditions in the Jordan Valley, Israel, it was just more than 3 months (Bernstein & Klein, 1957); and in Wagga Wagga, Australia, it was also 3 months (Smith & Holzapfel, 2012).

Several authors reported that the highest sugar concentration in dormant buds occurs during the coldest part of the winter (Uys, 1981; Kaplan *et al*., 2004; Mohamed *et al*., 2012; Smith & Holzapfel, 2012; Rubio *et al*., 2016). This high concentration corresponds with the cold hardiness (freezing tolerance) the grapevine develops at low temperatures in winter/full endodormancy, enabling it to survive temperatures as low as -40°C (Mills *et al*., 2006; Keller, 2010; Ferguson *et al*., 2011). Low temperatures exposure of buds induces development of cold hardiness, characterised by the breakdown of starch, accumulation of soluble sugars and up-regulation of dehydrin genes (Kaplan *et al*., 2004; Mohamed *et al*., 2012; Smith & Holzapfel, 2012; Rubio *et al*., 2016; Köse & Ateş, 2017). The conversion of starch to soluble sugars results in an increased

negative osmotic potential and decreased freezing point of cellular sap (crypto protection) (Holzapfel *et al*., 2010).

Holzapfel *et al.* (2009) studied the seasonal dynamics of carbohydrate reserves of grapevines in two regions of Australia, by excavating whole vines, and results are depicted in Figures 2.2a-b. Root carbohydrate reserve concentrations were higher than in the above-ground parts of the vine. In the warmer region (Figure 2.2a), the post-harvest period (harvest to leaf fall) was longer and the maximum increase in root carbohydrate reserve concentration was higher compared to the cooler region (Figure 2.2b). In the same study, it was found that root starch was the most predominant carbohydrate, with the highest peak reached towards the beginning of leaf fall. At this stage (before pruning), the root system contained 57% of the total vine carbohydrates, with medium-small sizes containing higher concentrations than large-sized roots. The canes contained 12% of the total vine carbohydrate reserves, followed by the trunk and cordons, which collectively represented 26%. The remaining 5% was in the rootstock shank. Hunter (1998) obtained similar results in which roots contained the highest percentage of carbohydrates, namely 51% of the total vine reserves.

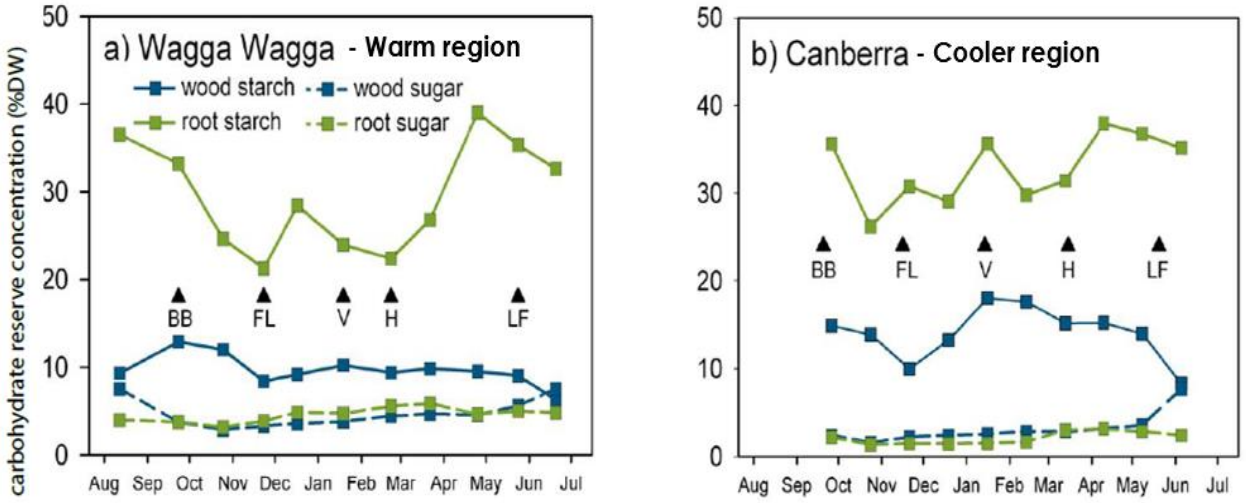

Figure 2.2: Seasonal pattern of carbohydrate reserve dynamics in the wood and roots of mature own-rooted Shiraz vineyards at two locations during the 2006/07 season. (a)- Wagga Wagga (warm region) and (b)- Canberra (cooler region) (adapted from Smith & Holzapfel, 2012).

In a review of literature on dynamics of grapevine carbohydrate reserves spanning from 1893 to 2009, Holzapfel *et al*. (2010) summarised the seasonal course of the concentration of carbohydrate reserves (on a dry weight basis) in the major storage organs (roots, trunks and canes), as well as the shoots and buds, as follows:

(i) The concentration of nonstructural carbohydrates in roots declines from ca. 22% to 25% at bud break to ca. 5 to 16% at anthesis.

(ii) Root starch concentrations generally decrease from ca. 18% to 22% at bud break to ca. 3% to 11% at anthesis or later, increases thereafter and the concentrations reached at leaf fall are conserved or decline slightly during winter (dormancy).

(iii) Total soluble sugar concentration in roots decreases from ca. 3% to 6% at bud break to 2% to 4% at anthesis and increases to peak levels, ca. 3 to 6%, near fruit maturity. Through autumn and winter, changes in the soluble sugar concentration in roots are minor.

(iv) The concentration of nonstructural carbohydrates in trunks declines from ca. 18% to 20% at bud break to ca. 10% to 12% between anthesis and véraison; increasing thereafter to peak levels, ca. 15% to 18%, after fruit maturity.

(v) Trunk starch concentration decreases from ca. 10% to 14% at bud break to minimum levels of ca. 6% to 12% at anthesis or later and increases thereafter to peak levels, ca. 10% to 16%, after fruit maturity. In contrast to roots, trunk starch concentrations decrease to a midwinter minimum, attributed to interconversions with sugars.

(vi) Total soluble sugar concentration in trunks decreases from a peak of ca. 4% to 12% in midwinter, to a minimum of ca. 1% to 4% at anthesis or several weeks later, whereafter it increases during mid and late summer until midwinter.

(vii) The total nonstructural carbohydrate concentration in the current season's shoot tips increases rapidly from ca. 4% at bud break to 14% at 2 weeks after bud break, decreases to ca. 5% near véraison, and increases to ca. 19% at leaf fall, when the shoot, which has completed lignification, is regarded as a cane.

(viii) After the transition from shoot to cane, the total nonstructural carbohydrate concentration in canes decreases slightly (by 2 to 4%) from dormancy to bud break. The total nonstructural carbohydrate concentration in canes decreases from ca. 8% to 12% at bud break to ca. 3% to 6% around anthesis, whereafter it increases to ca. 13% to 16% by leaf fall.

(ix) Cane starch concentration decreases from ca. 5% to 16% to midwinter levels of ca. 5% to 8%, whereafter it increases due to interconversion with soluble sugars, to ca. 7% to 15% at bud break. It then decreases to ca. 1% to 2% between anthesis and véraison, whereafter it increases to ca. 6% to 8% in late summer and may decline to ca. 5% at leaf fall.

Climate is one of the major factors that affect grapevine production (Winkler *et al*., 1974) and the carbohydrate reserve pattern of grapevines (Field *et al*., 2009; Rogiers et al., 2011; Sawicki *et al*., 2015; Dahal *et al*., 2018). Accumulation of reserves will be negatively affected by continuous vegetative growth in the post-harvest period, which often occurs in warmer production regions, such as the LOR. In the LOR, vegetative growth often continues into late autumn, due to prevailing high temperatures, with an average of above 19°C for April and May (Source: Ileaf:

www.ileaf.co.za). Sudden cold and/or frost occurring in April or May, defoliate these vines, forcing them into dormancy, without the natural process of reserve accumulation having occurred (Van der Westhuizen *et al*., 2001, Volschenk & Hunter, 2009). Several authors linked continuous vegetative growth in autumn to insufficient reserve accumulation and the occurrence of growth arrest phenomenon (GAP) or restricted spring growth (RSG) in the following spring (Van der Westhuizen *et al*., 2001; Volschenk, 2005; Holzapfel, 2009; Volschenk & Hunter, 2009). Reductions in carbohydrate reserves were associated with reduced vegetative growth and yield in the following season (Hunter *et al*., 1995; Bennett *et al*., 2005; Smith & Holzapfel, 2012).

Most of the previous studies on the seasonal pattern of non-structural carbohydrate reserves in grapevines, were conducted in mediterranean regions. The work done in South Africa by Conradie (1980, 1981, 1990, 1992, 2005) and Uys (1981) on grapevine seasonal nutrient and reserve status was conducted in the Western Cape under cool climate conditions and on wine grape cultivars. The work done by Saayman (1983) in the LOR region on 'Sultanina' investigated the causes of GAP and was limited to cane sampling for determining starch and sugar concentrations at only four phenological stages and not over the whole season. The study of Volschenk (2005), investigated the causes of die-back of young vines in the LOR region and the only carbohydrate analysis conducted was the starch concentration of young, grafted vines grown in a glasshouse under controlled conditions, determined over 10 weeks.

2.4 FACTORS AFFECTING PRODUCTION AND ACCUMULATION OF CARBOHYDRATE RESERVES

Factors affecting carbohydrate reserve accumulation and mobilisation include: Genetic factors (cultivar) (Bates, *et al*., 2002; Holzapfel *et al*., 2010) and vine age (Bates *et al*., 2002), phenological development (Holzapfel *et al*., 2010; Köse & Ateş, 2017), abiotic and biotic environmental factors (Field *et al*., 2009; Holzapfel *et al*., 2010, Rogiers *et al*., 2011; Sawicki *et al*., 2015; Dahal *et al*., 2018), as well as viticultural practices, including pruning/leaf removal (Scholefield *et al*., 1978; Marangoni *et al*., 1980; Rühl & Clingeleffer, 1993; Candolfi-Vasconcelos *et al*., 1994; Clingeleffer & Sommer, 1995; Bennett *et al*., 2005; Vršič *et al*., 2009; Ikinci, 2014; Greven *et al*., 2016), crop control (Holzapfel, 2009; Smith & Holzapfel. 2012) and irrigation (Deloire *et al*., 2004, Holzapfel, 2009; Pellegrino *et al*., 2014; Rossouw *et al*., 2017). In sections 2.4.1. to 2.4.4, an overview of factors affecting grapevine carbohydrate status is presented.

2.4.1 Genetic factors

Holzapfel *et al*. (2010) reported that grape cultivars (Semillon and Ungi blanc, Muscat d'Alexandrie, Syrah, Arriloba and Chardonnay) show no difference in maximum photosynthetic rates in response to varying light intensities under optimum conditions with sufficient water supply.

However, cultivar differences do occur in response to environmental factors that influence photo assimilation, including water deficits, impacting stomatal conductance. Ershadi *et al*. (2016) reported significant differences in soluble carbohydrates among 12 grapevine cultivars (’Red Sultana’, ’Fakhri’, ‘Shahani’, White Sultana, ‘Tabarzeh’, ‘Gaznei’, ‘Thompson Seedless’, Laal, ‘Sahebi’, ‘Rishbaba’, ‘Yaquti’ and ‘Ruby Seedless’) at four sampling dates in Western Asia, Iran (November, January, March and April). In a study to evaluate the relationship between bud death and soluble carbohydrates after winter cold, higher concentrations were found in ’Bronx Seedless’ and ’Cardinal’ compared to ’Autumn Royal’ and ’Superior Seedless’ (Kaya, 2020).

2.4.2 Phenological development

Post-harvest period and dormancy

Once the grapes have been harvested, the vine continues to undergo vegetative growth for as long as the environmental conditions are favourable (Bates *et al*., 2002; Keller, 2010). This period is very important as replenishment of carbohydrates in perennial parts occurs (Smith & Holzapfel, 2009; Pellegrino *et al*.,2014; Hall *et al*., 2016). The length of this period differs between cultivars and climate conditions, namely in cool climatic areas, the period is shorter compared to warmer areas (Bates *et al*., 2002; Holzapfel *et al*. 2010; Holzapfel & Smith, 2011). According to Holzapfel *et al*. (2006), post-harvest practices may alter the vine’s capacity to replenish nutrient reserves (see Section 2.4.4).

As day length and temperatures start decreasing, vegetative growth rate gradually declines due to the decrease of gibberellin in the shoot elongation zone (Keller, 2010) and accumulation of carbohydrate (starch and soluble sugars) reserves starts to slow down. Bud break of compound buds is prevented by apical dominance from the growth tip during this stage and is known as paradormancy, summer rest, or pre-dormancy (Figure. 2.3) (Lavee & May, 1997; Anderson *et al*., 2005; Rohde & Bhalerao, 2007; Fracheboud *et al*., 2009; Keller, 2010; Pérez & Noriega, 2018). According to Williams (2000), Tanaka *et al*. (2006), and Keller (2010), dormant compound buds can burst due to lateral shoot removal, severe leaf removal and/or shoot tip removal. However, prompt or lateral buds are not dormant and can emerge to form lateral shoots (Keller, 2010).

Leaf senescence, as indicated by red and/or yellow colouration, depending on the cultivar, occurs during this period (Keller, 2010). The colour change is due to the degradation of chlorophyll and formation of yellow/orange carotenoids (Keller, 2010). Because chlorophyll is being degraded, photosynthesis slows down and fewer carbohydrates are stored in permanent parts. The initiation of leaf senescence is mainly affected by decreasing day length (Keller, 2010). Low temperatures, however, speed up the process compared to higher temperatures (Fracheboud *et al*., 2009; Keller, 2010). Thomas & Stodart (1980) reported that heat stress of ≥ 45°C accelerates

senescence. Carbohydrate reserve accumulation slows down as carbohydrate production decreases due to decreased day lengths and temperature (Keller, 2010).

As days shorten and temperatures decrease (Rubio *et al*., 2016) the vine transitions from paradormancy to endodormancy (Figure 2.3), which is induced by an increase in abscisic acid (ABA) within the buds (Rohde & Bhalerao, 2007; Keller, 2010). At this stage, carbohydrate concentrations are at a peak, due to the accumulation in the post-harvest period, when the leaves were still photosynthesising (Rubio *et al*., 2016). Although respiration takes place in the roots during endodormancy, the quantity of carbohydrates used for this process is not significant (Keller, 2010).

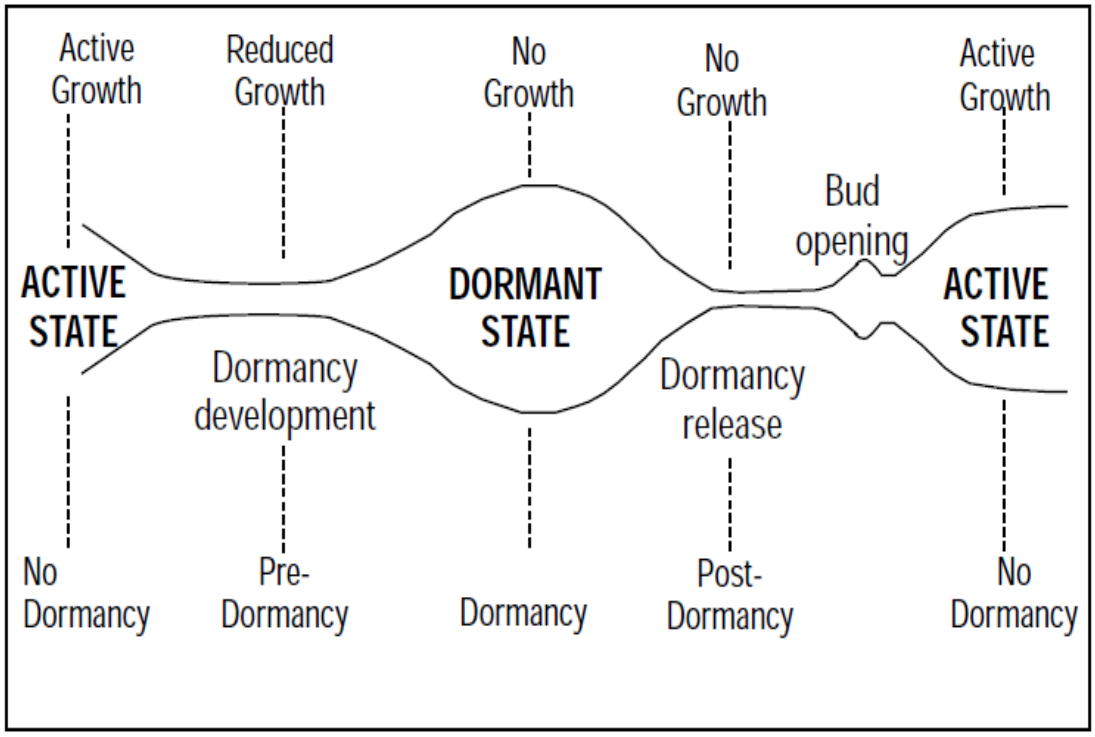

Figure 2.3: Schematic description of dynamics of the annual growth cycle of the grapevine in relation to shoot growth and bud dormancy (after Lavee & May, 1997).

Mohamed *et al*. (2012) and Rubio *et al*. (2016) reported a high starch concentration in dormant buds compared to non-dormant buds. As dormancy is lifted due to increased temperatures at the end of winter, a reverse conversion of sugars to starch occurs (Williams, 1996).

Bud break

The vine must be exposed to a minimum of 200 chilling units, a daily average temperature of less than 10°C for seven consecutive days, to be released from endodormancy (Keller, 2010). In regions with warm winters, insufficient chill unit accumulation results in prolonged endodormancy, and bud break tends to be delayed, uneven and reduced, leading to decreased shoot and cluster counts per vine, as well as poor uniformity of berry development, ultimately reducing yield

(Dokoozlian & Williams, 1995; Keller, 2010; Mohamed *et al*., 2012). To alleviate this, hydrogen cyanamide, a rest-breaking agent that inhibits catalase activity, resulting in the accumulation of hydrogen peroxide, is applied (Lombard *et al*., 2006; Keller, 2010; Mohamed *et al*., 2012). This agrochemical is used to induce early and even bud break (Carreño *et al*., 1999; Keller, 2010; Mohamed *et al*., 2012). In cold winter regions where sufficient chilling units are attained naturally, bud break occurs earlier, more even, and at a higher rate (Dokoozlian, 1999).

In a study on Superior Seedless, Mohamed *et al*. (2012) reported a rapid decline of starch content of buds to its lowest concentration 5 days after treatment with hydrogen cyanamide, while untreated buds reached their lowest concentration after 15 days from when treatment was applied. In the buds treated with hydrogen cyanamide, sucrose and glucose concentrations accumulated temporarily and decreased rapidly when bud break commenced (Mohamed *et al*., 2012).

Mean daily temperatures ≥ 8°C promote bud break, but the exact requirement differs between cultivars. Reserve uptake and assimilation remain low for a few weeks after bud break (Conradie, 1980; Löhnertz, 1988). The onset of bud break is marked by the plant exuding xylem sap, also known as "bleeding" (Keller, 2015). This "bleeding" is caused by root pressure, which in turn is caused by reserve remobilisation into the xylem (Keller, 2015). During the first stages of bud break, starch stored in the primordial shoot and surrounding bud scales during the previous season supports development, whereafter starch from canes emerging from dormancy is utilised (Holzapfel *et al*., 2010).

Shoot and inflorescence development

Shoot and inflorescence development occurs for the first 8 to 10 weeks after bud break. As new growth commences, carbohydrate reserves are mobilised and concentrations start to decrease rapidly as they are used by the developing tissues (Bates *et al*., 2002; Smith & Holzapfel, 2012). The developing tissues depend on the carbohydrate reserves until they can start producing their own, when the leaves have reached 50% of their final size (Bates *et al*., 2002; Bennett, 2002; Keller, 2015). Keller (2010) stated that sugars stored in the roots and trunk are the first to be used for new growth, with sucrose being the major form transported. The prevailing temperatures determine the rate of shoot growth and development during this growth period.

According to Keller (2010), there is a rapid differentiation and development of flowers of the inflorescences following bud break. The induction of inflorescence primordia is promoted by temperatures ranging from 25 to 30°C, amongst other factors including adequate nutrient supply. Low temperatures (< 20°C) promote tendril formation, whereas temperatures > 35°C contribute to a reduced number of primordia, resulting in unfruitful buds (Keller, 2010).

Flowering to fruit set

Both vegetative and reproductive growth utilise stored reserves (Bates *et al*., 2002; Smith & Holzapfel, 2012). Carbohydrate reserves decrease to a minimum until flowering (Smith *et al*., 2009). Slow pollen tube growth, caused by starch accumulation interference before pollination, may lead to poor fruit set (Keller, 2010; Sharafi & Bahmani, 2011). Carbohydrate reserves highly affect berry set and development after set (Buttrose, 1966; Sharafi & Bahmani, 2011).

Berry growth and ripening

The pattern of grape berry development follows a double sigmoid curve (Figure 2.4) consisting of three stages (Coombe, 2000; Ollat *et al*., 2002), however, seedless cultivars' phases are not as clearly defined as those of seeded cultivars (Friend, 2005; Keller, 2010).

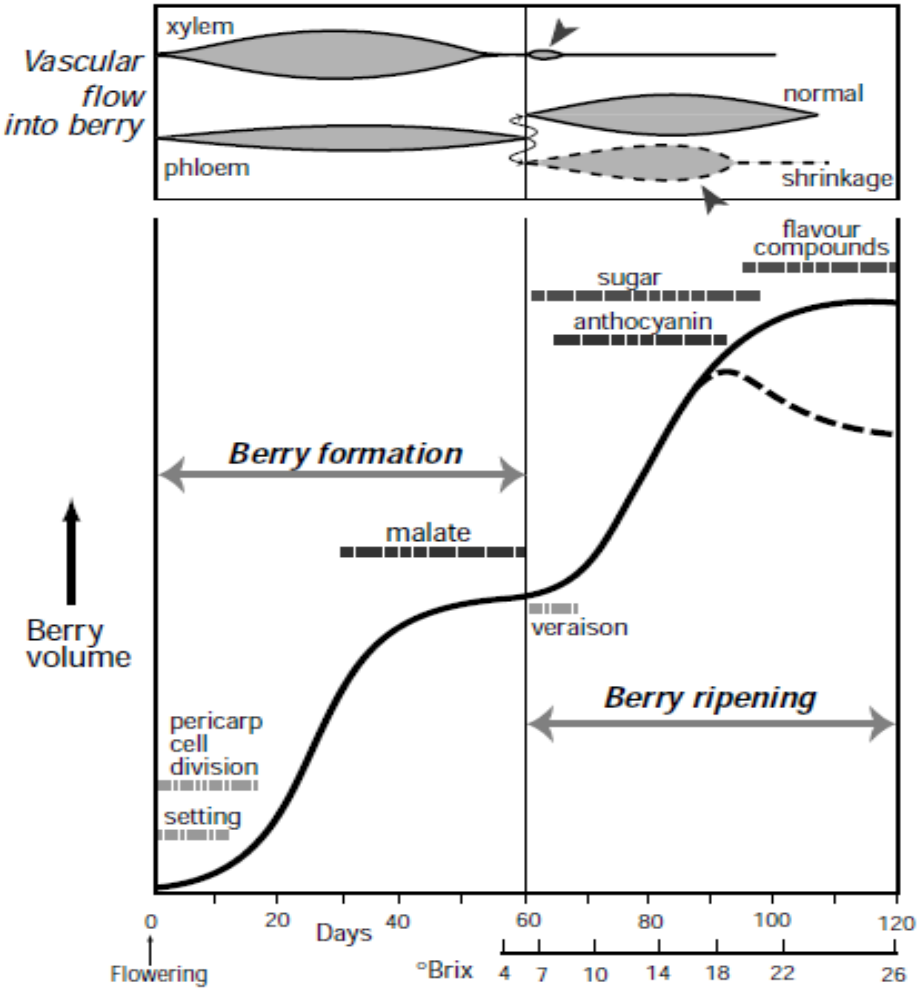

Figure 2.4: Illustration of grape berry development (adapted from Coombe & McCarthy, 2000).

After seed formation, the seeds and the berries are very small, green and hard. The greenness marks the availability of chlorophyll, allowing the berries to undergo photosynthesis, producing

carbohydrates in small quantities which cannot solely supply the berries' needs (Ollat *et al*., 2002; Ristic & Iland, 2005). Although the berries can take up carbon, the main supply for growth and development comes from leaves and reserves in perennial tissues (Ollat *et al*., 2002). The carbon import rate was reported to be 77 mmoles per berry per day in the first growing phase (Ollat *et al*., 2002). In addition, 30% of the imported carbon is retained in the seeds, 43% is retained in the pericarp and 47% is used for respiration (Figure. 2.5). During this period, the skin cells also divide, and this phase of berry development takes six to nine weeks before the lag phase begins. The lag phase is when the embryo grows and the seed eventually reaches its final size (Ristic & Iland, 2005; Keller, 2010). The growth of the berry is slow at this stage, exhibiting a hard, green character (Coombe & McCarthy, 2000).

The ripening period starts with a change in berry skin colour, *i.e*. véraison (Figure 2.4). According to Ollat *et al*. (2002), the rate of carbon import per berry per day during ripening was shown to be 3.5 times that reported in the first growth phase (266 mmoles). The berry starts to soften, sugar and phenolic content increase, and acidity decreases (Ollat *et al*., 2002; Friend, 2005). Berry size initially increases rapidly as the flesh develops and then slows down as the berry reaches maturity (Coombe & McCarthy, 2000; Ollat *et al*., 2002). During ripening, grape berries become stronger sinks for carbohydrates than before véraison, when shoots compete for assimilates (Ollat *et al*., 2002). However, Holzapfel *et al*. (2009) reported that ripening berries did not prevent reserve replenishment before harvest. During ripening, respiration is responsible for the loss of 13% of imported carbon. At maturation, fructose and glucose reach high concentrations, representing 60% of solutes in berry juice. Berries do not store starch (Ollat *et al*., 2002). The fruit is harvested once it reaches the desired ripening parameters (sugars, acidity, colour and flavour). If the bunch is left longer on the vine to over ripen or for the drying-on-vine system of raisins, the berries lose water and the sugars and flavours concentrate (Ollat *et al*., 2002).

The phase length and total duration of fruit development and growth differ between cultivars and are affected by carbohydrate supply (Ollat *et al*., 2002). The duration of fruit development and the final berry size obtained are modified by factors such as environmental conditions, rootstock (scion-rootstock relations), nutrients (specifically carbohydrates) and water supply (Ollat *et al*., 2002).

2.4.3 Environmental factors

2.4.3.1 **Abiotic factors**

Light

For the vine to produce the maximum quantity of photosynthetic products, the maximum surface of leaves per hectare should be exposed to photosynthetically active radiation (PAR) (Uys, 1991). The light saturation point of a leaf is defined as the photosynthetic photon flux density (PPFD)

value beyond which photosynthesis does not increase, and it ranges between 600 and 1200 µmol m^{-2} s^{-1} (Poni & Intrieri, 2001). Light compensation point is the PPFD value at which net photosynthesis is equal to zero. (Poni & Intrieri, 2001).

The proportion of photosynthetically active radiation (PAR) reflected, absorbed and transmitted by leaves, depends on leaf age (Holzapfel *et al*., 2010). Dokoozlian and Kliewer (1995) reported that in canopies of low leaf area density (< 4 m^2 leaf area.m^{-1} canopy of length), sun flecks illuminated 20% to 40% of the surface area. This illumination was reported to have contributed to the photosynthesis in the canopy. In the dense canopy (> 8 m^2 leaf area m^{-1} canopy length), however, the sun flecks were reported to be almost absent and their contribution to the photosynthesis in the canopy was minimal (Dokoozlian & Kliewer, 1995). When a leaf receives direct radiation, 10% is reflected, 9% transmitted, and 81% absorbed (Dokoozlian & Kliewer, 1995; Poni & Intrieri, 2001; Strever, 2012). From the absorbed light, 60% is used for transpiration and convection, 20% emitted as infrared, and about 1% is used for photosynthesis (Strever, 2012).

The penetration of light into the canopy is affected by the number of leaf layers in the canopy (Strever, 2012). The level of photosynthesis differs between sun-exposed and shaded leaves. Due to the longer palisade parenchyma cells or an additional cell layer, sun-exposed leaves are characteristically thicker and more absorption efficient (Strever, 2012). Leaves developed in shade exhibit maximum photosynthesis of 30% to 50% less than those developed in the sun (Poni & Intrieri, 2001). The age of a leaf determines how much light is transmitted, as the chlorophyll content and overall photosynthetic efficiency are affected (Poni & Intrieri, 2001; Strever, 2012). The photosynthesic activity of a vine leaf rapidly increases until up to 40 to 45 days, whereafter, it exhibits a decline (Poni & Intrieri 2001; Keller, 2015).

Temperature

The duration of continued vegetative growth in the post-harvest period is determined by climate and cultivar (Bates *et al*., 2002; Smith & Holzapfel, 2012): In cool climatic regions, this period is short and vegetative growth does not last long before leaf fall commences, photosynthesis approaching a halt and the vine becoming dormant. Bates *et al*. (2002) indicated the short duration after harvest as a limitation in the cooler regions since the accumulation of reserves only takes place for a short while. In Western New York, where frost is experienced, Concord grapes are sometimes harvested a month before leaf fall, or just after frost has commenced. Due to this, the recovery period is very limited, as photosynthesis is reduced by decreasing photoperiod and radiation (Bates *et al*., 2002; Keller, 2010). More carbohydrate accumulation is experienced in warmer regions after harvest, due to a longer period of vegetative growth, made possible by favourable conditions for photosynthetic activity (Smith & Holzapfel, 2012).

Hendrickson *et al*. (2004) reported a small (1 to 3°C) consistent air temperature difference over the period to result in a large mid-season growth differential between vines on a slope in a warmer and cooler microclimate. From these results, he speculated that in the long term, warmer microsites located at higher elevation, had a slight growth advantage over the cooler microsites at lower elevation. Soil temperatures were observed to impact the mobilisation of carbohydrate reserves in roots (Rogiers *et al*., 2011). Field *et al*. (2009) found warm soil temperature (23°C) to increase starch catabolism in roots, resulting in depletion of carbohydrates. Rogiers *et al*. (2011) reported that the mobilised reserves in warm soils (± 26°C) tend to accelerate structural growth, but there is a delay in their restoration.

In the study of Rubio *et al*. (2016), low starch levels in dormant buds were induced by exposure to a low temperature (5°C) for three weeks, while exposure to ambient temperatures (14°C) for the same period reduced the starch level by only 20% of the impact of low temperatures. However, the exposure to low temperatures resulted in an increase in soluble sugars (sucrose, d-fructose, d-glucose) in the dormant buds. Sawicki *et al*. (2015) found that cold nights induced the accumulation of sugars.

Water

Water plays an important role in grapevine carbohydrate dynamics *via* its impact on the photosynthetic activity of the vine. Van Leeuwen *et al.* (2009) and Myburgh (2018) proposed the following thresholds for stem water potential as an indication of grapevine stress: > -0.6 MPa (no stress) -0.6 to_-0.9k MPa (low), -0.9 to -1.1MPa (moderate), -1.1 to -1.4 MPa (high) and > -1.4 MPa (severe). Lovisolo *et al*. (2010) reported a midday to afternoon decrease of leaf water potential, under sufficient soil water availability, results in a depression of stomatal conductance and net photosynthesis (A_N) in the same period. A midday depression occurs in the most exposed leaves of the canopy in irrigated plants. In the same study, three general stages of photosynthesis regulation in grapevines as subjected to progressive soil water stress were reported, as defined by Flexas *et al*. (2002b) and Medrano *et al*. (2002b):

a) Stage 1- Mild water stress: Stomatal conductance (g_s) decreases from a maximum (between 200 and 500 mmol H_2O m^{-2} s^{-1}) to 150 mmol H_2O m^{-2} s^{-1}.
   There is no inhibition of photosynthetic enzymes as well as on the photosynthetic capacity. A small decline of A_N occurs and this is caused by diffusional limitations. These are stomatal closure and restricted diffusion of CO_2.
b) Stage 2- Moderate water stress: g_s ranges between 50 and 150 mmol H_2O m^{-2} s^{-1}.
   A further decline of A_N due to both stomatal and non-stomatal limitations including decreased evapotranspiration and impaired Rubisco. Stomatal limitations still dominate at this stage.

c) Stage 3- Severe water stress: g_s drops below 50 mmol H_2O m^{-2} s^{-1}.
Non-stomatal limitations dominate as A_N cannot be restored even at high CO_2 concentrations. This is most evident at very high temperature and irradiance.

Monosaccharides (glucose and fructose) represented 38% and sucrose 62% of daily total soluble sugars in well-watered plants and 53% and 47% in drought-stressed grapevines respectively (Rodrigues *et al*., 1993). Sucrose and starch concentrations were significantly higher in well-watered grapevines compared to stressed plants. Rodrigues *et al*. (1993) further reported that factors other than leaf water status play a key role in the control aperture and possibly exert non-stomatal effects on carbon assimilation under reduced soil water availability.

2.4.3.2 **Biotic factors**

Diseases

Pathogens impact the metabolism of plants (Berger *et al*., 2007). Once a plant gets in contact with a pathogen, a defense mechanism is triggered, which comes at the cost of plant assimilates, because the pathogen competes with the plant for these assimilates to survive inside the plant (Berger *et al*., 2007; Gamm *et al*., 2011). The plant's photosynthetic activity is usually the most affected as pathogens often cause leaf damage (necrosis, chlorosis). Carbohydrates are the most affected as carbohydrate production and metabolism are altered (Berger *et al*., 2007).

Downy mildew, caused by *Plasmopara viticola*, one of the grapevine's major diseases (Gamm *et al*., 2011), requires living tissues to grow and reproduce, thriving on the vine's nutrients, including glucose (Keller, 2010; Gamm *et al*., 2011). It has been reported that it represses photosynthesis due to damaged leaves, affecting the accumulation of reserves, fruit ripening, yield and cold hardiness of the vine (Keller, 2010; Gamm *et al*., 2011). Gamm *et al*. (2011) found that infected leaves have an abnormally high starch, glucose and fructose accumulation, compared to healthy leaves at 7 days post-inoculation of *P. viticola*. Sugars have been reported to play essential roles in plant-pathogen interactions, including nutrition, providing energy for defense reactions and involvement in regulating gene expression (Gamm *et al*., 2011).

Esca, a disease caused by several fungi in different parts of wood, primarily affects mature vines (Petit *et al*., 2006). One of the symptoms of this disease is chlorosis of the leaves, which affects the photosynthetic activity, thus disrupting metabolism/accumulation of carbohydrates and its export to storage organs (Petit *et al*., 2006). Petit *et al*. (2006) reported asymptomatic and symptomatic canes of diseased vines to have lower starch content than healthy canes.

Rühl and Clingeleffer (1993) inoculated Cabernet Franc with leafroll and yellow speckle virus from two Sultana clones (H4 and H5) to investigate the inocula's effect on carbohydrate and nitrogen

status of infected vines. The healthy vines accumulated more carbohydrates (starch and sugars) in one-year-old canes, wood and roots. Both viruses resulted in delayed sugar accumulation during ripening, with the virus isolated from clone H5 causing a more severe effect.

2.4.4 Viticultural practices

2.4.4.1 **Pruning**

Pruning is a practice done to maintain the vine structure, vigour, productivity, fruit quality, promote uniform bud break, fruit quality and to maintain a practical number of shoots and fruit for labour (Sommer *et al*., 1995; Keller *et al*., 2004; Keller, 2010). There are two main pruning times, namely pruning practiced in winter and the other in summer (post-harvest). Winter pruning is generally applied to grapevines during the dormancy period. The fertility of the cultivar determines the pruning system that should be applied (Lombard *et al*., 2006). Spur pruned cultivars are those with fertile basal buds. Those cultivars that are fertile in the middle of the shoot are pruned to half-long bearers (4 to 8 buds), while those that are fertile at bud positions located more apically are pruned as canes (10 to 16 buds) (Christensen, 2000; Keller, 2010).

Minimal and mechanical pruning techniques were developed to reduce labour and production costs (Rühl & Clingeleffer, 1993; Silvestroni *et al*., 2018). Pruning technique was found not to influence the total concentration and composition of carbohydrates in wood and roots of Cabernet Franc and Shiraz within seasons (Clingeleffer & Sommer, 1995; Pellegrino *et al*. 2014). Minimal pruning converted from spur pruning (MPCT-spur), as well as minimal converted from cane pruning (MPCT-cane), was found not to affect the carbohydrate concentration of one-year-old canes, determined five years after the conversion (Rühl & Clingeleffer, 1993). Spur pruning resulted in lower reducing sugars in roots and old wood (Rühl & Clingeleffer, 1993). Comparing the three techniques, spur-pruned vines had more carbohydrates accumulated in one-year-old canes than MPCT vines (Rühl & Clingeleffer, 1993).

Post-harvest summer pruning is often practiced in warm, arid/semi-arid areas on vigorous, cane-pruned cultivars. It is applied aiming at channeling reserves to shoots selected to be bearers in the new season, contributing to increasing bud break and fertility for next season, as well as controlling vegetative growth in the following spring (Smith & Holzapfel, 2012; Raath & Du Plessis, 2012; Van der Merwe, 2017). This practice is applied by removing young, weak, or poor-quality shoots to improve carbohydrate reserve accumulation into the main shoots identified to be selected as canes during winter pruning. (Van der Merwe, 2017). Post-harvest summer pruning could also be considered a pre-winter pruning technique (Keller, 2010; El-Boray *et al*., 2018).

Several studies reported that the removal of either shoots or leaves in the post-harvest period, resulted in a reduction in the concentration of carbohydrates. Holzapfel & Smith (2012), having

cut all shoots to five nodes after harvest, reported reduced carbohydrate concentrations by 14% after two seasons and 27% in the third season. Due to this reduction, limited vine growth was observed in the next season, accompanied by a decrease in the number of inflorescences per shoot and flowers per inflorescence, resulting in decreased yields (Bennett, 2002; Bennett *et al*., 2005; Holzapfel *et al*., 2006; Smith & Holzapfel, 2009; Smith & Holzapfel, 2012). However, Links (2014) did not obtain any significant effect with 33% and 66% post-harvest pruning on starch and sugars in canes in two consecutive seasons.

Alteration in nutrient reserve replenishment, specifically carbohydrates, may affect vine fruitfulness (Sommer *et al*., 2000; Bennett, 2002). In a study on Sauvignon Blanc by Trought *et al*. (2011), post-harvest pruning had little effect on trunk carbohydrates and no effect on bud break. When El-Boray *et al*. (2018) applied this practice, it did not affect bud fertility percentage in the first season and this was reported to be since the inflorescence primordia had already been formed in the previous season. In the second season, bud fertility and the fertility coefficient increased. Ashraf & Ashraf (2014) reported that summer pruning increased flower bud formation and return bloom in apple trees.

According to two-year average results, summer pruning and standard winter pruning significantly reduced water-soluble reducing sugars and starch in almond (Ikinci *et al*., 2016), but tree fruit quality effects were only observed in the season after treatment application. In a study conducted in Turkey, post-harvest summer pruning resulted in the lowest total sugar content in July (summer) and increased starch content of apricot shoots in January, March and October (Demirtas *et al*., 2010). This practice reduced the cold hardiness of flower buds, delayed defoliation and reduced carbohydrate levels in peach trees (Ikinci, 2014).

2.4.4.2 **Defoliation**

Leaf/shoot removal is usually applied to manipulate the source-sink ratio as it lowers and favours it (Hunter *et al*., 1995). It is also applied to improve light intensity in/through the canopy which results in higher photosynthetic activity, to modify the grape composition, as well as to open up the canopy for practical purposes such as making it easier to reach the bunches for crop control (Poni *et al*. 2009; Pallioti *et al*. 2011; Osrečak *et al*. 2016) and spray applications (SATI, 2021). Scholefield *et al*. (1978) stated that leaf removal at harvest could reduce yield by over 50% in Sultana grapes in the following year. Smith and Holzapfel (2009) reported that defoliation at harvest reduced crop production by 22% after one season of treatment and 50% after two seasons. It also reduced reserves in the trunk, which resulted in reduced inflorescences per shoot, flowers per inflorescence and eventually lower yield in the following seasons (Holzapfel *et al*., 2006; Holzapfel & Smith, 2012). Complete defoliation immediately after harvest reduced vine growth and yield, although the impact on carbohydrate reserves was only established after two consecutive years of defoliation treatments (Greven *et al*., 2016). In contrast to this, Smith and Holzapfel (2009) reported that total non-structural carbohydrates were decreased and yield reduced by up to 22% after one season of complete defoliation at harvest. After two seasons, yield was reduced by 50%.

According to Hunter *et al*. (1995) and Hunter and Visser (1990), the earlier (from bud break to véraison) and severe (66%) the defoliation, the more shoot growth is reduced. After defoliating grapevines at pepper-corn size for two consecutive years, Martínez-Lüscher and Kurtural (2021) reported that retaining 33% of leaves decreased starch content of roots when compared to no defoliation (100% leaves retained). This was seen from mid-ripening to harvest. At pruning, however, both treatments had the same starch content.

Partial defoliation (33%) at pea-size and véraison resulted in approximately 11% and 59% higher yields respectively (Hunter *et al*., 1995). In contrast, severe defoliation (66%) reduced yields, which was attributed to decreased photosynthetic activity (Hunter & Visser, 1990). Bennett *et al*. (2005) did not find effects of defoliation (at 4, 8 or 12 weeks post-bloom) on cluster number per vine, as well as weight. Candolfi-Vasconcelos and Koblet (1990) reported that defoliation before bloom reduced berry set and mass. When leaves were removed just after fruit set, there was a reduction in berry growth, whereas removal after veraison impaired the accumulation of sugars (Ollat *et al*., 2002).

2.4.4.3 **Crop control**

Crop control (bunch removal) is an essential viticultural practice for the production of high-quality table grapes (Fallahi, 2007; Xi *et al*., 2020; SATI, 2021) and is recommended for all table grape cultivars produces in SA (SATI, 2021). Crop control alters source-sink relations, thereby affecting

carbohydrate dynamics (Pellegrino *et al*., 2014). When bunches are removed, the sinks are reduced and this causes the remaining crop to obtain more photosynthetic products (Petrie & Clingeleffer, 2006; Palliotti *et al*., 2011). Depending on the cultivar, crop control of table grapes is applied either before 5% set, or after set, when natural berry abscission has occurred (SATI 2021).

Higher crop loads require more carbohydrates to ripen the bunches, thereby affecting photosynthesis (Holzapfel, 2009; Smith & Holzapfel, 2012). Most carbohydrates produced during this period are used to sustain growth of developing shoots and ripening of bunches (Smith & Holzapfel, 2012). In warm climatic regions, due to the high temperatures in spring and summer, the focus is on protecting bunches from sunburn, thereby keeping enough shoots. Holzapfel (2009) applied two levels of bunch thinning, 33% and 66%, just before véraison on Shiraz and Chardonnay and found a higher yield and berry sugar in the 33% crop removal treatment for both cultivars. Both treatments resulted in an increased rate of sugar accumulation and colour development in Shiraz bunches compared to the control.

Holzapfel *et al*. (2009) and Smith and Holzapfel (2012) stated that the importance of reserve accumulation during the post-harvest period depends strongly on crop load, including conditions that affect accumulation that takes place before harvest. High crop loads are associated with a delay in the accumulation of root reserves in the post-harvest period (Smith & Holzapfel, 2012). Retaining 33% crop level at pepper-corn size resulted in approximately twice the root soluble sugars content compared to retaining 100% of the crop (Martínez-Lüscher & Kurtural, 2021). In the carry-over year, in which treatments were not applied, 100% crop level resulted in higher soluble sugars than 33% crop level when measured at mid-ripening. The opposite was recorded before leaf fall where the soluble sugars associated with the 33% crop level were approximately two times higher than that of the 100% crop level (Martínez-Lüscher & Kurtural, 2021).

2.4.4.4 **Girdling**

Girdling entails removing/cutting a thin ring of phloem around the trunk, shoot, or cane to temporarily disrupt the downward flow of carbohydrates and hormones through the phloem (Roper & Williams, 1989; Caspari *et al*., 1998; Keller, 2010; Williams *et al*., 2000). This practice is done to improve fruit set, berry size, enhance maturation, colour development and yield (Roper & Williams, 1989; Caspari *et al*., 1998; Williams *et al*., 2000; Keller, 2010; Carrillo *et al*., 2020; SATI, 2021) and is a recommended practice for berry size improvement of Sultanina H5 (SATI, 2021).

Application of stem girdling, which permanently disrupts phloem function without affecting that of the xylem, resulted in total independence of young shoots and their inflorescences, preserving

stored reserves (Lang & Thorpe, 1989; Eltom *et al*., 2013). Eltom *et al*. (2013) further reported that the restriction of carbohydrate reserves by girdling before bud break resulted in no lateral shoot growth. Higher carbohydrate levels were recorded in girdled shoots than in non-girdled shoots (Caspari *et al*., 1998; Carrillo *et al*., 2020). Furthermore, improved berry set was attributed to the increased available carbohydrates in shoots due to girdling. In contrast to this, trunk and cane girdling reduced leaf net CO_2 assimilation rate, resulting in reduced photosynthetic activity (Roper & Williams, 1989; Williams *et al*., 2000).

2.4.4.5 **Irrigation**

Deficit irrigation impacts carbohydrate reserve status by reducing photosynthetic activity (Deloire *et al*., 2004; Holzapfel *et al*., 2009; Smith & Holzapfel, 2012; Pellegrino *et al*., 2014), resulting in a relative decline in net photosynthesis, reported to be three-fold (Pellegrino *et al*., 2014). Deficit irrigation lowered leaf and trunk starch concentration over the day and the season respectively (Pellegrino *et al*., 2014). Prolonged water shortages resulted in low root reserves. Water stress has a negative long-term effect on carbohydrate accumulation, mainly when induced in the post-harvest period. Smith and Holzapfel (2009) stated that although reserves could be reduced by prolonged water shortage, they are not depleted within a season as canopy growth and yields are reduced. Under these conditions, sugar concentrations, are higher, which could be a result of higher sugar mobilisation (Pellegrino *et al*., 2014).

Rossouw *et al*. (2017) recorded a significant reduction in root starch and TNC content during rapid berry sugar accumulation (véraison to véraison + 27 days) under reduced water supply (50% irrigation) compared to higher water supply (100% irrigation). Less carbohydrates were allocated to the perennial tissues under moderate to severe water constraints. Water status of a vine significantly influences berry growth and ultimately, yield (Deloire *et al*., 2004). Moderate to severe water deficit from flowering to veraison, resulted in decreased size and volume of berries (Ollat *et al*., 2002; Deloire *et al*., 2004). Berry sugar accumulation was affected by water deficit (Deloire *et al*., 2004).

Deficit irrigation of Sultanina H4 grapevines at various pre- and post-harvest phenological phases over four seasons in the Upington area, did not have a significant effect on vegetative growth and yield (Myburgh, 2003b). In contast, vegetative growth and yield of Sultanina H4 were significantly reduced by irrigation at 90% plant-available water (PAW) depletion compared to 30% PAW depletion applied from September to May near Upington (Myburgh, 2003a). Reduced irrigation significantly induced root starch depletion during rapid berry sugar accumulation (Rossouw *et al*., 2017).

Falchi *et al*. (2020) reported that petioles of water-stressed vines had significantly lower starch concentration compared to the control and accumulation of maltose/sucrose in petioles of water-stressed vines occurred as a result of decreased water potential.

2.5 GRAPEVINE ABNORMALITIES LINKED TO CARBOHYDRATE RESERVE STATUS

2.5.1 Growth arrest phenomenon

Due to the climate in the Lower Orange River region (LOR), an extended post-harvest period is conducive to continued growth that proceeds until late autumn, resulting in a short period available for accumulation of carbohydrate reserves needed for cold hardiness in winter and new growth after bud break, contributing to the occurrence of Growth Arrestment Phenomenon (GAP) (Volschenk & Hunter, 2015). Saayman (1983) reported that carbohydrate reserves affected the occurrence of GAP in the LOR: Before bud break, canes of healthy vines contained significantly higher concentrations of starch than canes of GAP-affected vines, which indicated an inability of GAP vines to synthesise and accumulate sufficient carbohydrate reserves.

2.5.2 Bud necrosis

Localised carbohydrate deficiency in tissues contributes to bud necrosis, a condition where the latent bud cells die off (Vasudevan *et al*., 1998; Bennett, 2002). It is usually observed in the primary bud, although it can occur in the secondary bud (Vasudevan *et al*., 1998). This condition, due to the primary bud being the most fertile, results in low bud break percentage and thus reduced yields (Links, 2014). Vasudevan *et al*. (1998) correlated low carbohydrate levels associated with bud necrosis, with shading.

2.6 MEASUREMENT OF CARBOHYDRATE RESERVES

2.6.1 Destructive methods

2.6.1.1 Colometric methods

The Anthrone method (Dreywood, 1946) has been used for several decades to determine soluble carbohydrate concentration in grapevine tissues and an adapted method is described in Section 3.2. This method is, however, not user-friendly, mainly due to the sample preparation and extraction procedures which are tedious and time-consuming. The iodine starch test used in the apple and forestry industries has been studied on grapevine wood by a few researchers (Zapata *et al*., 2004; Rustioni *et al*., 2017). This test entails collecting wood material (roots, trunks, canes and/or shoots), cutting the material in cross sections and staining the cuttings with iodine solution (Zapata *et al*., 2004; Rustioni *et al*., 2017). Because this test only gives a visual estimate or

location of starch in wood tissues (roots, trunks, canes and/or shoots), Rustioni *et al*. (2017) used reflectance spectroscopy and Partial Least Squares (PLS) regression for quantification.

2.6.1.2 **Chromatographic methods**

According to Magwaza and Opara (2015), high-performance liquid chromatography (HPLC) is the most effective method of carbohydrate analysis. Because sugars do not absorb ultraviolet, an evaporative light-scattering detector (ELSD) is used to quantify these in HPLC analysis (Shanmugavelan *et al*., 2013). It is important to note that the calibration response of ELDS is non-linear and to get a linear relationship, the log10 values must be plotted on both axes (Magwaza & Opara, 2015). Pulsed amperometric detector (PAD) can also be used to quantify sugars due to its most highly sensitive and reliable detection (Magwaza & Opara, 2015).

2.6.2 Non-destructive methods

All the destructive methods described in Section 2.5.1 are time-consuming, costly and not simple, although accurate. Methods that are not laborious, are rapid and affordable would be of advantage to the industry. Optical methods such as visible and near-infrared spectroscopy (Vis/NIR), attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FT-IR) and chemometrics or x-ray micro-computed tomography (microCT) could be used to quantify or predict carbohydrates (Schmidtke *et al*., 2012; Magwaza & Opara, 2015; Rustioni *et al*., 2017; De Bei *et al*., 2017; Călugăr *et al*., 2019; Jones *et al*., 2020).

According to De Bei *et al*. (2017), NIR does not require to be done in a laboratory and is fast. It can therefore be used as a practical tool to determine starch and carbohydrates in leaves and trunks. The method, however, still requires destructive sampling of tissues. A database of calibrations is required for validation of the results obtained from the method (De Bei *et al*., 2017). Jones et al. (2020) investigated the feasibility of NIRS to predict starch reserves in intact and ground grapevine cane wood. A partial least squares regression was used on the spectral data of the samples and compared against conventional wet chemistry starch analysis. Prediction of starch in intact canes, with or without the bark resulted in low correlations (r^2= 0.19 and r^2= 0.34, respectively). However, the root mean square error of cross-validation values were low (0.75-0.86 $mg.g^{-1}$). This indicated good predictability of the model indicating the potential of the technology to predict starch reserves in intact canes.

According to Schmidtke *et al*. (2012), ATR-FT-IR with chemometric modelling is a method used to determine a range of analytes. The method entails collecting infrared spectra of samples and correlating the absorbance at specific wavelengths to the concentration of the analytes with predictive models constructed to determine the concentration of the analytes in other samples. ATR-FT-IR has been used in determining organic acids and carbohydrates in fruit (Schmidtke *et*

al., 2012). X-ray micro-CT is used to visualise internal structures of small objects and has been used in seed research (Gargiulo *et al*., 2019). It is used to characterise whole seeds and their anatomy and has been used on maize and quinoa (Gargiulo *et al*., 2019). This method could be evaluated for studying grapevine tissues, anatomically and physiologically.

2.7 CONCLUSION

Studies conducted to date on seasonal dynamics of grapevine carbohydrate reserves have provided insight into the carbon economy of the grapevine as well as biotic and abiotic factors that affect it. Climate is one of the primary factors that affect the carbohydrate reserve pattern in a season. In SA, research on grapevine carbohydrate reserve status has focused mainly on the Mediterranean region and mostly on wine grape cultivars. Thus, a need for studies in other major production regions such as the LOR and for the table grape industry was identified. The impact of the longer continuous vegetative growth in the post-harvest period, as well as the influence of higher late-summer, autumn and winter temperatures on the seasonal carbohydrate reserve pattern in the warm climates, such as the LOR is not known. Theoretically, it is expected that this will negatively affect carbohydrate reserve accumulation.

In warm regions of SA, post-harvest summer pruning has been applied based on theoretical knowledge and anecdotal evidence. This calls for a scientific study on the effect of this practice on grapevine carbohydrate reserves and aspects affected by these reserves. Such a study on table grapes will provide the producer with scientific evidence whether post-harvest summer pruning has practical and economical benefits, confirming whether it is a required practice.

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Chapter 3: Comparing the seasonal dynamics of total non-structural carbohydrates of *Vitis vinifera* L. cv. Sultanina H5 in a winter rainfall and a semi-arid summer rainfall region of South Africa

3.1 ABSTRACT

Climate plays a major role in vine growth, both vegetative and reproductive. This entails that due to different climates, the grapevine phenological development and growth cycle may differ. In this trial, a fully randomised design was used to establish the seasonal total non-structural carbohydrate (NSC) dynamics of Sultanina H5 in two major table grape-producing regions, the Lower Orange River (LOR) and the Hex River Valley (HRV). These regions were labelled according to their characteristic climates, the Semi-arid (LOR) and Mediterranean (HRV) regions. Sampling of roots, stems, canes and shoots was done monthly. The Anthrone method was used to analyse soluble sugars and starch, while enzymatic analysis was used to quantify specific sugars (sucrose, d-fructose and d-glucose). Starch and sucrose were the most abundant carbohydrates in all tissues studied. Soluble sugars peaked at dormancy, while starch peaked just before bud break in both regions. A steep decrease was recorded from dormancy to flowering in both regions, indicating a vital dependency of the vine on carbohydrate reserves during that period. Accumulation of reserves began after flowering to the post-harvest period, reaching their second peaks in autumn. Significantly higher soluble sugar concentrations were recorded in the tissues of the Mediterranean region. TNC reserves in the roots and canes of the Mediterranean region were also significantly high. The significantly high positive correlations between starch and soluble sugar content of roots, trunks and shoots and canes indicate that the latter two tissues could be sampled and analysed to indicate the overall NSC status of the vine.

3.2 INTRODUCTION

Carbohydrates provide the building blocks for plant structure, as well as resources for metabolic processes (Iland *et al*., 2011; Hartmann & Trumbore, 2016). Structural carbohydrates are molecules that provide building blocks for biomass, utilised for building and solidifying structures, whereas non-structural carbohydrates (NSC) are critical substrates for plant growth and metabolism (Hartmann & Trumbore, 2016; Han *et al*., 2020). Carbohydrate reserves entail the carbohydrate fractions available for use in respiration or translocation to areas where they are required (Uys, 1981; Cheng *et al*., 2004). Carbohydrates are produced *via* photosynthesis and are either used in metabolism and growth or stored as reserves in the woody structures of the

vine (Candolfi-Vasconcelos *et al*., 1993; Bouard, 1996; Lebon *et al*., 2008; Wojnarowiez *et al*., 2008; Iland *et al*., 2011; Rogiers *et al*., 2011). Grapevines require a supply of carbohydrates and nutrients from stored reserves to support root growth in the month before bud break, as well as to support shoot growth (Bates *et al*., 2002; Bennett, 2002; Smith & Holtzapfel, 2012) and final differentiation of inflorescences between bud break and flowering in spring (Bennett *et al*., 2005).

Carbohydrate reserves are stored in the form of starch and sugars (Bates *et al*., 2002; Zapata *et al*., 2004; Holzapfel, 2009). These sugars are soluble and consist primarily of sucrose, d-glucose and d-fructose (Candolfi-Vasconcelos *et al*., 1994; Jones *et al*., 1999; Bates *et al*., 2002; Lebon *et al*., 2008). Starch is the most important and main reserve compound in plants storage tissues (Winkler & Williams, 1945; Vaillant-Gaveau *et al*., 2014; Dayer *et al*., 2020). The seasonal dynamics of carbohydrates in grapevines has been studied and reported for various cultivars and regions in the world, including the work of Winkler (1929), Winkler & Williams (1938), Picket & Cowart (1941), Winkler & Williams (1945), Bernstein & Klein (1957), Eifert *et al*. (1961), Marutyan (1962), Eifert & Eifert (1963, 1966), Kliewer (1965), Kliewer & Nassar (1966), Scholefield *et al*. (1978), Baines *et al*. (1981), Loescher *et al.* (1990), Bates *et al*. (2002); Zapata *et al*. (2004), Bennett *et al*. (2005), Holzapfel (2009), Smith & Holzapfel (2012), Zufferey *et al*. (2012) and Philips *et al*. (2015). In Section 2.3 the main findings of these studies are reviewed.

Factors reported to affect carbohydrate reserve accumulation and mobilisation include, amongst others, cultivar and vine age (Bates *et al*., 2002), climate (Field *et al*., 2009; Rogiers *et al*., 2011; Sawicki *et al*., 2015; Dahal *et al*., 2018), cultural practices including defoliation/pruning (Scholefield *et al*., 1978; Marangoni *et al*., 1980; Rühl & Clingeleffer, 1993; Candolfi-Vasconcelos *et al*., 1994; Clingeleffer & Sommer, 1995; Bennett *et al*., 2005; Vršič *et al*., 2009; Ikinci, 2014; Greven *et al*., 2016), crop load (Holzapfel, 2009; Smith & Holzapfel. 2012), irrigation (Deloire *et al*., 2004, Holzapfel, 2009; Pellegrino *et al*., 2014; Rossouw *et al*., 2017), as well as the occurrence of diseases/disorders (Rühl & Clingeleffer, 1993; Petit *et al*., 2006; Berger *et al*., 2007; Gamm *et al*., 2011). In Section 2.4 these factors are discussed.

Climate is one of the major factors that affect grapevine production (Winkler *et al*., 1974) and the carbohydrate reserve pattern of grapevines (Field *et al*., 2009; Rogiers *et al*., 2011; Sawicki *et al*., 2015; Dahal *et al*., 2018). Climate comprises four levels, namely macroclimate, mesoclimate, microclimate and nanoclimate. Macroclimate refers to the climate of the region/country, mesoclimate is that of where the vineyard is located, microclimate is the climate within the canopy and nanoclimate is within the bunch (Hunter & Archer, 2002; Kurtural *et al*., 2006; Stoutjesdijk & Barkman, 2014).

The five major table grape production regions of SA represent four climatic regions, namely sub-tropical (Northern Provinces), semi-arid with summer rain (Orange River region) semi-arid to arid with winter rain (Olifants River region) and mediterranean (Berg River region, Hex River Valley) (Avenant & Lombardt, 2018; SATI, 2021).

Accumulation of reserves will be negatively affected by continuous vegetative growth in the post-harvest period, which often occurs in warmer production regions, such as the LOR. In the LOR, vegetative growth often continues into late autumn, due to prevailing high temperatures, with a long-term average of above 19°C for April and May (Source: Ileaf: www.ileaf.co.za). Sudden cold and/or frost occurring in April or May, defoliate these vines, forcing them into dormancy, without the natural process of reserve accumulation having occurred (Van der Westhuizen *et al*., 2001, Volschenk & Hunter, 2009). Several authors linked continuous vegetative growth in autumn to insufficient reserve accumulation and the occurrence of growth arrest phenomenon (GAP) or restricted spring growth (RSG) in the following spring (Van der Westhuizen *et al*., 2001; Volschenk, 2005; Holzapfel, 2009; Volschenk & Hunter, 2009). Reductions in carbohydrate reserves were associated with reduced vegetative growth and yield in the following season (Hunter *et al*., 1995; Bennett *et al*., 2005; Smith & Holzapfel, 2012).

Most of the previous studies on the seasonal pattern of non-structural carbohydrate reserves in grapevines, were conducted in mediterranean regions. The work done in South Africa by Conradie (1980, 1981, 1990, 1992, 2005) and Uys (1981) on grapevine seasonal nutrient and reserve status was conducted in the Western Cape under cool climate conditions, with a different grapevine seasonal cycle and on wine grape cultivars. The work done by Saayman (1983) in the LOR region on 'Sultanina' investigated the causes of GAP and was limited to cane sampling for determining starch and sugar concentrations at only four phenological stages and not over the whole season. The study of Volschenk (2005), investigated the causes of die-back of young vines in the LOR region and the only carbohydrate analysis conducted was the starch concentration of young, grafted vines grown in a glasshouse under controlled conditions, determined over 10 weeks.

The impact of the longer continuous vegetative growth in the post-harvest period, as well as the influence of higher late-summer, autumn and winter temperatures on the seasonal carbohydrate reserve pattern in the warm climate of the LOR is not known. Theoretically, it is expected that this will negatively affect carbohydrate reserve accumulation. The study aimed to: (i) Establish the seasonal dynamics of non-structural carbohydrate reserves of *Vitis vinifera* L. cv. Sultanina H5 in the two major table grape production regions of South Africa; and (ii) establish a basis for the sampling of grapevine tissues for qualitative assessment of carbohydrate grapevine reserve

status (establish the optimal sampling time and type of tissue to be sampled to obtain reliable indicators of grapevine carbohydrate reserve status).

3.3 MATERIALS AND METHODS

3.3.1. Site and cultivar description

The study was conducted in two South African table grape production regions with different climates and growing seasons (Table 3.1). Sultanina was selected for this study, because it is one of the major cultivars produced both in South Africa and internationally (SATI, 2019).

Table 3.1: Experimental site details of the Sultanina H5 carbohydrate reserve dynamics trial.

Descriptor	Experimental site 1	Experimental site 2
Climate	Semi-arid	Mediterranean
Region	Lower Orange River	Hex River Valley
Province	Northern Cape	Western Cape
Coordinates	28°39'05.3"S, 21°06'38.9"E	33°47’S, 19°67'E
Location	Kanoneiland	De Doorns
Farm	Yarona, Karsten Boerdery	Hex River Experimental Farm, ARC
Cultivar	*Vitis vinifera* L. cv. Sultanina	
Clone	H5	
Rootstock	Ramsey	
Year established	2013	2003
Size	5 ha	0.78 ha
Vine spacing	3.3 m × 1.8 m	3.0 m × 2.0 m
Irrigation system	Micro sprinkler	
Pruning method	Cane (14 buds per cane)	
Trellis system	Gable	Trentina

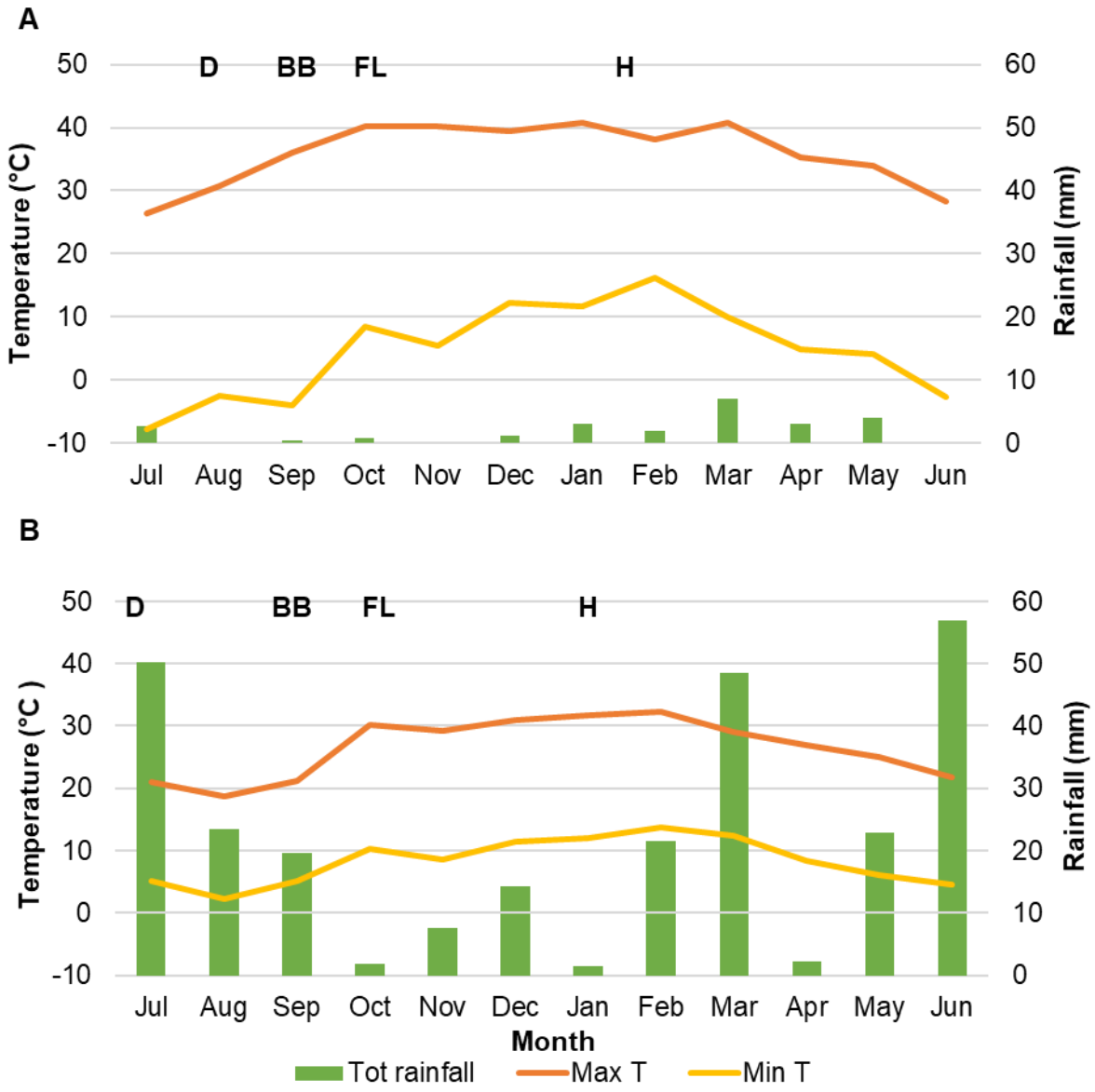
Site 1 (Semi-arid region):

An aerial view of Site 1 is given in Figure 3.1A. The automatic weather station (AWS) at Yarona, located about 500 m from the experimental site, was used as the source of weather data for Site 1 (Source: Ileaf: www.ileaf.co.za). In the 2018/2019 season, the mean minimum and maximum temperatures were 5°C and 36°C respectively (Source: Ileaf: www.ileaf.co.za). Based on the heat summation method over the growing season (Winkler *et al.,*1974), Saayman (1981) reported the LOR to be in the class V (3 022) climatic region for viticulture. Calculations done with the 2018/2019 season's AWS data, confirmed this site to be classified as Region V. Total rainfall for 2018/2019 was 24 mm, with a mean minimum and maximum relative humidity of respectively 8% and 86% (Figure 3.2A).

Site 2 (Mediterranean region):

An aerial view of Site 2 is given in Figure 3.1B. The AWS Hex River Valley, located about 50 m from the experimental site, was used as the weather data source for Site 2 (Source: ARC Institute for Soil Water and Climate). In the 2018/2019 season, the mean minimum and maximum temperatures were 8°C and 27°C, respectively. Based on the growing degree days (GDD) from September to March (Winkler *et al.,*1974), Saayman (1981) reported the Hex River Valley to be in the class V climatic region for viticulture. Calculations done with the data of the 2018/2019 season's AWS data, confirmed this site to also be classified as Region V. Total rainfall for 2018/2019 was 271 mm, with a mean minimum and maximum relative humidity of respectively 26% and 89% (Figure 3.2B).

Figure 3.2: An aerial view of the experimental sites for the Sultanina H5 carbohydrate reserve dynamics trial. (A)- Site 1- Lower Orange River/Semi-arid region and (B) Site 2- Hex River Valley/Mediterranean region.



	Date	
Key stage	**Semi-arid**	**Mediterranean**
Dormancy (D)	July- August	June- August
Bud Break (BB)	10 September	05 September
Flowering (FL)	23 October	25 October
Harvest (H)	04 February	21 January

Figure 3.3: Mean monthly minimum and maximum air temperatures and rainfall for the Sultanina H5 carbohydrate reserve dynamic trial sites. A- Lower Orange River (Semi-arid/Site 1) and B- Hex River Valley (Mediterranean/ Site 2) regions in the 2018/2019 season. (Sources: Site 1- Ileaf: www.ileaf.co.za; Site 2: ARC ISCW). Key phenological stages of the 2018/2019 season are also presented.

3.3.2 Experimental layout and sampling

The trial was laid out with a fully randomised design. Since this trial aimed to establish the seasonal pattern of carbohydrate reserves in two of the major table-grape-growing regions with different climates, no treatments were applied.

Sampling was done at both sites once a month (in the first week of the month and as far as possible, on the same date), for 12 months. Sampling commenced at the beginning of July 2018 and ended in June 2019. The sampling procedure described by Holzapfel (2009) was followed in the manner illustrated in Figure 3.3. Five replicates (panels), containing five vines per panel in Site 1 and four vines in Site 2 were sampled on each sampling date. Four panels were located towards the corners or within the four quarters of the block, while the fifth panel was located in or near the center of the block. These panels were chosen randomly and recorded not to be sampled more than once. Based on the results of Holzapfel *et al*. (2009), it was decided that at each sampling date, two shoots (green shoots or dormant shoots/canes, depending on date), a wood sample from the trunk, as well as medium-class roots (3-7 mm) were collected from each vine in the selected panel for determining carbohydrate reserve status. Trunk tissue samples were collected by drilling (BOSCH, GSR 180-LI Professional) with a 5 mm drill bit to a depth visually estimated as the center of the trunk and root samples were collected within 50 cm from the vine's base, using a spade. The basal three internodes of the shoot (cane) samples were used for analyses.

Sample preparation for chemical analysis

The roots were washed with a non-phosphate containing dishwashing liquid and rinsed with de-ionised water. All tissues were oven-dried at 60°C for 14 days (two weeks). Once dry, these tissues were ground to powder using a grinder (Retsch, MM 400) and kept in a -80°C freezer until extraction.

Figure 3.3: Illustration of the relative positions of panels used for sampling (five replicates) in the Sultanina H5 experimental vineyards in both regions for the carbohydrate reserve dynamics trial during the 2018/2019 season.

3.3.3 Extraction

To extract soluble sugars, the Anthrone method described by Dreywood (1946), Morse (1947), Loewus (1952), Windell (2012) and Ershadi *et al*. (2016) was used: 100 mg of each of the different ground tissues (roots, trunks, canes and shoots) was weighed out into 10 ml glass Kimax tubes and then 5 ml of 80% aqueous ethanol solution (EtOH) was added and the tubes were vortexed. These mixtures were placed in a heating block (Grant QBD4) for 60 minutes at 80°C and then centrifuged (Eppendorf Centrifuge 5810 R by Merck) for 10 minutes at 3 500 rpm with the temperature set at 20°C. The supernatant was decanted into marked glass vials. This was followed by another wash of 5 ml of 80% EtOH and placed in the heating block for 30 minutes at 80°C and centrifuged. The supernatant was decanted into the same glass vials for the second time. Finally, 5 ml distilled water (dH_2O) was added to the samples, vortexed and immediately centrifuged for 10 minutes at 3500rpm (20°C) and the supernatant was decanted into the glass vials. The three combined supernatants resulted in the extract. The extract was filtered through 0.45 µm Millipore filters into newly marked 2 ml vials.

To extract starch, the method of Windell (2012) was used, with amendments: in the same Kimax tubes where sugars were extracted, two ml acetate buffer (340 g/L at pH 4.5) was added. After the tubes were closed and vortexed, these tubes were placed in the heating block for 60 minutes at 100°C. The tubes were taken out of the block to cool and the heating block temperature was lowered to 60°C. Once cooled, 2 ml Amyloglucosidase enzyme in acetate buffer solution (0.25

mg/ml at pH 4.5) was added to each tube, the mixture was vortexed, and incubated in the 60°C heating block for 18 hours. After incubation, the temperature of the heating block was increased to 100°C and the tubes were placed in the heating block for another 5 minutes to stop the enzyme activity. The mixtures were centrifuged for 12 minutes at 4 000rpm with the temperature set at 20°C. The supernatant was decanted into newly marked glass vials and 5 ml dH_2O was added to the supernatant, making up the extract. The extract was filtered through 0.45 µm Millipore filters into marked 2 ml vials.

For the enzymatic analysis with the Enzyme robot, Arena 20XT (Thermo Electron Oy), to extract sucrose, d-fructose and d-glucose, a similar method to Windell (2012) was used: 200 mg of each of the different ground tissues was weighed out into 1.5 ml vials and 1 ml of 80% EtOH was added to the sample in each vial and vortexed. These samples were wrapped with parafilm and placed in the heating block for 60 minutes at 80°C. Subsequently, the samples were centrifuged for 5 minutes at 12 000 rpm and the supernatant was decanted into marked glass vials. The same step was followed with another 1 ml of 80% EtOH added to the same samples (tissues) and placed in the heating block for 30 minutes at 80°C. After centrifuging for 5 minutes at 12 000 rpm, the supernatant was decanted into the same glass vials for the second time. Finally, 1 ml dH_2O was added to the samples, whereafter it was vortexed and centrifuged for 5 minutes at 12 000rpm. This time, there was no heating. The supernatant was decanted into the glass vials and this resulted in the extract, which was a combination of three supernatants.

3.3.4 Analysis

The Anthrone method (described by Windell, 2012) was used to determine soluble sugars and starch, while enzymatic analysis with the Enzyme Robot, Arena 20XT (Thermo Electron Oy), was used to determine sucrose, d-fructose and d-glucose.

Soluble sugars and starch

From each of the filtered extracts, 20 µL of soluble sugars or 30 µL of the starch extraction was pipetted out into new marked Kimax tubes in triplicates and a dilution of 480 µL or 470 µL dH_2O was added to make up a 500 µL sample. A total of eight d-glucose standards were pipetted out according to Appendix 1 and both samples and standards were placed in ice water. In each tube (samples & standards), 1 ml Anthrone in sulphuric acid (2 g/L) was added (Dreywood, 1946; Morse, 1947), vortexed and placed in the heating block for 5 minutes at 100°C. Thereafter, the tubes were placed in ice water for cooling. Each mixture was transferred to 2 ml cuvettes.

Absorbance of all samples and standards were read at 620nm using a UV-Visible spectrophotometer (ThermoScientific, EVOLUTION 220) with one zero-d-glucose standard used as a blank (Ershadi *et al.* 2016). The preparation of standards is indicated in Appendix 1. The

TNC concentration is presented as the sum of soluble sugars and starch concentrations in this study.

Sucrose, d-fructose and d-glucose

To prepare the extract for enzymatic analysis, a dilution of 300 µL of the extract and 670 µL dH_2O was prepared, vortexed and centrifuged for three minutes at 12 000 rpm. The sample was decanted into enzyme robot cuvettes. Standards for the enzyme robot were prepared as indicated in Appendix 2 (A-C) The sample vials were placed in the enzyme robot, the Arena 20XT (Thermo Electron Oy) and readings were obtained.

3.3.5 Statistical analysis

The data were subjected to an ANOVA to compare months for each site and tissue type, a second ANOVA to compare months and sites for each tissue type and a third ANOVA where tissue type was added as a subplot factor to the model, using the General Linear Models Procedure (PROC GLM) of SAS software (Version 9.4; SAS Institute Inc, Cary, USA). The Shapiro-Wilk test on the standardised residuals from the model verified normality (Shapiro & Wilk, 1965). In cases where there was significant deviation from normality, outliers were replaced by a predicted (model) value (An observation is an outlier when the standardised residual for an observation deviated with more than three standard deviations from the model value). Levene's test verified homogeneity of site, month and tissue variances (Levene, 1960). Fisher's least significant difference (LSD) was calculated at 5% level to compare means of the factors (main effects) and factor interaction means (Ott & Longnecker, 2010). A probability level of 5% was considered significant for all significance tests. Pearson's product-moment correlation was performed between variables using the Correlation Procedure (PROC CORR) of SAS software (Version 9.4; SAS Institute Inc, Cary, USA).

3.4 RESULTS AND DISCUSSION

ANOVA results showing the impact of treatments, region and sampling date for the four types of plant tissues are presented in Appendix 3. For soluble sugars, starch and TNC concentration, significant differences were recorded between regions, as well as between sampling dates (months), but there was no significant interaction between region and sampling date. For sucrose, d-fructose and d-glucose, significant differences between regions and months occurred and there were several significant interactions between region and month.

Soluble sugars

Results presented in Appendix 4A indicate a decreasing trend in soluble sugars of all sampled tissue types and of both regions from the July to December sampling date, with significant reductions in no particular order or trend.

The seasonal dynamics of total soluble sugars, starch and total non-structural carbohydrates (TNC) of 'Sultanina H5' determined monthly during the 2018/2019 season in the two experimental sites are presented in Figure 3.4 A-F. For most of the sampling dates (July, August and February for both regions, September, December, March and April in the Mediterranean and November, January and June in the Semi-arid region), the trunks contained the highest soluble sugar concentration compared to the other tissues and this was most evident in the Mediterranean region. Soluble sugars in permanent tissues (roots, trunks, canes) reached their highest concentration during dormancy (June- July in the Mediterranean and June in the Semi-arid regions) and showed a steep decrease from dormancy through bud break (September), to flowering (October) in both regions (Figure 3.4A-B). A further decrease followed this in all tissues in the Semi-arid region until the December sampling date (6 weeks after flowering), as well as for canes, roots and shoots in the Mediterranean region. Another decrease was recorded for the shoots and canes of the Semi-arid region just after harvest. The decrease in soluble sugars accounted for an average of 89% calculated over all permanent tissues.

Soluble sugars in newly growing shoots of both regions generally increased in concentration after flowering (November in the Semi-arid and October in the Mediterranean region) until the May (Semi-arid region) and the June sampling dates (Mediterranean region) respectively. In the Semi-arid region, soluble sugars in trunks and roots gradually increased from the December (before véraison) to the February (2 days after harvest) sampling dates. After harvest, the soluble sugars increased in both regions. Tissues (roots, canes and shoots) from the Semi-arid region exhibited a decrease in soluble sugars from May to June, in contrast with the increase in all the tissues of the Mediterranean region in the same period.

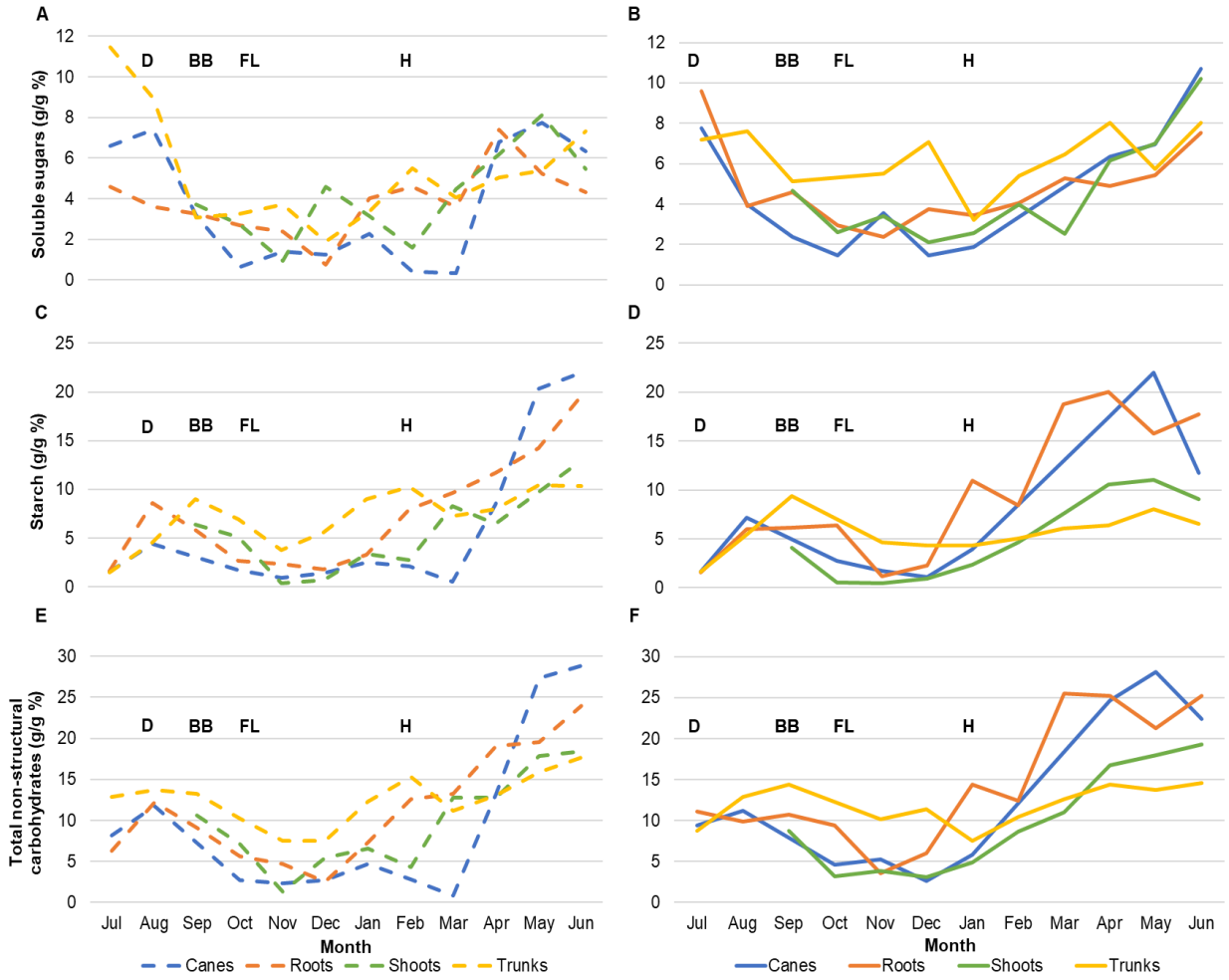


Figure 3.4: Soluble sugars, starch and total non-structural carbohydrate reserve dynamics in roots, trunks, canes and shoots of *Vitis vinifera* L. cv. Sultanina H5 in the Semi-arid region (A, C, E- dashed lines) and the Mediterranean region (B, D, F- solid lines) of South Africa during the 2018/2019 season. D- Dormancy, BB- Bud break, FL- Flowering, H- Harvest.

The high soluble sugar concentration in trunks throughout the season was also reported by Bennett (2002). The steep decrease that took place as bud break approached is ascribed to the onset of root as well as vegetative growth, as also reported by Uys (1981), Bates *et al*. (2002), Zapata *et al*. (2004), Holzapfel (2009) and Smith & Holzapfel (2012). Holzapfel (2009) reported that the decrease of carbohydrate concentrations from dormancy to flowering was just under 60% of the total grapevine reserves in the Wagga Wagga region, which has a warm climate. More reserves (89% soluble sugars) were mobilised in the current study between dormancy and flowering as stated earlier. This indicates the dependency of the grapevine on carbohydrate reserves to sustain new growth, both vegetative and reproductive.

In our study, the decrease in soluble sugars in November and December in both regions can be explained by the fact that during those periods, the bunches, which are strong sinks, may have been utilising the reserves for development and growth. Similar results were reported by Ollat *et al*., 2002, Lebon *et al*., 2005 and Vaillant-Gaveau *et al*., 2014. The increase from January in the

Mediterranean region and March in the Semi-arid region may be attributed to the removal of these sinks and thereby promoting the accumulation of reserves. The concentration increase in the Semi-arid region may also have been due to favourable environmental conditions for photosynthesis to take place, resulting in increased carbohydrate reserve accumulation (Volschenk & Hunter, 2009). In May, the concentration decrease in the Semi-arid region was expected as this region has a longer period of continued active vegetative growth in the post-harvest period (Volschenk & Hunter, 2009), linked to temperatures favourable for physiological activity (minimum temperatures above 10°C). This continuous active growth may have induced mobilisation of reserves, rather than accumulation. Vines of the site in the Semi-arid region still had mature green leaves and actively growing shoots until the occurrence of sub-zero temperatures and frost events in June, namely -3.9°C on 14/6/2019; -4.3°C on 15/6/2019; -4.4°C on 16/6/2019; according to Ileaf AWS data (Ileaf.co.za).

The decrease in soluble sugar concentrations during dormancy (July-August for the Mediterranean and July-mid-September for the Semi-arid region) may be due to respiration taking place during this period. This is in support of Winkler and Williams (1945) and Keller (2010) who stated that the reduction of carbohydrates during the dormancy period resulted from respiration. Keller (2010) reported this reduction, although it was not significant, in contrast with the results obtained in the current study. The decrease during the dormancy period was also observed in a study by Holzapfel (2009).

Because of early winters, grapevines in cool regions reach dormancy earlier than in warm regions (Bates *et al*., 2002; Smith & Holzapfel, 2012). This would mean that freezing tolerance is developed in early winter due to low winter temperatures. Sugars accumulate towards full endodormancy from the breakdown of starch and up-regulation of dehydrin genes (Kaplan *et al*., 2004; Holzapfel, 2009; Mohamed *et al*., 2012; Smith & Holzapfel, 2012; Rubio *et al*., 2016). The accumulation occurred in late autumn (May to June) in the Mediterranean region in the current study.

3.4.1. Starch

Starch was the most abundant form of carbohydrates in all tissues (roots, trunks, canes & shoots) (Figure 3.4A-D, Appendix 4A). In both regions, the starch concentration was low in all tissues in winter (July), during grapevine dormancy (Figure 3.4C-D, Appendix 4A). The concentration then increased in the Semi-arid region to a peak occurring in August (before bud break) in all the permanent tissues (roots, trunks & canes). A second peak was recorded in May for the Mediterranean region and in June for the Semi-arid region.

From August/September, all tissues showed a decrease in starch concentration. The decrease continued in all tissues, through flowering to December. The lowest concentration of starch occurred shortly after the flowering stage in all tissues in the Semi-arid region (Figure 3.4C-D, Appendix 4A). The same decreasing trend was also observed in the Mediterranean region, although the starch in the trunks was not at its minimum. The starch accumulated from December through to the post-harvest period in both regions.

The low starch concentrations (1.5- 1.7 g/g%) in both regions in winter (July) may be due to freezing tolerance. Numerous studies have reported that freezing tolerance is directly correlated with a .decrease in starch (Jones *et al*., 1999; Fennell, 2004; Mills *et al*., 2006; Ruelland *et al*., 2009; Janská *et al*., 2010; Ferguson *et al*., 2011). These studies reported that starch is broken down from which sugars accumulate in the vacuole and reduce intercellular ice crystals formation. The reverse conversion of sugars to starch may have been the reason for the increase that resulted in low peaks in August. Jones *et al*. (1999) and Ershadi *et al*. (2016) also reported the conversion of sugars to starch and correlated it to the increase in temperatures during late winter.

According to Bates *et al.*, (2002), Holzapfel, (2009) and Smith & Holzapfel, (2012), mobilisation of reserves takes place from late winter, before bud break, to supply newly developing tissues which are dependent on reserves for nutrition and growth. The reduction of starch as the flowering period approached recorded in this study, was also reported in a study by Holzapfel (2009). Zapata *et a*l. (2004) reported that starch concentration was reduced by 70% from dormancy to early bloom. In numerous other studies, the starch concentration was reported to reach minimum levels at the end of flowering (Bates *et al*., 2002; Zapata *et al*., 2004; Holzapfel, 2009). According to these studies, there is an increased mobilisation of carbohydrate reserves as new competitive sinks (inflorescences) develop during this period. The same may have occurred in the current study as flowering took place in late October (23rd in the Semi-arid and 25th in the Mediterranean regions). This could mean that the flowering period extended into November (3 and 9 days after flowering in the Semi-arid and Mediterranean regions respectively), hence the minimum concentrations recorded for these periods.

The accumulation of starch in the post-harvest period can be ascribed to the majority of photosynthetic products being stored as reserves (Bates *et al*., 2002; Smith & Holzapfel, 2012). According to Smith and Holzapfel (2012), the accumulation of reserves starts as soon as the growing tissues, especially leaves, are able to photosynthesise to provide nutrition for the developing sinks. This was also observed in the current study as starch concentrations began to increase, following the flowering period, in agreement with Holzapfel's (2009) and Keller (2010) results. The high concentration of starch in the roots of the Mediterranean region supports the

findings of other studies where roots contained the most starch (Zapata *et al*., 2004; Holzapfel, 2009).

3.4.2. Total non-structural carbohydrates (TNC)

Regarding TNC, the patterns for both regions (Figure 3.4E-F) resemble those of starch, TNC concentration in all measured tissues decreased from dormancy to bud break, with a further general decreasing trend from bud break until December. From then, a general increase was recorded.

After harvest in the Semi-arid region, TNC decreased for a short while, followed by a gradual increase and then a steep increase (Figure 3.4E). In the Mediterranean region, the increase began after harvest except in roots where a similar trend for the Semi-arid region was found (Figure 3.4F). These increases continued through to May, from where the Mediterranean region exhibited a decrease in canes.

In this study, accumulation of TNC commenced between one and two months after flowering in both regions, in contrast with the results of Lebon *et al*. (2008), that carbohydrate reserves begin to accumulate in the perennial organs for storage from flowering. The growing clusters can photosynthesise due to the presence of chlorophyll but because they are strong sinks, they also utilise photosynthates produced by new sources, the leaves (Ollat *et al*., 2002; Ristic & Iland, 2005). Candolfi-Vasconcelos *et al*. (1994) and Lebon *et al*. (2008) reported that mobilisation of reserves for supplying inflorescences and other sinks stops at flowering, whereafter the main sources (young fully mature leaves) are actively supplying nutrients.

In Table 3.2 the mean concentrations of soluble sugars, starch and TNC over all 12 sampling dates are presented for the four tissue types sampled. The soluble sugars in roots, trunks and canes, as well as starch in roots and canes of the Mediterranean region were significantly higher than in the Semi-arid region. Starch in the Semi-arid region trunks and shoots was significantly lower than the Semi-arid region.

Table 3.2: Mean soluble sugars, starch and total non-structural carbohydrate concentrations (g/g%) in roots, trunks, canes and shoots of the Semi-arid and Mediterranean regions.

Soluble sugars (g/g %)	Semi-arid	Mediterranean
Roots	3.85 b*	4.75 a
Trunks	5.11 b	6.15 a
Canes	3.52 b	4.44 a
Shoots	4.27 a	4.60 a
Starch (g/g %)		
Roots	7.56 b	9.03 a
Trunks	7.13 a	5.52 b
Canes	5.71 b	7.28 a
Shoots	5.62 a	4.99 b
Total non-structural carbohydrates (g/g %)		
Roots	12.02 b	14.23 a
Trunks	12.54 a	11.96 a
Canes	7.95 b	11.06 a
Shoots	10.32 a	9.66 b

*Means with the same letter in rows do not differ significantly from each other ($p < 0.05$).

Sucrose

Results presented in Appendix 4B show significant differences ($p < 0.0001$) between regions in different tissue types for some of the sampling dates for sugars (sucrose, d-fructose and d-glucose). These did not show any particular trend. Sucrose in roots showed a significant difference between regions for only one sampling date, June ($p < 0.0001$) (Appendix 4B).

Amongst the three sugars in both regions, sucrose was the most abundant (Figures 3.5A-F), with the highest concentrations recorded for the roots. Comparing the two regions, the tissues of the Mediterranean region tended to contain the most sucrose throughout the season (Figures 3.5A-B). At dormancy, the Mediterranean region had a higher concentration in roots than the Semi-arid region but it was the opposite for the trunks. The canes and roots in both regions showed an increase in sucrose from August, whereas the trunks showed a decrease as bud break approached.

In the Semi-arid region, all tissues experienced a decrease from after the flowering period to December, when the minimum concentration in all tissues was reached. In the Semi-arid region, the roots and, to a lesser extent the trunks and canes, began increasing in sucrose from December, while sucrose in the shoots showed an increase a month later. In the Mediterranean

region, the decrease to the minimum sucrose concentration occurred earlier (November) in roots and a month later (December) in canes and shoots. Both regions showed a general increasing trend amidst the fluctuations from the minimum concentrations. The increase continued into early winter in the Semi-arid region, while the concentrations decreased from mid to late autumn in the Mediterranean region.

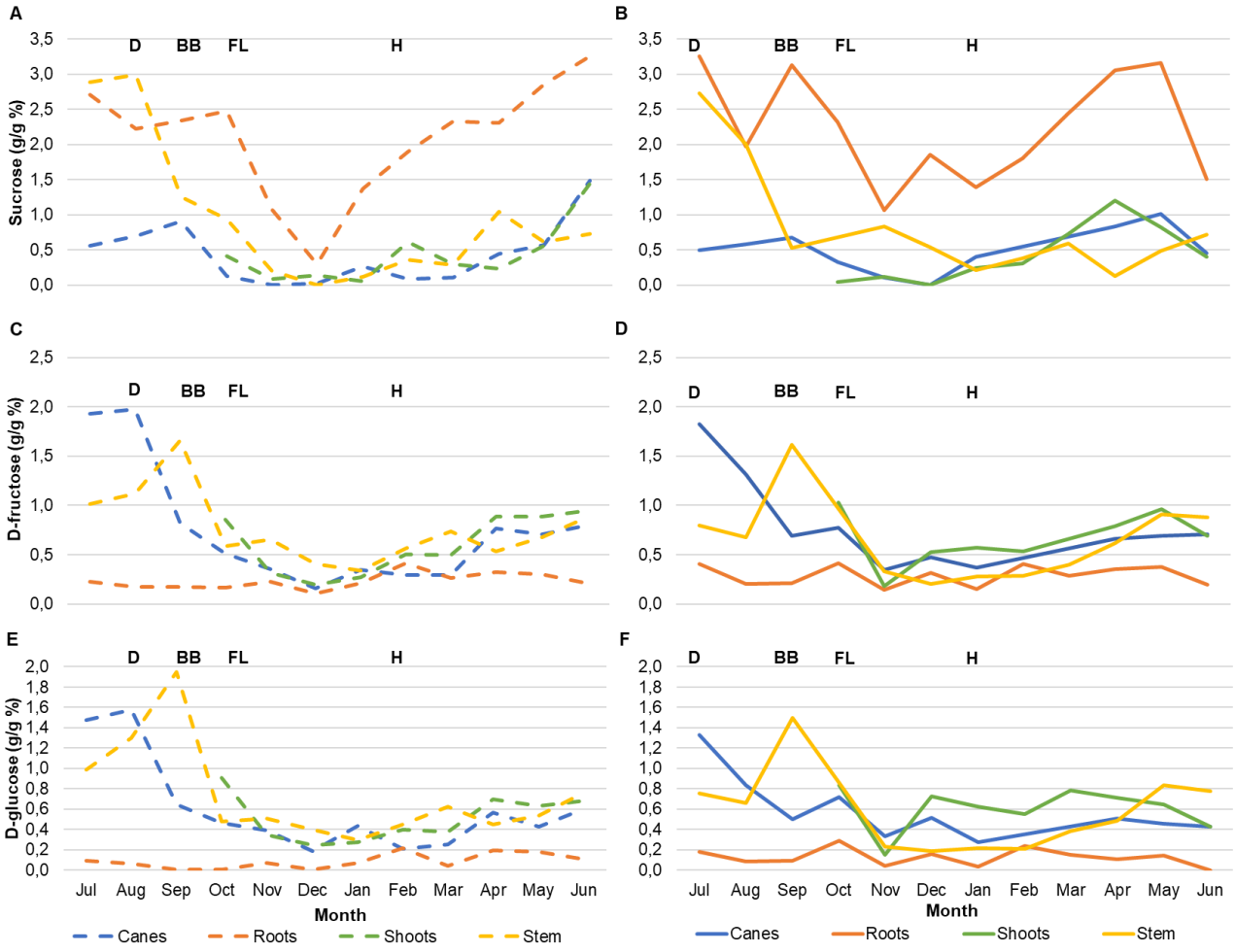


Figure 3.5: Non-structural carbohydrate reserve dynamics in roots, trunks, canes and shoots of the Semi-arid region (A, C, E- dashed lines) and the Mediterranean region (B, D, F- solid lines) of South Africa during the 2018/2019 season. D- Dormancy, BB- Bud break, FL- Flowering, H- Harvest.

The steep decrease to a minimum (after flowering) in the Semi-arid region observed in our study could be due to the impact of the high demand of the developing crop as well as shoots on reserve mobilisation as reported by Pellegrino *et al*. (2014). The reductions toward harvest might be because of ripening as Ollat *et al*. (2002) reported an increased carbon import by berries during this period. Silva *et al.* (2017), Keller (2010) and Hunter *et al*. (1995) also reported that roots contained the most sucrose.

Sucrose generally accumulated after harvest in both regions. For the Semi-arid region, the continuous increase in sucrose as winter approached, might be due to grapevines' continued

vegetative growth activity in this particular climate (Volschenk & Hunter, 2009) as discussed in Section 2.3. This period is essential for carbohydrate replenishment, especially for grapevines with high crop loads in warm climates like the Semi-arid region (Holzapfel & Smith, 2012). Grapevines in the Mediterranean region have a shorter post-harvest period; therefore, vegetative growth and consequently, photosynthesis does not continue as long, due to unfavourable environmental conditions in preparation for the winter period (Bates *et al*. 2002). Due to this, the accumulation of reserves stops earlier, as seen in Figures 3.5A-B.

3.4.3. D-fructose and d-glucose

The d-fructose and d-glucose patterns over the season were similar, although differences occurred between the regions. The d-fructose concentration was slightly higher than d-glucose in both regions. According to Figures 3.5C-F, the Mediterranean region had overall higher concentrations of the two sugars than the Semi-arid region, in all tissues excluding the trunks. The canes and trunks had the highest peaks at dormancy and bud break in both regions, respectively. Of all tissues studied, the roots had the lowest concentration of the two sugars in both regions throughout the season, with values below 0.5% for all 12 sampling dates (Figures 3.5C-F).

In the Semi-arid region, d-fructose and d-glucose in canes decreased from dormancy to bud break, while they increased in trunks. From bud break to flowering both sugars decreased in all measured tissues. Similar to the soluble sugars, the accumulation of d-fructose and d-glucose in shoots, canes and roots of the Semi-arid region commenced from December when these tissues were at their minimum concentrations.

From bud break to flowering, d-fructose and d-glucose in canes and roots of the Mediterranean region increased. D-fructose and d-glucose in trunks of the Mediterranean area showed a decrease from bud break until about 2 months after flowering (December), while in shoots, canes and roots a decrease occurred from flowering until about 1 month after flowering (November). From after flowering until harvest, all tissues of both regions showed a general increase in the two sugars. According to Figures 3.5C-F, trunks, canes and shoots increased after harvest (February) in both regions, while the roots showed a decrease. The decrease in roots continued until the onset of winter (June). In mid-autumn (April), both d-fructose and d-glucose showed a low peak in canes and shoots of the Semi-arid region while this peak occurred early autumn (March) in trunks. On the other hand, the Mediterranean region's roots, trunks and shoots showed peaks in d-fructose late autumn (May), while the d-glucose peaks occurred at different sampling dates. Smith and Holzapfel (2012) reported that trunks and canes had the highest sugar concentration while roots contained the least. The current study's results support this.

In contrast to the current study, Silva *et al*. (2017) found d-glucose to be higher than d-fructose. The two sugars peaked in canes at dormancy for both regions, which may result from low temperatures in buds inducing freezing tolerance. Starch is converted to soluble sugars during this period, as discussed in Section 3.4.1 (Kaplan & Guy, 2005; Mohamed *et al*., 2012; Smith & Holzapfel, 2012; Rubio *et al*., 2016). In the same tissue, the concentration of the sugars decreases before bud break as a reverse conversion of soluble sugars to starch occurs when temperatures start to increase (Williams, 1996). Similar to soluble sugars, d-fructose and d-glucose concentrations in both canes and trunks in the two regions decreased from bud break due to commencement of new vegetative and reproductive growth (Bates *et al*., 2002; Bennett, 2002; Smith & Holzapfel, 2012).

In Table 3.3 the mean concentration of sucrose, d-fructose and d-glucose over all 12 sampling dates are presented for the four tissue types sampled. There was no significant difference in sucrose concentration in all the tissues of the two regions. D-fructose in roots and d-glucose roots and shoots of the Semi-arid region were significantly lower than in the Mediterranean region. D-fructose and d-glucose in trunks of the Semi-arid region were significantly higher compared to the Mediterranean region.

Table 3.3: Non-structural carbohydrate concentrations in roots, trunks, canes and shoots of the Semi-arid and Mediterranean regions. (Means over 12 monthly sampling dates during the 2018/2019 season).

Sucrose (g/g %)	Semi-arid	Mediterranean
Roots	2.09 a*	2.25 a
Trunks	0.95 a	0.83 a
Canes	0.44 a	0.49 a
Shoots	0.43 a	0.43 a
D-fructose (g/g %)		
Roots	0.23 b	0.29 a
Trunks	0.76 a	0.63 b
Canes	0.75 a	0.79 a
Shoots	0.60 a	0.66 a
D-glucose (g/g %)		
Roots	0.09 b	0.13 a
Trunks	0.73 a	0.57 b
Canes	0.60 a	0.59 a
Shoots	0.51 b	0.61 a

*Means with the same letter in rows do not differ significantly from each other ($p < 0.05$).

3.4.4. Correlations between internal carbohydrate concentrations of different vine tissues

Table 3.4 presents correlation coefficients and the significance of the relationships between non-structural carbohydrate reserves in roots, trunks, canes and shoots for the Semi-arid region. Soluble sugars in canes showed high positive correlations between canes and trunks (0.73) and shoots (0.80). For starch, roots had significantly high positive correlations (correlation coefficients ranging from 0.65 to 0.92; $p < 0.001$) with all above-ground parts of the vine. Starch in shoots and canes was highly positively correlated with all tissues (0.70 to 0.92 and 0.60 to 0.89 respectively). With sucrose, significantly positive correlation coefficients were recorded between roots and shoots, canes and trunks and canes and shoots (0.65, 0.67 and 0.82 respectively). For d-fructose, a significantly high positive correlation of 0.85 was recorded between shoots and canes whereas with d-glucose, the relationship between roots and the above-ground parts of the vine was not significant.

Table 3.5 presents correlation coefficients and the significance of the relationships between non-structural carbohydrate reserves in roots, trunks, canes and shoots for the Mediterranean region. Shoots had high and significant positive correlations with roots and canes for soluble sugars (0.79

and 0.95 respectively). For starch, canes correlated highly and significantly with all tissues (0.79 to 0.96) and shoots with roots and canes (0.88 and 0.96 respectively). Canes correlated highly and significantly with roots and shoots (0.71 and 0.81 respectively) with sucrose. Canes also correlated highly with shoots with d-fructose (0.81).

Table 3.4: **Correlation coefficients and significance of the relationship between NSC reserves in roots, trunks, canes and shoots of the Semi-arid region during the 2018/2019 season.**

Semi-arid	Roots	Trunks	Canes	Shoots
Soluble sugars (g/g %)				
Roots	1	0.47 **	0.60 **	0.53 **
Trunks	0.47 **	1	0.73 ***	0.43 *
Canes	0.60 **	0.73 ***	1	0.80 ***
Shoots	0.53 **	0.43 *	0.80 ***	1
Starch (g/g %)				
Roots	1	0.65 ***	0.88 ***	0.92 ***
Trunks	0.65 ***	1	0.60 ***	0.70 ***
Canes	0.88 ***	0.60 ***	1	0.89 ***
Shoots	0.92 ***	0.70 ***	0.89 ***	1
Sucrose (g/g %)				
Roots	1	0.40 *	0.46 **	0.65 ***
Trunks	0.40 *	1	0.67 ***	0.37 ns
Canes	0.46 **	0.67 ***	1	0.82 ***
Shoots	0.65 ***	0.37 ns	0.82 ***	1
D-fructose (g/g %)				
Roots	1	-0.13 ns	-0.12 ns	0.36 ns
Trunks	-0.13 ns	1	0.49 **	0.51 **
Canes	-0.12 ns	0.49 **	1	0.85 ***
Shoots	0.36 ns	0.51 **	0.85 ***	1
D-glucose (g/g %)				
Roots	1	-0.29 ns	-0.01 ns	0.21 ns
Trunks	-0.29 ns	1	0.51 **	0.34 ns
Canes	-0.01 ns	0.51 **	1	0.60 ***
Shoots	0.21 ns	0.34 ns	0.60 ***	1

[a] *, **, *** and ns indicate significance at $p < 0.05$, < 0.01, < 0.001, and not significant, respectively. Values presented in this table represent correlation coefficients of means.

Table 3.5: Correlation coefficients and significance of the relationship between NSC reserves in roots, trunks, canes and shoots of the Mediterranean region during the 2018/2019 season.

Mediterranean	Roots	Trunks	Canes	Shoots
Soluble sugars (g/g %)				
Roots	1	0.52 **	0.69 ***	0.79 ***
Trunks	0.52 **	1	0.43 ns	0.47 *
Canes	0.69 ***	0.43 ns	1	0.95 ***
Shoots	0.79 ***	0.47 *	0.95 ***	1
Starch (g/g %)				
Roots	1	0.51 **	0.82 ***	0.88 ***
Trunks	0.51 **	1	0.79 ***	0.50 *
Canes	0.82 ***	0.79 ***	1	0.96 ***
Shoots	0.88 ***	0.50 *	0.96 ***	1
Sucrose (g/g %)				
Roots	1	0.12 ns	0.71 ***	0.68 ***
Trunks	0.12 ns	1	0.01 ns	-0.36 ns
Canes	0.71 ***	0.01 ns	1	0.81 ***
Shoots	0.68 ***	-0.36 ns	0.81 ***	1
D-fructose (g/g %)				
Roots	1	-0.07 ns	0.27 ns	0.57 **
Trunks	-0.07 ns	1	0.28 ns	0.62 **
Canes	0.27 ns	0.28 ns	1	0.81 ***
Shoots	0.57 **	0.62 **	0.81 ***	1
D-glucose (g/g %)				
Roots	1	-0.09 ns	0.38 *	0.42 *
Trunks	-0.09 ns	1	0.16 ns	0.03 ns
Canes	0.38 *	0.16 ns	1	0.60 **
Shoots	0.42 *	0.03 ns	0.60 **	1

[a] *, **, *** and ns indicate significance at $p < 0.05$, < 0.01, < 0.001, and not significant, respectively. Values presented in this table represent correlation coefficients of means.

Sampling of roots is a cumbersome process, more time-consuming and less practical compared to sampling above-ground tissues, in this case, canes and shoots. The highly significant positive correlations between starch and soluble sugar content of roots and all above-ground parts of the vine in the Semi-arid region indicate that any of these tissue types could be sampled and analysed to indicate the overall carbohydrate reserve status of the vine. In the Mediterranean region, any of the above-ground tissue types could be analysed for starch, while shoots and canes are considered reliable tissue types to be sampled for assessment of soluble sugars. In both regions, highly significant correlations between sucrose content of roots and shoots were recorded. Holzapfel (2009) reported significant correlations ($p < 0.05$ and $p < 0.01$) between medium roots and above-ground tissues for starch and non-significant correlations for sugars in Wagga Wagga, a warm region in Australia.

3.5 CONCLUSION

The seasonal dynamics of non-structural carbohydrate reserves of *Vitis vinifera* L. cv. Sultanina H5 in the two major table grape production regions of South Africa was established. Starch was the most abundant form of carbohydrates in all tissues in both regions. In both regions, soluble sugars in permanent tissues (roots, trunks, canes) reached their highest concentration during dormancy (June- July). The starch concentration was low in all tissues in winter (July), during grapevine dormancy, whereafter it increased to a peak occurring in August (before bud break) in all the permanent tissues in the Semi-arid region (roots, trunks and canes) and in canes and roots of the Mediterranean region. The Mediterranean region had higher concentrations of soluble sugars in roots, trunks and canes as well as starch in roots and canes compared to the Semi-arid region.

Sucrose was the highest form of sugar and was mostly contained in the Mediterranean region tissues than the Semi-arid region. The minimum concentrations were recorded in December in all tissues of both the Semi-arid and Mediterranean regions except for the roots of the latter region whose concentration reached a minimum a month earlier. The Mediterranean region, again, contained the most d-glucose and d-fructose. All sugars began to increase from minimum concentrations at post-flowering to harvest.

A basis was established for sampling grapevine tissues for qualitative assessment of grapevine carbohydrate reserve status, linking sampling time to occurrence of peaks in soluble sugars and starch concentrations. It is recommended that sampling for qualitative assessment of soluble sugars should be done after leaf fall, during dormancy (June-July under the conditions of this study). Starch concentrations should be assessed before bud break (August under the conditions of this study). In the Semi-arid region, any of the above-ground tissue types could be sampled and analysed to indicate the overall carbohydrate reserve status of the vine. In the Mediterranean region, any of the above-ground tissue types could be analysed for starch, while shoots and canes are considered reliable tissue types to be sampled for assessment of soluble sugars. In both regions, highly significant correlations between sucrose content of roots and shoots were recorded. Canes and/or shoots are considered the most practical tissue types to sample for determination of grapevine non-structural carbohydrate reserve status.

Analysis of TNC is usually done using wet chemistry methods, which although accurate, are time-consuming and costly. Further research should be done to establish feasible and rapid methods for quantifying carbohydrate reserve status of grapevines. It is recommended that in a further phase of this project:

i. NIR spectroscopy should be used for collecting NIR spectra from grapevine cane tissues, whereafter the TNC concentration of the same samples must be determined using a wet chemical method and the results should be compared, using multivariate data analysis. Through this process, it could be established whether NIR spectroscopy could be used as a practical tool for rapid screening of TNC concentrations of grapevine tissues.

ii. The starch iodine test (already commercially used in the apple and forestry industries), should be evaluated as an additional tool for quantifying starch status of grapevine tissues (canes and roots), as an indicator of TNC status.

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Chapter 4: Effect of post-harvest summer pruning on carbohydrate reserve status, bud break and fertility of *Vitis vinifera* L. cv. Sultanina H5 in the Lower Orange River region.

4.1 ABSTRACT

Carbohydrate dynamics are affected by several viticultural practices. The effects of post-harvest summer pruning on carbohydrate reserve status, bud break and fertility were evaluated in this trial. The experimental layout was a randomised block design, with five treatments replicated in six blocks. These entailed 33% and 66% shoot removal one day after harvest (33_1dAH and 66_1dAH respectively), 33% and 66% shoot removal 45 days after harvest (33_45dAH and 66_45dAH respectively) and a control (Ctr). Perennial tissues (cane, trunk and roots) were destructively sampled at four sampling dates and non-structural carbohydrates were extracted and quantified to assess the effect of post-harvest summer pruning on carbohydrate reserve status. Both 66% pruning treatments significantly increased photosynthetically active radiation (PAR) and percentage transmittance immediately after application. Pruning did not have a significant effect on photosynthetic rate. No clear trend trends were recorded regarding the effect of the treatments on TNC reserves. Cumulative bud break was not significantly influenced by post-harvest summer pruning, although 66_1dAH resulted in an increased bud break rate. Return potential fertility was also not affected by post-harvest summer pruning but actual fertility was increased by 66_45dAH. Based on this one season's results, post-harvest pruning did not have overall practical benefits, apart from improving light penetration. A continuation of this trial is recommended as second and third seasons' data would contribute to confirming the effect of these pruning treatments on bud break and fertility.

4.2 INTRODUCTION

Pruning during the post-harvest period is a cultivation practice widely used by several table grape producers in South Africa. This practice is done to improve post-harvest reserve accumulation in the current season and bud break as well as bud fertility in the following season (Van der Merwe, 2017). Increased input costs of table grape production, force producers to evaluate the return on investment of each cultivation practice, such as post-harvest pruning. Any manipulation should only be done if it is scientifically proven to be necessary and economically justified.

Management practices are important as they alter the vine source-sink relations, affecting metabolism and growth (Silva *et al*., 2017). Pruning is applied to manage or maintain vine vigour and structure, as well as to promote uniform bud break, productivity, fruit quality and to maintain a practical number of shoots and fruit (Sommer *et al*., 1995; Keller *et al*., 2004) for manual manipulation practices that need to be applied, impacting on the labour requirements required for these practices. During the application of winter pruning, different techniques could be followed and, in a few situations, it is not applied at all. The pruning system to be applied is determined by the position of fertile buds on the canes (Lombard *et al*., 2006). Spur pruned cultivars are those with fertile basal buds. Those cultivars that are fertile in the central part of the shoot are pruned to half-long bearers (4 to 8 buds), while those that are fertile at higher bud positions are pruned as canes (10 to 16 buds) (Christensen, 2000; Keller, 2010).

Strategies applied during winter pruning include minimal/light, as well as mechanical pruning (Rühl & Clingeleffer, 1993). Minimal/light pruning could be applied to manipulate the vine into producing a large number of bunches, contributing to decreased set. The goal with this is to reduce bunch compactness in naturally compact bunch-producing cultivars (Keller, 2010). According to long-term observations, Zheng *et al*. (2017) reported that minimal pruning induced higher yields. Mechanical pruning could be used as a pre-pruning practice that would later be followed up by manual pruning. It can also be applied as a normal winter pruning practice, usually on cordon-trained vines. In a study conducted by Keller *et al*. (2004), machine pruning resulted in a higher bunch number per vine and higher yields. With this practice, the producer does not have control over how many buds are left compared to other methods (Keller, 2010). Machine pruning is considered cost-effective as it requires less labour and low costs (Rühl & Clingeleffer, 1993).

Post-harvest summer pruning is often practiced in warm, arid/semi-arid areas. Since these areas have a long post-harvest period compared to cool climates, summer pruning reduces the quantity of carbohydrate reserves utilised by continuously growing tissues in this period (Keller, 2010; Hall *et al*., 2016). This practice is carried out by removing young, weak or poor-quality shoots to improve carbohydrate reserve accumulation into the main shoots identified to be selected as canes during winter pruning. (Van der Merwe, 2017). Post-harvest summer pruning could be considered a pre-winter pruning technique (Keller, 2010; El-Boray *et al*., 2018). According to Christensen (2000), canes exposed to the sun must be kept during winter pruning of Thompson Seedless grapevines as they are more fertile than those in the shade.

Removing only a part of the shoot, especially topping, breaks apical dominance. The phenomenon of apical dominance entails the suppression of lateral growth by the shoot apexes (Tanaka *et al*.,2006; Shimizu-Sato *et al*., 2009). These shoot apexes produce auxins which are hormones that repress cytokinin biosynthesis (Nordström *et al*., 2004; Shimizu-Sato *et al*., 2009).

Therefore, the removal of shoot apexes promotes the biosynthesis of cytokinins and their transportation to lateral buds, resulting in lateral shoot growth (Nordström *et al*., 2004; Tanaka *et al*.,2006; Shimizu-Sato *et al*., 2009). Removing a shoot tip, or part of a shoot could result in either more uptake of carbohydrate reserves by new growth or an increase in replenishment by newly photosynthesising leaves.

Carbohydrate reserves in the form of starch and sugars, are products of photosynthesis (Candolfi-Vasconcelos *et al*., 1994, Bouard, 1996, Bates *et al*., 2002, Wojnarowiez *et al*., 2008, Holzapfel, 2009, Rogiers *et al*., 2011, Smith & Holzapfel, 2012; Greven *et al*., 2016). These reserves are stored in permanent parts of the vine. According to Smith & Holzapfel (2009), Pellegrino *et al*. (2014) and Hall *et al*. (2016), the post-harvest period is essential for replenishing carbohydrate reserves and root growth, especially for high yielding vineyards in warm climatic areas.

According to Holzapfel *et al*. (2006), post-harvest practices may alter the vine capacity to replenish nutrient reserves. Alteration in nutrient reserve replenishment, specifically carbohydrates, may also affect vine fruitfulness (Sommer *et al*., 2000; Bennett, 2002). The impact of shoot/leaf removal on carbohydrates and, therefore, bud break and fertility, have been studied by numerous researchers. In a study on Sauvignon Blanc by Trought *et al*. (2011), post-harvest pruning had little effect on trunk carbohydrates and no effect on bud break. When El-Boray *et al*. (2018) applied this practice, it did not affect bud fertility percentage in the first season, and this was reported to be since the inflorescence primordia had already been formed in the previous season. In the second season, bud fertility and the fertility coefficient increased. Complete defoliation immediately after harvest reduced vine growth and yield, although the impact on carbohydrate reserves was only established after two consecutive years of defoliation treatments (Greven *et al*., 2016).

In contrast to this, Smith and Holzapfel (2009) reported that total non-structural carbohydrates were decreased and yield reduced by up to 22% after one season of complete defoliation at harvest. After two seasons, yield was reduced by 50%. Holzapfel & Smith (2012), having cut all shoots to five nodes after harvest, reported reduced carbohydrate concentrations by 14% after two seasons and 27% in the third season. Links (2014) did not obtain any significant effect with 33% and 66% post-harvest pruning on starch and sugars in canes in two consecutive seasons. In the same study, bud break percentage was significantly reduced by 33% post-harvest pruning in the season following treatment application, while none of the treatments was significant in the following season.

According to two-year average results, summer pruning and standard winter pruning significantly reduced water-soluble reducing sugars and starch in almond trees (Ikinci *et al*., 2016). The tree

and fruit quality effects were only observed in the season after treatment application. Post-harvest summer pruning resulted in the lowest total sugar content in July (summer) and increased starch content of apricot shoots in January, March and October (Demirtas *et al*., 2010). This practice reduced the cold hardiness of flower buds, delayed defoliation, and reduced carbohydrate levels in peach trees (Ikinci, 2014). It was also reported in the same study that early summer pruning resulted in the lowest carbohydrate concentrations. Ashraf & Ashraf (2014) reported that summer pruning increased flower bud formation and return bloom in apple trees.

Taking the above-mentioned into consideration, this study aimed to investigate whether post-harvest pruning results in practical and economic benefits for the producer, *i.e.* increased carbohydrate reserve status and improved performance regarding bud break and fertility? The objective of this study was to determine the effect of post-harvest pruning, early and late, severe (66%) and less severe (33%), on carbohydrate reserve status, bud break and fertility of *Vitis vinifera* L. cv. Sultanina H5 in the semi-arid Lower Orange River region.

4.3 MATERIALS AND METHODS

4.3.1 Site and cultivar description

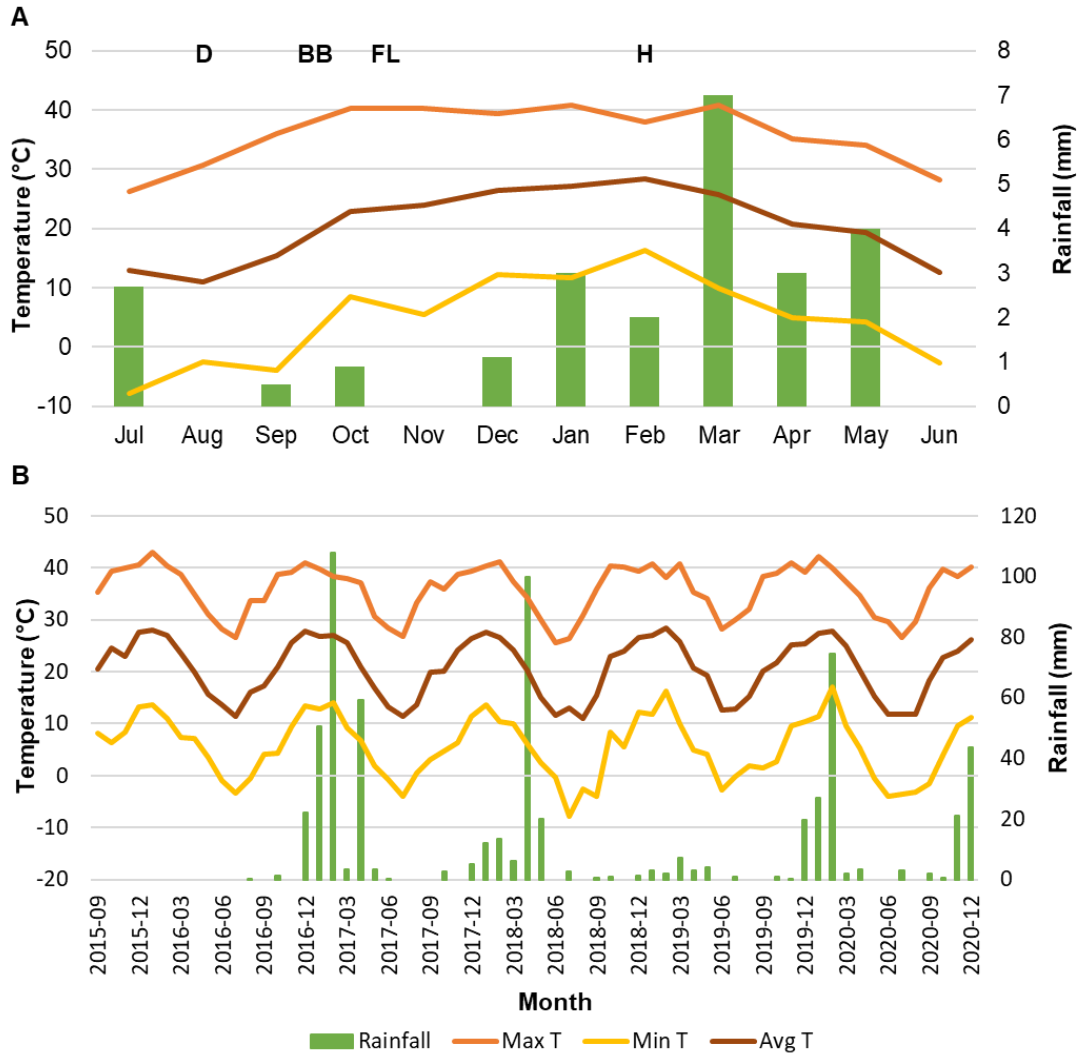
The field trial block details are presented in Table 4.1 and Figure 4.4. The study was conducted in a five-year-old vineyard, with *Vitis vinifera* L. cv. Sultanina H5 grafted onto Ramsey (*Vitis champini*), on the commercial farm Yarona, near Kanoneiland. The soil form was classified as an Augrabies soil. The experimental vineyard's soil texture and chemical analyses results are presented in Appendix 5. The vines were spaced 3.3 m x 1.8 m and trained onto a gable trellis system, with the rows orientated in an east-west direction. The site was considered representative of the soil type and irrigation system mainly utilised for table grapes in the region. The vineyard was irrigated using scheduled micro-irrigation. Vines were fertilised according to standard practices for the region, cultivar and vine leaf petiole analyses during the growing season. All other viticultural treatments were as recommended for the production of export quality 'Sultanina' grapes. in the region (Van der Merwe, 2017), including allocating 8 canes per vine (14 buds per cane), during winter pruning in July 2018 and application of hydrogen cyanamide (2.5%) on 8 August 2018. Plastic sheets were installed over the canopy 6 weeks before harvest, to protect grapes from possible rain damage.

Table 4.1: Details of the Sultanina H5 experimental block of the post-harvest pruning trial in the Lower Orange River region.

Descriptor	Experimental site
Climate	Semi-arid
Region	Lower Orange River
Province	Northern Cape
Coordinates	28°39'05.3"S, 21°06'38.9"E
Location	Kanoneiland
Farm	Yarona. Karsten Boerdery
Cultivar	*Vitis vinifera* L. cv. Sultanina
Clone	H5
Rootstock	Ramsey
Year established	2013
Size	5 ha
Vine spacing	3.3 m × 1.8 m
Row direction	East-West
Irrigation system	Micro sprinkler
Pruning method	Cane (14 buds per cane)
Trellis system	Gable
Soil type	Augrabies

Figure 4.1: Location of the Sultanina H5 block where the post-harvest pruning field trial was conducted (highlighted in yellow) on the commercial farm Yarona, near Kanoneiland in the Lower Orange River region.

Based on the growing degree days (GDD) from September to March (Winkler *et al.,*1974), Saayman (1981) reported the LOR to be in the class V climatic region for viticulture. The region is Semi-arid (Hunter & Bonnardot, 2011). In the season of the study, the mean minimum, average and mean maximum temperatures were 5°C, 21 °C and 36°C respectively. Total rainfall was 24 mm, with a mean minimum and maximum relative humidity of 8% and 86% respectively. The heat summation calculations (Winkler index) for the 2018/2019 season gave a result of 3 022 GDD (growing degree days). This value is similar to the value of 3 064 GDD reported by Hunter & Bonnardot (2011) for the same region. The maximum rainfall was 7 mm in March (Figure 4.2A), supporting the long-term rainfall results reported by Harmse *et al*. (2019) for the Northern Cape province. On the 7th of July 2018, which was leaf fall, frost occurred. Hydrogen cyanamide (2.5%) was applied on 8 August 2018. In the season of the study, corresponding with long-term trends, the coldest month in this region was July and minimum temperatures in this particular month were often below 0°C. The LOR is a summer rainfall region, with a long-term average rainfall of 10 mm (Source: www.ileaf.co.za).



Key stage	Date
Dormancy (D)	July- August
Bud Break (BB)	10 September
Flowering (FL)	23 October
Harvest (H)	04 February

Figure 4.2: Long-term and monthly (2018/2019 season) temperature and rainfall data for the experimental site in the semi-arid Lower Orange River region with key phenological stages of the experimental block (2018/2019 season).

4.3.2 Experimental layout and sampling

The experiment for the MSc study formed part of an extensive project, which included ten treatments, as indicated in Figure 4.3. The experimental layout was a randomised block design with ten treatments, replicated in six blocks (Figure 4.3). The MSc study focused on treatments 1 to 5. An experimental panel consisted of 6 experimental vines. The two central vines were used as data vines, which were surrounded by two buffer vines on both ends of the panel. Five post-

harvest pruning treatments were applied. These included an early 33% and a 66% shoot removal pruning treatment one day after harvest (33_1dAH and 66_1dAH respectively), a late 33% and a 66% shoot removal pruning treatment 45 days after harvest (33_45dAH and 66_45dAH respectively) and a control (Ctr), in which no post-harvest summer pruning was applied. The 33% shoot removal treatment applied 45 days after harvest was based on industry guidelines (Van der Merwe, 2017). Treatments where 66% shoot removal were applied, as well as treatments applied at 1 day after harvest were included, to evaluate the effects of more severe pruning and a more extended period available for reserve accumulation.

Row	Panel	Block	Treatment number	Row	Panel	Block	Treatment number
31	1	1	9	32	1	3	3
31	2	1	6	32	2	3	8
31	3	1	8	32	3	3	2
31	4	1	10	32	4	3	9
31	5	1	4	32	5	3	4
31	6	1	5	32	6	3	1
31	7	1	7	32	7	3	5
31	8	1	2	32	8	3	7
31	9	1	3	32	9	3	10
31	10	1	1	32	10	3	6
31	11	2	6	32	11	4	9
31	12	2	10	32	12	4	7
31	13	2	7	32	13	4	2
31	14	2	4	32	14	4	10
31	15	2	3	32	15	4	6
31	16	2	2	32	16	4	1
31	17	2	8	32	17	4	4
31	18	2	1	32	18	4	3
31	19	2	9	32	19	4	8
31	20	2	5	32	20	4	5
31	21	5	1	32	21	5	6
31	22	5	2	32	22	5	7
31	23	5	3	32	23	5	8
31	24	5	4	32	24	5	9
31	25	5	5	32	25	5	10
31	26	6	3	32	26	6	4
31	27	6	1	32	27	6	5
31	28	6	2	32	28		

1	0% Summer Pruning: Control
2	33% Summer Pruning: After harvest
3	66% Summer Pruning: After harvest
4	33% Summer Pruning: 45 days after harvest
5	66% Summer Pruning: 45 days after harvest

Figure 4.3: The experimental layout of the post-harvest summer pruning trial on Sultanina H5 in the Semi-arid Lower Orange River region.

During treatment application, shoots were first counted, where after the applicable percentage shoots was removed. Green shoots (non-lignified) were first to be removed, where after some of the mature (lignified) shoots were removed to obtain the required percentages. This was done so that only mature shoots remained on the vines. The green shoots were identified as fully green, or if only one or two basal internodes had lignified. Mature shoots were defined as lignified from the third bud to completely lignified. For the 33% pruning treatment, the first two shoots were counted and retained on the vine, while the third was removed. This was done on all fruiting canes of the data vines of the 33% treatment panels. For the 66% pruning treatment, one shoot was counted and retained on the vine, while the second and third were removed. This was done on all fruiting canes of the data vines of the 66% treatment panels. Figure 4.4 shows the canopies resulting from two pruning treatments (33% and 66%) applied.

Figure 4.4: Sultanina H5 vines after application of post-harvest pruning treatments; A- 33% pruning and B- 66% pruning.

4.3.3 Sampling, sample preparation and measurements

Quantifying leaf area removed:

To quantify pruning severity, the number and length of removed shoots, as well as the number of leaves and leave area removed were determined at the time of the post-harvest summer pruning treatment application. The sampled shoots and leaves used to measure the area were selected randomly as representatives of each treatment. Leaf area was measured using a LI-3100 area meter (LI-COR Inc., Lincoln, Nebraska USA). After winter pruning was applied, the removed canes and shoots were measured to calculate the overall shoot length and leaf area removed per vine.

Photosynthetically active radiation (PAR):

The day after pruning treatments were applied, photosynthetically active radiation (PAR) transmission measurements were taken in each panel to quantify the impact of the applied

treatments on PAR transmission through the canopy. The measurements were done using a LI-COR Model LI-250 Line Quantum Sensor (LI-COR Inc., Lincoln, Nebraska, USA).

Due to the summer rainfall occurring in this region (as depicted in Figure 4.2), plastic covers were installed over the canopy during the ripening period in the 2018/19 season and were still present during the application of early pruning treatments and measurements taken in the week after harvest. PAR measurements were taken under the canopy and, above the canopy (which was under plastic covering), as well as outside, where there was no plastic covering. The instrument was hung 30 cm beneath the third canopy wire to measure PAR under the canopy. The results are expressed as PAR and percentage transmittance.

Photosynthesis

Measurements of the photosynthetic activity and related physiological parameters of treated and untreated panels were taken 1 day after applying of the pruning treatments, to evaluate the immediate impact of the applied treatments on the photosynthetic activity of the vines. These were done on five mature, healthy leaves per panel, which were fully exposed to the sun. Net carbon assimilation rates as an indicator of photosynthesis rate, intercellular CO_2 concentration, stomatal conductance and transpiration rate were measured with an infrared gas analyser (LI-6400XT, Li-Cor, Lincolin, Nedbraska, USA). The measurements were done at four times during the day, namely in the morning (10h00), midday (12h00) and in the afternoon (14h00 and 16h00).

Total non-structural carbohydrates

Sampling of cane or shoot (depending on the phenological stage), stem and root tissue, for assessing the effect of pruning treatments on grapevine carbohydrate reserve status was done 45 days after the first pruning, 45 days after the second pruning, during dormancy (1 month before bud break) and at flowering. The sampling methods described by Holzapfel (2009) and in Section 3.2.2 were used in this study. To extract and analyse soluble sugars and starch, the methods described in Sections 3.2.5 and 3.2.6 were used.

4.3.4 Bud break and fertility

Bud break and fertility were assessed through forced bud break in a glasshouse and bud dissection for potential bud break and fertility, while actual bud break and fertility were assessed in the vineyard. Since the block was pruned to canes consisting of 14-15 buds in winter, the bud break and fertility were evaluated on either 14 or 15 buds per cane.

Forced bud break in a glasshouse

The method used to assess bud break in the glasshouse, was described by Avenant & Avenant (2014). For each treatment, 12 representative dormant canes were sampled during winter pruning from the experimental site. From each cane, single-node cuttings were prepared. These cuttings

were placed according to their positions on the canes in water trays in a glasshouse at 25°C. Buds were assessed three times per week, namely Monday, Wednesday and Friday, for a period of 44 days. The modified Eichhorn and Lorenz (E-L) system was used to assess bud break and stage four was considered to be the indicator of bud break (Coombe, 1995). This is the stage where the first green leaf tip or edge is visible. The dates on which bud burst occurred were recorded. Bud fertility was also assessed by counting and recording the visible number of inflorescences from each sprouted bud.

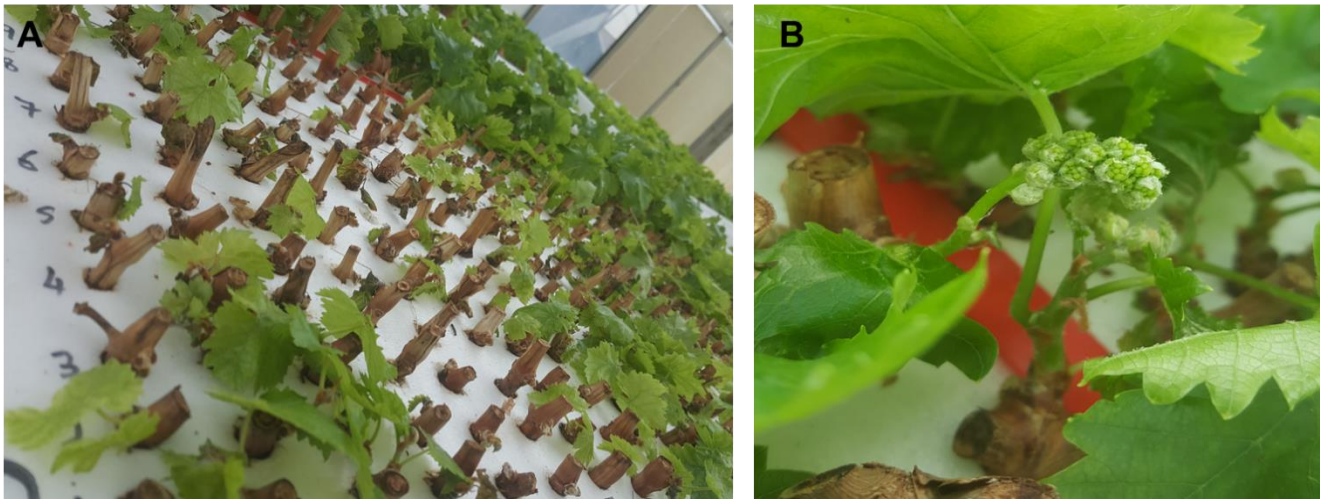


Figure 4.5: An indication of forced budding in a glasshouse. A- budded, delayed as well as dead buds. B- a fruitful bud with a bunch.

Bud dissection

For each treatment, 20 representative dormant canes were sampled during winter pruning from the experimental site. These were placed in plastic bags and stored in a cold room at 6°C to prevent them from drying out (Kavoosi *et al*., 2015). Before analysis, the canes were cut to single-node cuttings and fertility evaluation was done, using a microscope, Olympus SZ61-ILST (Olympus Corporation, Japan). Individual buds were dissected to the interior of the compound bud, in which inflorescence primordia were counted once detected under the microscope (Sommer *et al*., 2000).

Actual bud break and fertility

This assessment was done in the field on two 14-bud-canes (verified to be fertile) per data vine and the modified E-L system (Coombe, 1995) was used as a reference. The system was used to assess bud break, the progression of bud break and bud development after bud break. The assessment was done up to stage 13 of the E-L system namely, when six leaves have separated from the shoot and inflorescences were visible. Inflorescences were counted on the shoot that developed from each sprouted bud to assess.

4.3.5 Statistical analysis

The experimental design was a randomised block design. The data was analysed using SAS software's SAS-GLM (General Linear Models) procedure, version 9.4 (SAS Institute Inc, Cary, USA). Completely random ANOVA (Analysis of variance) was done for each tissue type separately. A probability level of 5% was considered significant for all significance tests. Fisher's least significant difference was calculated at the 5% level to compare means (Ott and Longnecker, 2010).

Gompertz functions were fitted over cumulative bud break percentages over time for each shoot (Fialho, 1999; Anzanello *et al*., 2018). The maximum bud break percentage was fixed at 100% as all canes reached 100% bud break. An ANOVA was done on all estimated parameters, namely: M- Maximum bud break (total percentage of sprouted buds), U- Uniformity (the period between 10% and 90%) and P- Precocity (number of days to reach the inflection point of the curve, at 37% of maximum bud break) as well as calculated days up to 10%, 50% and 90% bud break and number of days from 10% to 90%. ANOVA was done on the average number of bunches per bud to analyse fertility data.

4.4 RESULTS AND DISCUSSION

4.4.1 Leaf area measurements

The results presented in Table 4.2 show the total leaf area per vine, which remained on vines and the total leaf area removed per vine during post-harvest pruning. The aim was to remove respectively 33% and 66% of the total leaf area per vine as described in Section 4.2.2. According to the calculations, however, that was not achieved. With the 33% treatment, only 21% and 25% were removed for 33_1dAH and 33_45dAH, respectively, thus only 70% of what was supposed to be removed. For the 66% pruning treatment, 35% and 33% were removed for 66_1dAH and 66_45dAH, respectively, thus only 50% of what was intended to be removed. As expected, the removed main shoot leaf area per vine obtained with the two levels (33% & 66%) of pruning were significantly different from each other at both times (1dAH & 45dAH). However, the two less severe treatments (33_1dAH & 33_45dAH) were not significantly different from each other.

Early pruning treatments removed more total leaf area than those applied late (Table 4.2). There was no significant difference between the two severe (66%) treatments and the less severe treatments regarding removed total leaf area per vine. There was no significant difference in remained total leaf area per vine amongst all treatments.

Table 4.2: Comparison of leaf area measurements obtained as a result of pruning treatments in the post-harvest summer pruning trial on Sultanina H5 in the semi-arid Lower Orange River region in the 2018/2019 season.

Parameter	Treatment				
	Ctr	33_1dAH	66_1dAH	33_45dAH	66_45dAH
Removed main shoot leaf area per vine (m^2)	0	5.20 c	8.80 a	4.59 c	7.28 b
Removed lateral shoot leaf area per vine (m^2)	0	0.93 ab	1.07 ab	0.54 bc	1.34 a
Removed total leaf area per vine (m^2)	0	6.13 b	9.87 a	5.13 b	8.62 a
Remaining main shoot leaf area per vine (m^2)	16.25 a*	14.88 a	13.06 a	12.99 a	14.10 a
Remaining lateral shoot leaf area per vine (m^2)	6.08 ab	8.61 a	5.68 ab	4.80 b	4.62 b
Remaining total leaf area per vine (m^2)	22.33 a	23.50 a	18.73 a	17.79 a	18.72 a
Total main shoot leaf area per vine (m^2)	16.25 b	20.09 ab	21.85 a	17.58 ab	21.39 a
Total lateral shoot leaf area per vine (m^2)	6.08 b	9.54 a	6.75 ab	5.34 b	5.96 b
Total leaf area per vine (m^2)	22.33 a	29.63 a	28.60 a	22.92 a	27.34 a
Removed main shoot leaf area per vine (%)	0	88	91	92	86
Removed lateral shoot leaf area per vine (%)	0	12	9	8	14
Removed total leaf area per vine (%)	0	21 b	35 a	25 b	33 a

*Values with the same lower-case letter within rows do not differ significantly ($p < 0.050$).

Similar results were also obtained by Kliewer & Fuller (1973) and Hunter & Visser (1990), in which the theoretical (intended) level of pruning was not obtained after treatment application. The reason for this could be that with the method used, the majority of shoots removed were young, green and weak, which might have not made a large contribution to the overall percentage of shoot removal. Since the pruning was carried out according to shoot number and not leaf area, this could also account for the results obtained (Kliewer & Fuller, 1973; Hunter & Visser 1990). These

results indicate that what was theoretically meant to be 66% removal, "severe" pruning, proved to have not resulted in the expected degree of shoot removal. The two 66% pruning treatments were significantly different compared to the two 33% pruning treatments. In contrast, Links (2014) reported that there was no significant difference between 33% and 66% shoot removal after harvest. Another reason could be the occurrence of regrowth triggered by the application of treatments, as reported by Marangoni *et al*. (1980), where shoot growth was observed soon after leaves were removed.

Estimation of canopy leaf area before pruning may be the best way to accurately determine the amount that must be pruned. In a study to predict leaf area index (LAI), Arnó *et al*. (2012) used light detection and ranging (LiDAR) sensors in several fields to assess their feasibility. Tree area index (TAI), which is the ratio of the crop estimated area by the LiDAR sensor per unit ground area proved to be the best parameter to estimate LAI. It is recommended that to obtain reliable LAI maps, section row lengths of 1 meter in each sampling area should be scanned. This technology, however, does not distinguish leaves from other plant tissues and cannot compensate for non-random leaf positioning (Jonckheere *et al*., 2004).

4.4.2 Photosynthetically active radiation (PAR) and transmittance

All pruning treatments opened the canopies, increasing PAR and transmittance percentage through the canopies (Figure 4.6). This was most evident in the severely pruned (66%) panels at both times, compared to the 33% and the Ctr. The 66_1dAH and the 66_45dAH treatments significantly increased PAR when measured immediately after pruning compared to the Ctr and the 33_1dAH.

The transmittance results were similar to those of PAR (Figure 4.6). Percentage transmittance was less than 15% for all treatments. The panels that were pruned late had high transmittance percentages compared to other treatments, with the severe treatment resulting in the highest percentage. The interaction between the pruning treatments and the time of pruning was significant. The results presented in Figure 4.6 show that the PAR and transmittance through the Ctr canopies after late pruning were more than double that measured after early pruning. The canopies subjected to early pruning had lower PAR and transmittance compared to late pruned canopies.

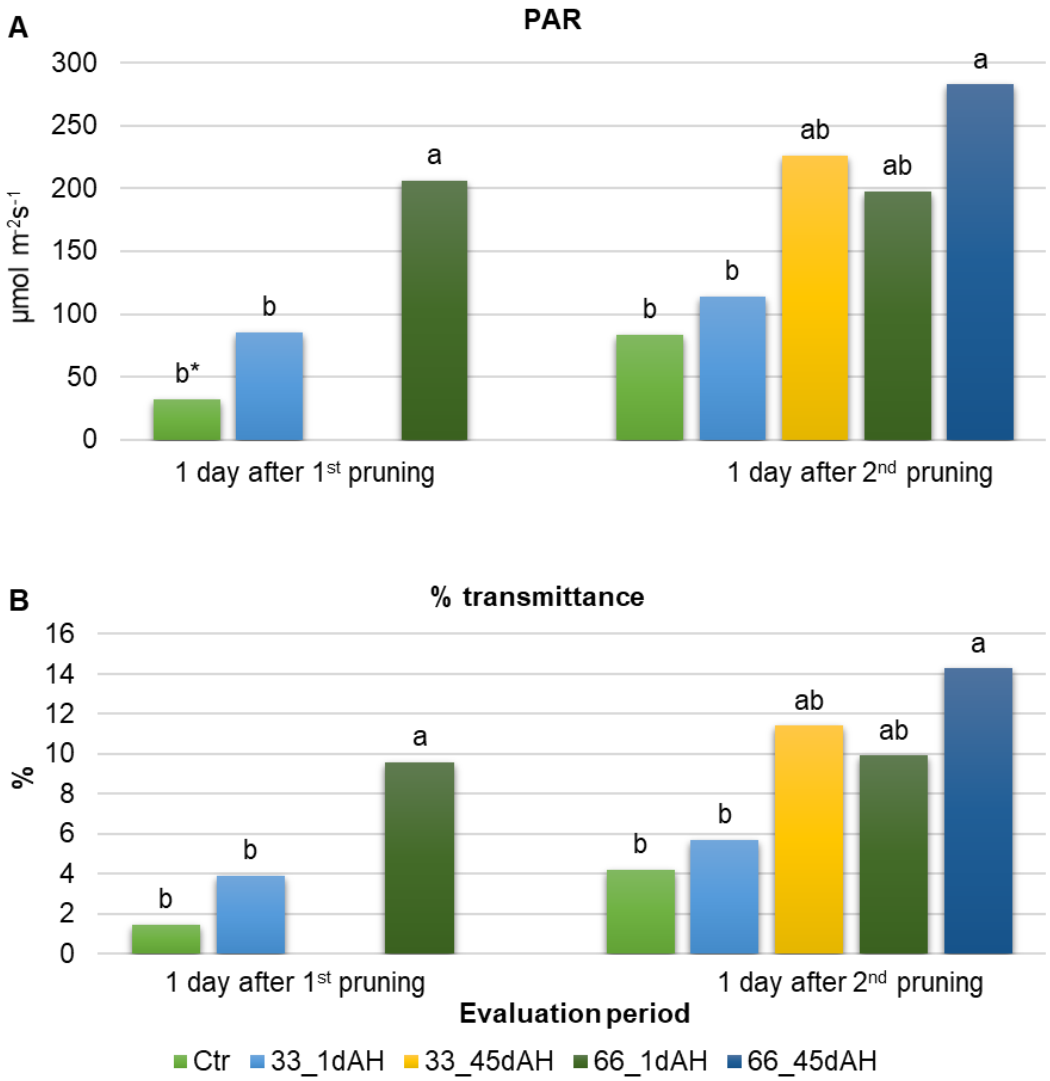


Figure 4.6: Effect of post-harvest summer pruning on PAR and transmittance throughout Sultanina H5 canopies in the 2018/2019 season. *Means with the same lower-case letter within a period of pruning do not differ significantly ($p < 0.05$).

The results of the shoot removal treatments contributed to the results of Hunter & Visser (1990) and Hunter *et al*. (1995) on partial defoliation, as well as what Smart (1982) and Ashraf & Ashraf (2014) reported. Wang *et al*. (2020) also reported that light interception was reduced with time after shoot thinning as in the current study. The low PAR and transmittance results in the Ctr panels (Figure 4.6) were expected as no pruning was applied in the post-harvest period. Also, the plastic coverings may have had a shading effect on the canopies. The significantly lower PAR and transmittance obtained with untreated grapevines and those pruned with 33_1dAH compared to the 66_1dAH-pruned grapevines (Figure 4.6A), can be ascribed to the high remained total leaf area per vine (Table 4.2), which might have impeded light penetration.

According to Shrestha & Fidelibus (2005), PAR of 500 µmol $m^{-2}s^{-1}$ is approximately 30 to 40% of full sun. This entails that the PAR values obtained in this study was below 30% of full sun. The overall low PAR values and percentages of transmittance obtained for all treatments may be due

to the effect of the plastic coverings that were installed. The vigorous growth character of Sultanina (Goussard, 2008) may also have had an effect on this. Another reason could be that all pruning treatments were not as severe in that the highest removed percentage of leaf area per vine was only 35%. It has been reported that approximately 85-90% of incident light in the PAR range falling on a grapevine leaf is absorbed by the leaf. From the remaining amount, only 9% of direct radiation is transmitted through a vine leaf, while 6% is reflected at the surface (Dokoozlian & Kliewer, 1995; Poni & Intrieri, 2001; Keller, 2010).

4.4.3 Photosynthetic activity

Physiological parameters (photosynthesis related) assessed one day after applications of post-harvest summer pruning treatments are presented in Figure 4.7 (A-C) and Appendix 6. The photosynthetic rate of the Ctr vines showed a decrease from 10h00 in the morning to 12h00 in the afternoon. The lowest photosynthetic rates (3.6 µmol CO_2 m^{-2} s^{-1}) were recorded at 12h00 for the 66_1dAH treated grapevines (Appendix 6). The Ctr and 33_1dAH treated grapevines reached their lowest photosynthetic rate at 18h00 with 3.5 µmol CO_2 m^{-2} s^{-1} and 2.4 µmol CO_2 m^{-2} s^{-1} respectively (Appendix 6). The photosynthetic rate reached minimum values late in the afternoon. Neither of the two treatments significantly impacted the photosynthetic rate compared to the untreated grapevines, except for the 33_1dAH treatment measured at 10h00.

Stomatal conductance was significantly high in Ctr grapevines compared to other treatments and the maximum level of 0.3 mol H_2O m^{-2} s^{-1} was recorded at 12h00 noon (Figure 4.7D & Appendix 6). At this time, the transpiration rate was also at a maximum level of 11.2 mmol H_2O m^{-2} s^{-1} (Appendix 6). Lower stomatal conductance and transpiration rates were recorded for all pruned grapevines.

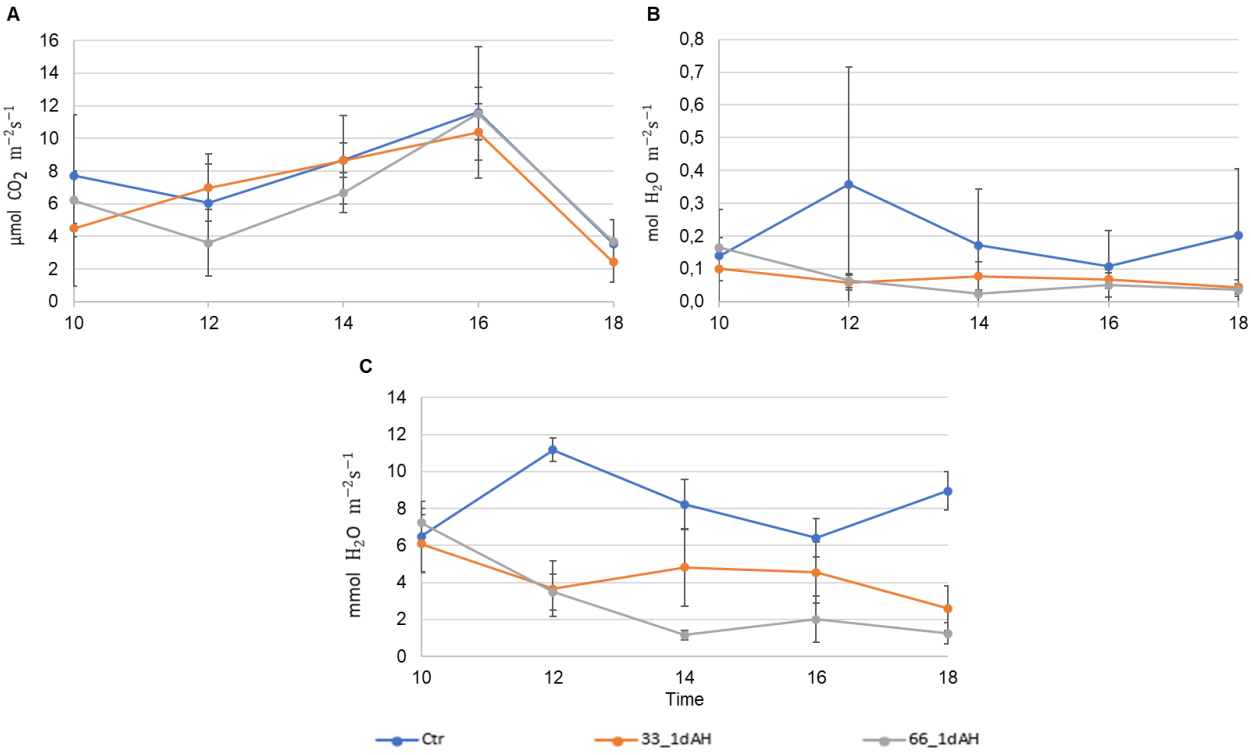


Figure 4.7: Physiological parameters (Photosynthesis related) of Sultanina H5 assessed one day after post-harvest summer pruning treatments in the semi-arid Lower Orange River region. (A) Photosynthetic rate, (B) Stomatal conductance and (C) Transpiration rate.

The decrease in photosynthetic rate from early morning to midday supports the results obtained by Downton *et al*. (1987) on Riesling with the lowest photosynthesis levels were recorded mid-afternoon between 14h00 and 15h00 while in this study, according to Figure 4.7A and Appendix. 6, the lowest levels were recorded at 12h00 and 18h00. The increasing rate from midday may be due to the increase in temperatures, which is the norm in this region. According to Weather Spark (2020), The highest temperatures usually occur between three and five in the afternoon in this region. The reduction observed late in the afternoon is also a result of decreasing light intensity associated with the onset of sunset. According to results obtained by Hendrickson *et al*. (2004), higher temperature (25°C) as well as increasing PAR, increased photosynthetic carbon fixation, transpiration and stomatal conductance.

According to several studies, photosynthetic activity increases when source size is reduced (Hunter *et al*., 1995; Koblet *et al*., 1996; Poni *et al*., 2006). The results obtained in this study contradict these findings. Post-harvest pruning may have induced partial closure of stomata, resulting in reduced conductance and transpiration rate, as reported by Iland *et al*. (2011) and Ashraf and Ashraf (2014). This was primarily distinguished in the grapevines that were 66% pruned.

4.4.4 Total non-structural carbohydrates

Soluble sugars obtained in the March sampling date were significantly lower in roots of grapevines pruned by 66_1dAH compared to untreated grapevines (Table 4.3). In the month of September, all treatments resulted in significantly lower soluble sugars than the untreated in roots. The same was recorded with starch although only with both early pruning treatments. For trunks, starch concentrations obtained with 66_1dAH (March and August) and 33_45dAH (May and September) were significantly lower compared to the untreated grapevines. The soluble sugars obtained from shoots with the 33_45dAH (May), 33_1dAH and 66_45dAH (September) treatments were significantly lower than all other treatments. Starch concentrations were significantly decreased by 33_45dAH in May and all the treatments in September.

Significantly higher concentrations in roots were obtained with the 33_1dAH and 33_45dAH treatments in August for soluble sugars and starch respectively. In trunks, 66_1dAH gave significantly higher concentrations of soluble sugars in March and starch in September while 66_45dAH resulted in increased concentrations in trunks for starch and soluble sugars (in May and September respectively). In shoots, increased soluble sugars were obtained in shoots in September with the 66_1dAH and 33_45dAH treatments.

There were significant differences in each of the tissue types between different sampling dates (Table 4.3). Starch concentrations were highly reduced from May to September and this was most prominent in the grapevines that were pruned less severely (33_1dAH & 33_45dAH). For a specific sampling date, significant differences occurred between tissue types.

Table 4.3: The impact of post-harvest summer pruning severity and time on soluble sugars and starch concentration in roots, trunks and shoots of Sultanina H5 in the Lower Orange River region.

		Roots		Trunks		Shoots	
Month	**Treatment**	**Soluble sugars (g/g%)**	**Starch (g/g %)**	**Soluble sugars (g/g %)**	**Starch (g/g %)**	**Soluble sugars (g/g %)**	**Starch (g/g %)**
	Ctr	6.53 a*	13.41 ab	4.99 b	9.70 a	4.12 a	7.90 a
March	33_1dAH	6.21 a	11.10 b	5.09 b	9.56 a	4.31 a	7.28 a
	66_1dAH	4.62 b	13.95 a	5.70 a	8.16 b	4.31 a	7.57 a
	Ctr	2.71 a	17.09 a	5.90 a	9.88 b	4.05 a	13.94 a
May	33_45dAH	2.60 a	13.61 b	5.65 a	8.87 c	3.56 b	12.97 b
	66_45dAH	2.98 a	14.97 ab	6.06 a	11.09 a	4.07 a	14.44 a
	Ctr	5.87 b	5.77 b	10.45 a	5.68 a	4.74 a	10.14 abc
	33_1dAH	6.73 a	6.02 b	10.63 a	5.50 a	5.12 a	11.28 a
August	66_1dAH	6.20 ab	5.49 b	10.95 a	4.49 b	5.22 a	9.31 c
	33_45dAH	5.98 ab	8.47 a	10.76 a	5.10 ab	5.13 a	9.58 bc
	66_45dAH	6.06 ab	5.67 b	10.93 a	5.31 a	5.03 a	10.77 ab
	Ctr	6.34 a	3.91 a	3.90 b	5.13 bc	2.39 b	0.83 a
	33_1dAH	5.36 b	2.23 bc	4.58 ab	5.33 b	1.16 c	0.61 b
September	66_1dAH	3.57 d	1.30 c	4.50 ab	6.40 a	3.94 a	0.57 bc
	33_45dAH	4.36 c	3.16 ab	3.99 ab	4.24 d	4.42 a	0.58 bc
	66_45dAH	3.71 cd	3.24 ab	4.88 a	4.45 cd	1.28 c	0.50 c

*Comparing treatments within columns and for each sampling date (month). Means with the same lower-case letter do not differ significantly ($p < 0.050$).

Results obtained in March (Table 4.3), which was 45 days after the application of treatments, indicated that pruning did not have a clear effect on NSC. Most severe pruning treatments resulted in increased concentrations. Reserves are produced *via* photosynthesis by leaves and are basipetally transported to perennial tissues for storage (Wojnarowiez *et al*., 2008; Holzapfel *et al*., 2009; Rogiers *et al*., 2011).

The month of May represents mid-autumn and leaf fall takes place in this period in most parts of South Africa (Kruger & Shongwe, 2004). This is not the case with semi-arid regions such as the LOR, where a longer post-harvest period of active vegetative growth occurs (Volschenk & Hunter, 2009). At this sampling date, leaves were still actively photosynthesising. The highest concentrations of starch recorded for this sampling date support the results obtained by Bennett *et al*. (2005). The low starch concentration recorded in May in the trunks of the grapevines pruned with 33_45dAH may be as a result of the low leaf area that remained on the grapevines after application of this treatment (Table 4.2). This is in contrast with what was reported by Hunter *et al*. (1995) and Koblet *et al*. (1996) that the grapevine responds to defoliation by undergoing photosynthesis at high rates due to increased sun exposure in the canopy. This low starch concentration may also be an indication of a low photosynthetic response of the grapevine to a less severe treatment (Figure 4.7A). Leaf fall was recorded on 9 July in the season of the study and it was forced by frost. This implies that the vegetative growth could have continued had the frost not occurred.

The two last sampling were done while the vines were still dormant. This is explained by the decreased starch concentrations in the roots and trunks as well as increased soluble sugars in these two perennial parts in August. The interconversion of starch to sugars discussed in Section 3.4.1 may have been the reason for the decreased starch concentrations. In the last sampling date, just before bud break, the reserves were already mobilised for new season growth as shoots and roots concentrations were taken up greatly. This is ascribed to the fact that with all treatments, significantly decreased soluble sugars and starch were recorded for both roots and shoots. This is discussed further in Section 3.4.

4.4.5 Bud break and fertility

The cumulative bud break curve for pruning treatments revealed differences in bud break rate among treatments (Figure 4.8). All treatments increased the bud break rate. The 66_1dAH treatment exhibited the most rapid bud break, particularly from day 25 to 36, in contrast with the Ctr, which had the lowest bud break rate. All treatments had reached 100% bud break by day 43.

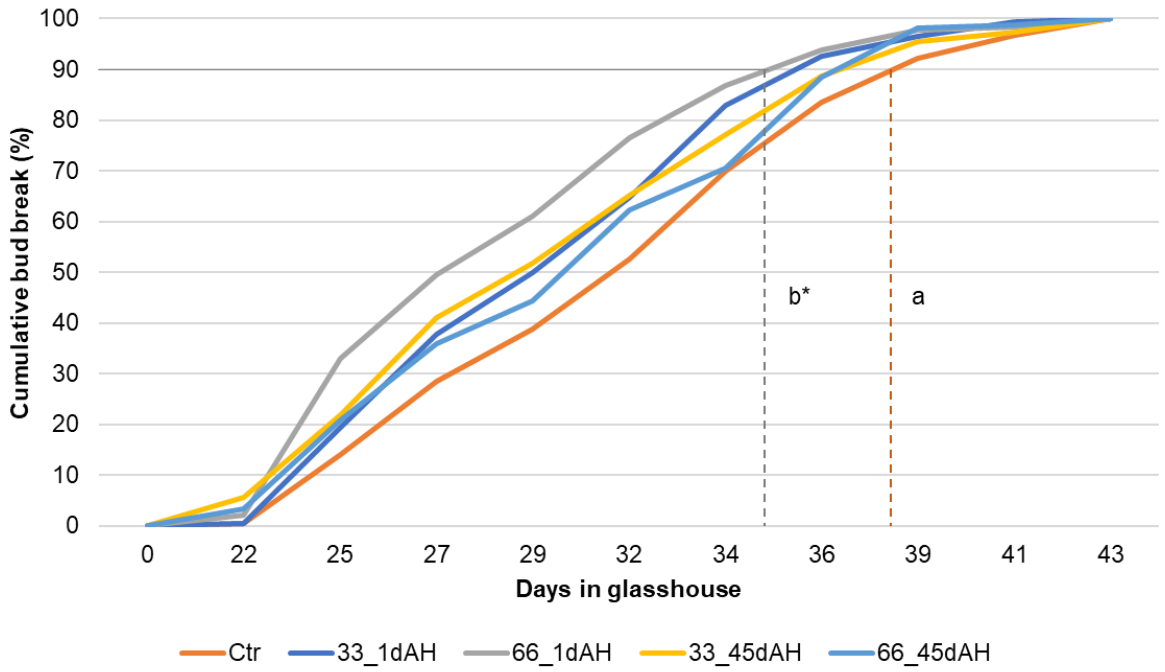


Figure 4.8: Cumulative bud break percentage of Sultanina H5 cuttings as a response to post-harvest summer pruning treatments applied on the experimental vineyard in the semi-arid Lower Orange River region in the 2018/2019 season. *Means with the same lower-case letter within a period of pruning do not differ significantly ($p < 0.05$).

Pruning treatments did not significantly affect bud break (Table 4.4). The 66_1dAH treated grapevines generally took the least number of days to reach all the levels of bud break presented, though only BBP90 and BBP10_90 were significantly lower than the Ctr. Ultimately, the Ctr grapevines took the longest to attain the bud break levels presented in Table 4.4.

Table 4.4: Bud break progression of Sultanina H5 cuttings after transfer to a 25°C glasshouse as a response to post-harvest summer pruning treatments applied on the experimental vineyard in the semi-arid Lower Orange River region in the 2018/2019 season.

Treatment	BBPP	BBP10	BBP50	BBP90	BBP10_90
Ctr	28.98 a*	25.77 a	30.38 a	37.63 a	11.86 a
33_1dAH	27.55 a	24.53 a	28.88 a	35.72 ab	11.20 a
66_1dAH	26.61 a	24.27 a	27.64 a	32.93 b	8.66 b
33_45dAH	27.63 a	24.73 a	28.91 a	35.46 ab	10.73 ab
66_45dAH	28.25 a	25.36 a	29.52 a	36.04 ab	10.68 ab

BBP10_90 - number of days from 10 to 90% BB
BBP10 - days up to 10% bud break
BBP50 - days up to 50% bud break
BBP90 - days up to 90% bud break
BBPP – bud break % at P (the inflection point) – always 37% due to nature of the re-parameterisation
*Means with the same lower-case letter within columns do not differ significantly ($p < 0.050$) in each data set.

Actual bud break results and phenological stages reached after bud break (expressed as E-L stages) are presented in Figure 4.9 and Table 4.5. Figure 4.9 shows evenness in bud break on day 40 of assessment. This evenness was in E-L stages one to eleven. Day 50 was variable, although it indicated that for the two severe treatments and the 33_1dAH in bud break and development thereafter were the most advanced. Day 63 also showed evenness in bud break and development after bud break. On this day, both less severe treatments had attained the highest percentages of E-L 12.

There were significant differences in bud break and phenological stages reached after bud break between evaluation dates (Table 4.5). On day 40, significantly high percentages of E-L 4 were recorded. All treatments resulted in low percentages of buds at E-L 5 on day 63. A significantly higher percentage of buds at E-L 7 was obtained by grapevines treated with 33_1dAH on day 50. At E-L 9, 66_1dAH pruned grapevines (buds) assessed on day 63 were significantly higher than those of day 40 and majority of day 50. The percentage of E-L 11 attained was significantly lower on day 40 than the other days. E-L 12 was the highest on day 60 in all treatments. The two severe treatments had significantly higher percentages of E-L 13 on day 50.

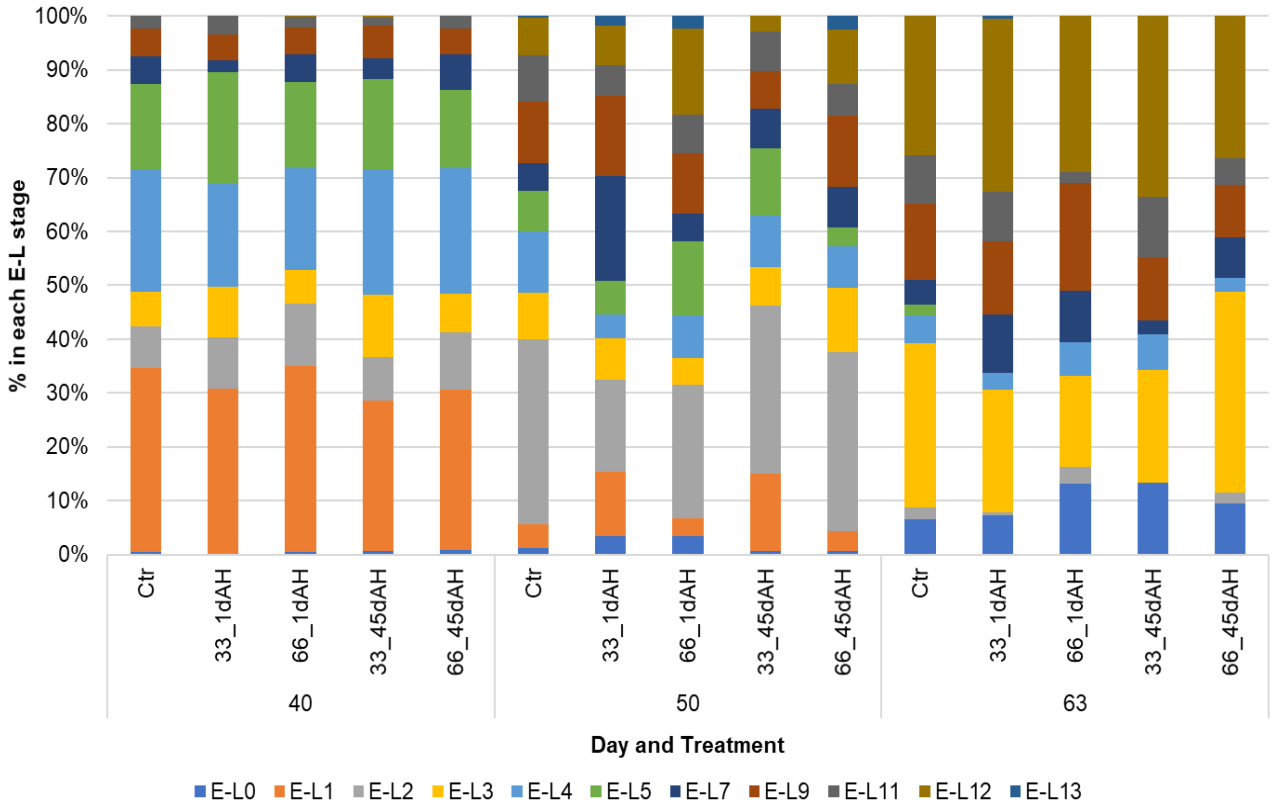


Figure 4.9: Actual bud break and phenological stages reached after bud break as a response to post-harvest summer pruning of Sultanina H5 in the semi-arid Lower Orange River region in the 2018/2019 season.

Table 4.5: Actual bud break and phenological stages reached after bud break as a response to post-harvest summer pruning of Sultanina H5 in the semi-arid Lower Orange River region in the 2018/2019 season.

Treatment	E-L0	E-L1	E-L2	E-L3	E-L4	E-L5	E-L7	E-L9	E-L11	E-L12	E-L13
	% in each E-L stage at assessment day 40										
Ctr	0.48 e*	34.18 a	7.67 cde	6.40 ef	22.76 cd	15.97 abcd	5.05 bcd	5.09 efg	2.42 bcdef	0.00 d	0.00 b
33_1dAH	0.00 e	30.78 a	9.49 cde	9.46 ef	19.05 d	20.81 A	2.24 d	4.75 efg	3.43 bcdef	0.00 d	0.00 b
66_1dAH	0.61 de	34.48 a	11.57 cd	6.14 ef	19.04 d	15.94 abcd	5.07 bcd	4.91 efg	2.05 bcdef	0.20 d	0.00 b
33_45dAH	0.70 de	27.97 a	7.96 cde	11.68 cdef	23.27 bcd	16.74 abc	3.79 cd	6.00 defg	1.56 cdef	0.33 d	0.00 b
66_45dAH	0.90 de	29.78 a	10.65 cde	7.12 ef	23.23 bcd	14.67 abcde	6.57 bcd	4.74 efg	2.36 bcdef	0.00 d	0.00 b
	% in each E-L stage at assessment day 50										
Ctr	1.29 de	4.45 bc	34.33 a	8.58 ef	11.34 e	7.56 defgh	5.12 bcd	11.38 bcde	8.66 abc	6.87 cd	0.42 b
33_1dAH	3.41 cde	12.04 bc	17.05 bc	7.74 ef	4.32 ef	6.20 efgh	19.49 a	14.99 ab	5.60 abcdef	7.44 cd	1.74 ab
66_1dAH	3.50 bcde	3.33 bc	24.73 ab	5.00 f	7.76 ef	13.93 abcde	4.98 bcd	11.18 bcde	7.27 abcde	16.03 b	2.29 a
33_45dAH	0.62 de	14.43 bc	31.17 a	7.174 ef	9.59 ef	12.43 abcdef	7.42 bcd	6.84 cdefg	7.43 abcd	2.90 cd	0.00 b
66_45dAH	0.62 de	3.70 bc	33.25 a	12.04 cdef	7.59 ef	3.49 Fgh	7.54 bcd	13.27 abcd	5.82 abcdef	10.23 bc	2.47 a
	% in each E-L stage at assessment day 63										
Ctr	6.62 bcd	0.00 c	2.24 de	30.30 ab	5.26 ef	2.04 gh	4.49 bcd	14.17 abcd	9.01 ab	25.87 a	0.00 b
33_1dAH	7.38 abc	0.00 c	0.44 de	22.79 bc	3.18 f	0.00 h	10.71 b	13.73 abcd	9.07 ab	32.27 a	0.44 b
66_1dAH	13.24 a	0.00 c	3.13 de	16.75 cde	6.28 ef	0.00 h	9.49 bc	20.02 a	2.06 bcdef	29.03 a	0.00 b
33_45dAH	13.31 a	0.00 c	0.00 e	20.95 bcd	6.73 ef	0.00 h	2.41 d	11.75 bcde	11.19 a	33.67 a	0.00 b
66_45dAH	9.51 ab	0.00 c	2.12 de	37.12 a	2.64 f	0.00 h	7.51 bcd	9.74 bcdef	4.86 abcdef	26.50 a	0.00 b

*Comparing the effect of pruning treatments on bud break and phenological stages reached after bud break (per E-L stage) across all evaluation dates. Means with the same lower-case letter within columns do not differ significantly ($p < 0.050$).

The first day of evaluation (Figure 4.9) showed evenness in bud break amongst all treatments. This coincides with the potential bud break data in Table 4.4, where the number of days up to 10% and 50% bud break and bud break percentage at the inflection point (37%) did not differ significantly between any of the treatments. The reason for this evenness may be due to the rest breaking agent, hydrogen cyanamide, applied as a standard practice in warm regions such as the Lower Orange River region (Avenant & Avenant, 2014). The application of this chemical seems to have promoted the initiation of bud break since bud break of all the treatments commenced at approximately the same time. These results support the study of Martínez-Lüscher and Kurtural (2021), where defoliation just before pea size did not significantly affect bud break date of Cabernet Sauvignon buds.

The more advanced bud break recorded on day 50 for the two severe treatments was expected, as grapevines pruned with these treatments had a low total leaf area remaining, resulting in significantly more sunlight penetration (Table 4.1 & Figure 4.6). Light exposed buds were reported to result in high bud break and fertility compared to shaded buds (Hopping, 1977). May and Antcliff (1963) also reported that reducing light intensity by 70% for at least six weeks between October and January (mid-spring and mid-summer) reduced yield in the same season. Furthermore, shading severely reduced fertility in the following season. As discussed previously, all treatments reached a final bud break of 100% with the forced budding assessment.

Potential fertility results (from both bud dissection and forced bud break) indicated no significant difference between the Ctr and any pruning treatments (Table 4.6). The bud dissection results indicated that the number of inflorescence primordia of the 66_1dAH pruning treatment was significantly higher than the 33_1dAH treatment. The actual fertility of the 66_45dAH treatment was significantly higher than the Ctr and the 66_1dAH and 33_45dAH treatments. There was no significant effect of the pruning treatments on the actual number of bunches per vine (Table 4.6), although a trend was observed that the earlier pruning treatments were associated with a lower number of bunches per vine, compared to the control and the later pruning treatments. Although pruning treatments increased light transmittance through the canopies, most treatments did not significantly affect (increase) bud fertility.

Table 4.6: The impact of post-harvest summer pruning on fertility of Sultanina H5 in the Lower Orange River region in the 2018/2019 season

Treatment	Nr bunches/sprouted bud		Nr primordia/bud	Nr bunches/vine
	Actual	Potential (forced)	Potential (dissection)	Actual
Ctr	0.74 b*	0.48 a	0.90 ab	47.69 a
33_1dAH	0.82 ab	0.47 a	0.82 b	44.94 a
66_1dAH	0.75 b	0.55 a	1.05 a	41.94 a
33_45dAH	0.76 b	0.63 a	0.92 ab	47.13 a
66_45dAH	0.87 a	0.49 a	0.98 ab	49.17 a

*Means with the same lower-case letter within columns do not differ significantly ($p < 0.050$) in each data set.

It has been reported that inflorescences and tendrils are structurally homologous (Buttrose, 1969; May, 2000; Lebon *et al*., 2008). Due to this, tendrils might have been counted as inflorescences during bud dissection analysis as they look similar, resulting in a higher number of primordia being recorded. The accuracy of bud dissection analysis should thus be evaluated and improved.
In contrast to these results, Ferrara and Mazzeo (2021) reported that the number of inflorescence primordia determined *via* bud dissection analysis is generally lower than the actual (observed in the field). Both forced bud break and bud dissection results confirmed the low fertility of basal buds in Sultanina (data not shown), which was reported by several studies (Sommer *et al*., 2000; Sánchez & Dokoozlian, 2005; Goussard, 2018; Doligez *et al*., 2010). This is why this cultivar is cane pruned (SATI, 2020).

The insignificance of the pruning treatments was expected as the development of inflorescence primordia; the initiation and first phases of differentiation had already taken place in the previous season (May, 2000; Sánchez & Dokoozlian, 2005; Deloire, 2009; Li-Mallet *et al*., 2016; Wang *et al*., 2020). This entails that the number of inflorescence primordia per bud was already been determined in the previous season (Li-Mallet *et al*., 2016; Wang *et al*., 2020). The impact of the pruning on fertility could be expected in the following season, and/or subsequent seasons. Sommer *et al*. (2000) too did not obtain significant fertility differences in the first season of pruning treatments compared to the subsequent seasons.

Bennett (2002), reported a significant reduction of inflorescence number per shoot due to severe (66% and 100%) defoliation carried out in the previous season. Severe (75%) defoliation did not affect clusters per vine in the same season (Bennett *et al*., 2005) and with early defoliation over

three seasons, a non-significant interaction between the years and the defoliation treatments on the number of clusters per shoot as well as per vine was reported (Palliotti *et al*., 2011). In a study by Eltom *et al*. (2014), winter pruning treatments did not significantly affect the bud fruitfulness of Sauvignon Blanc in two seasons of the trial.

The significantly higher actual fertility expressed as the number of bunches per sprouted bud recorded for the late and severe pruning treatment compared to the control cannot be ascribed to improved initiation of inflorescence primordia because initiation occurred in the previous season (around flowering in the 2018 season), which was before the application of the treatments. In studies in Australia (Noyce *et al*., 2015) and South Africa (Mchwango *et al.*, 2018), differentiation of inflorescence primordia was reported to be completed by May and April respectively. Based on these findings, it could be assumed that the improved light exposure of buds after application of this pruning treatment (45 days after harvest, which was in March 2019) could have impacted the final stages of inflorescence primordia differentiation, but that would also not have had any effect on the number of primordia per bud and eventually the number of bunches per sprouted bud. Therefore, this result is considered coincidental.

4.5 CONCLUSION

Both the early and late, 66% post-harvest summer pruning treatments increased PAR and transmittance but did not promote photosynthetic activity. Total non-structural carbohydrates were not affected by post-harvest summer pruning. Post-harvest summer pruning did not affect the final bud break percentage and the fertility of the following season. Based on this one season's results, post-harvest pruning did not have any practical benefits, apart from improving light penetration. It is recommended that this study be repeated as the second and third seasons' data would contribute to confirming the effect of these pruning treatments on bud break and fertility.

It is expected that repeating post-harvest pruning treatments for several seasons on the same data vines could result in a decrease in carbohydrate reserves accumulated. These reduced reserves could be insufficient to sustain new growth in the spring, resulting in low, delayed and uneven bud break. It could also contribute to the occurrence of bunch stem necrosis or delayed ripening.

Given that the actual total percentage leaf area per vine removed by the post-harvest pruning treatments was not as severe as theoretically calculated and anticipated, an improved method to accurately determine the severity of pruning should be established. Leaf area index

measurements before and after pruning could be done, to assess if the desired percentage leaf area removal was obtained following the procedure described in Section 4.3.2.

4.6 LITERATURE CITED

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Chapter 5: General conclusion and recommendations

5.1 GENERAL CONCLUSION

This study has contributed to available research results on the seasonal dynamics of TNC reserve status of grapevines, provided scientific evidence whether post-harvest pruning has practical benefits and contributed to establishing a base for quantifying and practically assessing TNC reserve status of Sultanina H5.

5.1.1 Seasonal dynamics of grapevine TNC reserves.

The seasonal dynamics of NSC recorded in this study supported the findings of previous studies on similar topics and confirmed the importance of carbohydrate reserves as the fuel for initiating new season growth until flowering. Although carbohydrate reserve accumulation is initiated after flowering, fluctuations take place as vegetative growth competes with reproductive growth. This study confirmed that the post-harvest period is the main recovery period of the grapevine during which majority of the carbohydrate reserves are accumulated and stored. During this period, differences between the two regions studied were also recorded: in the Semi-arid region, the main reserve accumulation period commenced later than in the Mediterranean region. This may have impacted the level of reserves accumulated in the former region.

Two carbohydrate reserve peaks, one in late autumn and the other in winter, were recorded in both regions, although they did not occur at the same time. Soluble sugars accumulated to reach maximum levels during dormancy, while starch levels decreased during the same period. There was more carbohydrate reserve accumulation in the Mediterranean region and this may be due to less reserves used for the development and ripening of bunches as the crop load was lower compared to the Semi-arid region.

5.1.2 Post-harvest summer pruning

Severe post-harvest pruning proved to be beneficial for light penetration, but it did not improve the photosynthetic rate of the leaves. Post-harvest summer pruning did not have a significant effect on total non-structural carbohydrate reserves, bud break and fertility of grapevines in the season following the treatment. Based on this one season’s results, post-harvest pruning did not have overall practical benefits, apart from improving light penetration.

5.1.3 Establishing a base for quantifying and assessing grapevine TNC reserve status

A basis was established for sampling grapevine tissues for qualitative assessment of grapevine carbohydrate reserve status, linking sampling time to occurrence of peaks in soluble sugars and starch concentrations. It is recommended that sampling for qualitative assessment of soluble sugars should be done after leaf fall, during dormancy (June-July under the conditions of this study). Starch concentrations should be assessed before bud break (August under the conditions of this study).

Based on significant positive correlations between NSC concentrations of different tissue types, tissue types that could be sampled for indication of the overall NSC status of the grapevine were identified. In both regions, canes and/or shoots could be considered to be the most practical tissue types to sample for determination of grapevine NSC reserve status.

With only one season's results, it was not yet possible to establish a range of values, or benchmark values, regarded as indicative of "good carbohydrate status". This study should be repeated, given that, the vine vigour, bud break and fertility obtained with the treatments applied are correlated with NSC status, for establishment of benchmark values associated with "good carbohydrate status".

5.2 RECOMMENDATIONS

The study has provided scientific insight regarding: (i) non-structural carbohydrate (NSC) reserve dynamics in perennial tissues of grapevines for two different climatic regions of South Africa; and (ii) the influence of post-harvest summer pruning on NSC reserves, bud break and fertility.
Some aspects that need to be amended or improved when similar studies are conducted in the future were identified. The following are recommended for future research:

- An accurate method of quantifying pruning severity should be established, especially when it is to be expressed as leaf area removed. Leaf area index measurements before and after pruning could be done, to assess if the desired percentage leaf area removal was obtained following the procedure described in Section 4.3.2.
- Results obtained with post-harvest treatments were for one season only. Repeating the treatments for two more seasons on the same data vines, would indicate whether there is a carry-over effect of the practice on NSC, bud break and fertility.
- Available rapid and accurate methods to quantify carbohydrate reserves should be used and/or evaluated for use in grapevine studies. It is recommended that in a further phase of this project:

i. NIR spectroscopy should be used for collecting NIR spectra from grapevine cane tissues, whereafter the TNC concentration of the same samples must be determined using a wet chemical method and the results should be compared, using multivariate data analysis. Through this process, it could be established whether NIR spectroscopy could be used as a practical tool for rapid screening of TNC concentrations of grapevine tissues.

ii. The starch iodine test (already commercially used in the apple and forestry industries), should be evaluated as an additional tool for quantifying starch status of grapevine tissues (canes and roots), as an indicator of TNC status.

APPENDICES

Appendix 1

Anthrone method of carbohydrate analysis

Table 1: D-glucose standard for sugar analysis. With a standard curve, total sugars (as d-glucose units for total sugar analysis) can be calculated with slope.

Concentration (µg/mL) in total volume (1.5mL)~	0	1.733	3.333	6.667	13.333	20	26.667	33.333
In 500µL (µg/mL)*	0	5.2	10	20	40	60	80	100
mg	0	2.6	5	10	20	30	40	50
Glucose µL	0	13	25	50	100	150	200	250
dH_2O µL	500	487	475	450	400	350	300	250

~ (25µL std solution taken x 200µg/mL glucose standard solution)/(1000µL anthrone + 500µL H2O)
* (25µL std solution taken x 200µg/mL glucose standard solution)/(500µL H2O)

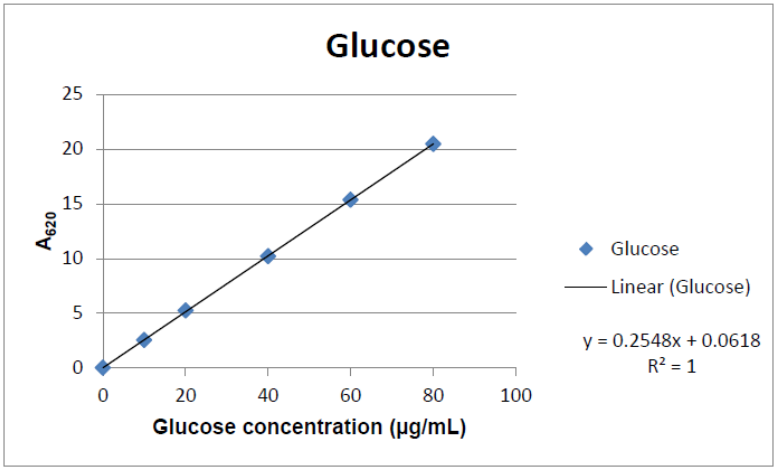

Figure 1: Anthrone reagent d-glucose standard curve. (Links, 2015).

From standard curve: ABS = a*CONC + b [a and b = values that are going to change for each spec output]

From moving equation: CONC (µg/ml d-glucose) = (ABS – b)/a

Appendix 2

A: Sucrose protocol

Summary

The photometric determination of Sucrose (g/L) in sample material (Roots, Shoots, Stems and Canes) based on the Enzytec™ *Liquid* Sucrose / D-glucose (E8180) method and performed on a Thermo Scientific Arena™ 20XT Analyzer. The method was adapted for lower Sucrose concentrations (0.1 – 1 g/L).

Principle

The test is based on an enzymatic test with ß-Fructosidase (Invertase), Hexokinase (HK) and D-glucose-6-Phosphate Dehydrogenase (G6P-DH). NADH is produced and is measured at 340 nm in the following reaction:

Sucrose + H_2O $\xrightarrow{ß - Fructosidase}$ D-glucose + D-fructose

D-glucose + ATP $\xrightarrow{HK}$ D-glucose-6-Phosphate + ADP

G-6-P + NAD^+ $\xrightarrow{G6P - DH}$ Gluconate-6-P + NADH + H^+

Assay Specifications

Chapter 3	Wavelength	340 nm
Chapter 4	Temperature	20 – 25 °C / 37 °C
Chapter 5	Optical path	1 cm
Chapter 6	Measurement	Against air or water
Chapter 7	Linearity	10 – 1000 mg/L

Reagents

Both reagents in the Enzytec™ Liquid Sucrose / D-glucose kit are ready to use.

- Reagent 1 NAD, ß-Fructosidase, ATP
- Reagent 2 HK, G6P-DH

Method

The test sequence was run automatically by the instrument as described in the Enzytec™ Liquid Sucrose / D-glucose (E8180) protocol. The instrument requires the use of low-volume cuvettes (< 250 µL) which means the reagent and sample volumes were reduced whilst keeping the same ratios than in the manual procedures. The incubation times were programmed for 10 min each, one for the ß-Fructosidase and one for the D-glucose reaction.

The particular feature of this assay is that 2 tests must be performed into 2 separate cuvettes:

- The Sucrose/D-glucose test (Total Sucrose) is in one cuvette and is described here (E8180)

- The D-glucose test is in the second cuvette, and is described separately (E8140)

The true Sucrose content is calculated by the subtraction of both assays:

- Sucrose (g/L) = Sucrose/D-glucose (g/L) – 1.9 x D-glucose (g/L)
    - Example:
        - Total-Sucrose (E8180) = 1.500 g/L
        - D-glucose (E8140) = 0.400 g/L
        - Sucrose = 1.500 g/L – 1.90 x 0.400 g/L = 0.740 g/

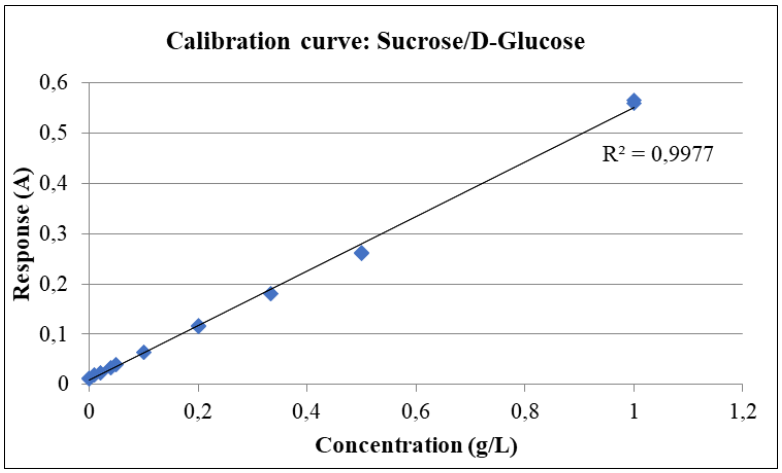

Figure 2: Calibration curve for Sucrose.

B: D-glucose protocol

Summary

The photometric determination of D-glucose (g/L) in sample material (Roots, Shoots, Stems and Canes) based on the Enzytec™ *Liquid* D-glucose (E8140) method and performed on a Thermo Scientific Arena™ 20XT Analyzer. The method was adapted for lower D-glucose concentrations (0.1 – 1 g/L).

Principle

The test is based on an enzymatic test with Hexokinase (HK) and D-glucose-6-Phosphate Dehydrogenase (G6P-DH). NADH is produced and is measured at 340 nm in the following reaction:

D-glucose + ATP $\xrightarrow{HK}$ D-glucose-6-Phosphate + ADP

G-6-P + NAD^+ $\xrightarrow{G6P-DH}$ Gluconate-6-P + NADH + H^+

Assay specifications

Chapter 8	Wavelength	340 nm
Chapter 9	Temperature	20 – 25 °C / 37 °C
Chapter 10	Optical path	1 cm
Chapter 11	Measurement	Against air or water
Chapter 12	Linearity	10 – 1000 mg/L

Reagents

Both reagents in the Enzytec™ Liquid Sucrose / D-glucose kit are ready to use.

- Reagent 1 NAD, Buffer
- Reagent 2 HK, G6P-DH

Method

The test sequence was run automatically by the instrument as described in the Enzytec™ Liquid D-glucose (E8140) protocol. The instrument requires the use of low-volume cuvettes (< 250 µL) which means the reagent and sample volumes were reduced whilst keeping the same ratios than in the manual procedures. After combining the sample with Reagent 1, the incubation time was programmed for 1 min, followed by 10 min after the addition of Reagent 2.

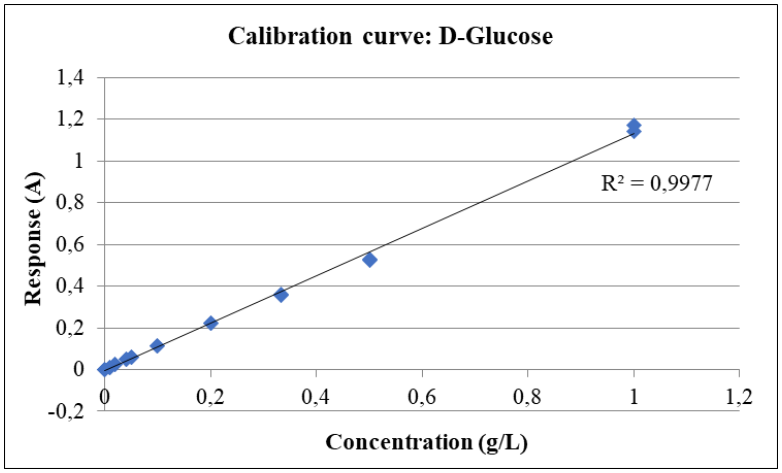

Figure 3: Calibration curve for d-glucose.

C: D-fructose protocol

Summary

The photometric determination of D-fructose (g/L) in sample material (Roots, Shoots, Stems and Canes) based on the Thermo Scientific D-fructose (984302) method and performed on a Thermo Scientific Arena™ 20XT Analyzer. The method was adapted for lower D-fructose concentrations (0.1 – 1 g/L).

Principle

The test is based on an enzymatic test with hexokinase (HK), phosphod-glucose isomerase (PGI) and d-glucose-6-phosphate dehydrogenase (G6P-DH). NADH is produced and is measured at 340 nm in the following reaction:

D-fructose + ATP $\xrightarrow{HK}$ D-fructose-6-phosphate + ADP

D-glucose + ATP $\xrightarrow{HK}$ D-glucose-6-phosphate + ADP

D-fructose-6-phosphate $\xleftrightarrow{PGI}$ D-glucose-6-phosphate

D-glucose-6-phosphate + NAD^+ $\xrightarrow{G6P-DH}$ Gluconate-6-phosphate + NADH + H^+

Assay Specifications

Chapter 13	Wavelength	340 nm
Chapter 14	Temperature	20 – 25 °C / 37 °C
Chapter 15	Optical path	1 cm
Chapter 16	Measurement	Against air or water
Chapter 17	Linearity	10 – 1000 mg/L

Reagents

The reagents R1, R2 and R3 are ready-to-use.

- Reagent 1 NAD, ATP, Buffer
- Reagent 2 HK, G6P-DH
- Reagent 3 PGI, Buffer

Method

The test sequence was run automatically by the instrument as described in the Thermo Scientific D-fructose (984302) protocol. The instrument requires the use of low-volume cuvettes (< 250 µL) which means the reagent and sample volumes were reduced whilst keeping the same ratios than in the manual procedures. The first incubation (R1 + sample + R2) was programmed for 5 min, followed by 7 min after the addition of Reagent 3.

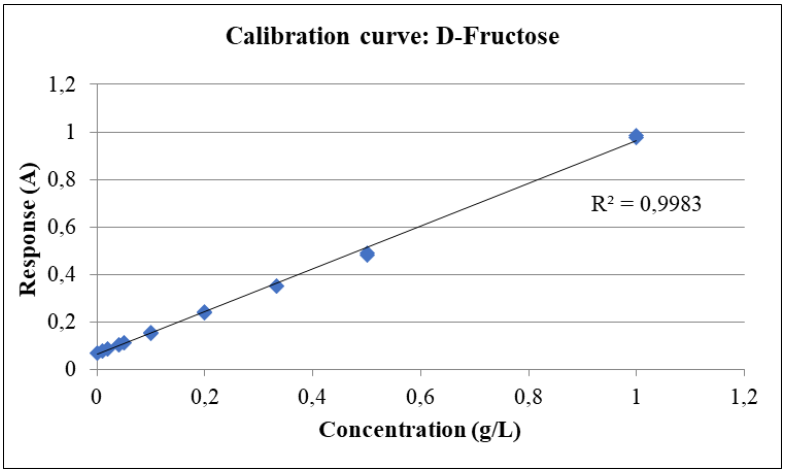

Figure 4: Calibration curve for d-fructose.

Appendix 3

Table 2: Analysis of variance to test farm (region) and month (sampling date) interaction of non-structural carbohydrate reserve patterns of Sultanina H5 in the semi-arid Lower Orange River and the Mediterranean Hex River regions of South Africa in the 2018/2019 season.

	Farm			Month			Farm x Month		
Tissue/Parameter	**DF**	**MS**	**P**	**DF**	**MS**	**P**	**DF**	**MS**	**P**
Roots									
Soluble sugars	1	5.97	0.16	11	18.76	<.0001	11	3.27	0.36
Starch	1	33.68	0.37	11	249.35	<.0001	11	23.19	0.84
TNC	1	11.29	0.60	11	340.92	<.0001	11	20.25	0.90
Sucrose	1	0.00	0.25	11	0.00	<.0001	11	0.00	0.00
D-fructose	1	0.00	<.0001	11	0.00	<.0001	11	0.00	<.0001
D-glucose	1	0.00	0.03	11	0.00	0.00	11	0.00	0.00
Stems									
Soluble sugars	1	25.03	0.01	11	18.79	<.0001	10	4.86	0.25
Starch	1	98.19	0.02	11	52.48	0.00	10	14.93	0.54
TNC	1	24.07	0.28	11	76.99	0.00	10	16.54	0.60
Sucrose	1	0.00	0.08	11	0.00	<.0001	10	0.00	<.0001
D-fructose	1	0.00	0.00	11	0.00	<.0001	10	0.00	0.01
D-glucose	1	0.00	<.0001	11	0.00	<.0001	10	0.00	0.00
Canes									
Soluble sugars	1	14.46	0.02	11	35.97	<.0001	9	5.22	0.05
Starch	1	133.44	0.05	10	365.69	<.0001	8	19.57	0.76
TNC	1	178.79	0.05	11	495.98	<.0001	9	14.17	0.96
Sucrose	1	0.00	0.23	11	0.00	<.0001	9	0.00	<.0001
D-fructose	1	0.00	0.09	11	0.00	<.0001	9	0.00	<.0001
D-glucose	1	0.00	0.62	11	0.00	<.0001	9	0.00	<.0001
Shoots									
Soluble sugars	1	4.60	0.22	9	30.19	<.0001	9	4.34	0.20
Starch	1	0.00	1.00	9	270.38	<.0001	9	24.74	0.03
TNC	1	4.65	0.60	9	476.37	<.0001	9	19.66	0.33
Sucrose	1	0.00	0.90	8	0.00	<.0001	8	0.00	<.0001
D-fructose	1	0.00	0.16	8	0.00	<.0001	8	0.00	0.06
D-glucose	1	0.00	0.07	8	0.00	0.00	8	0.00	0.01

DF- Degrees of freedom. MS- Mean square. P- Probability of F-ratio test.

Appendix 4A

Table 3: Non-structural carbohydrate reserve concentrations at different sampling dates in the roots, trunks, canes and shoots of the semi-arid Lower Orange River and Mediterranean Hex River regions in the 2018/2019 season.

Region	July	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun
Soluble sugars (g/g %)*												
Roots												
Semi-arid	4.61 bc	3.60 cdef	3.28 efg	2.68 fg	2.38 g	0.77 h	4.02 cde	4.58 bcd	3.59 def	7.38 a	5.25 b	4.24 bcde
Mediterranean	9.58 a	3.89 bcde	4.57 bcd	2.96 de	2.36 e	3.75 cde	3.45 cde	4.05 bcd	5.09 bc	4.90 bc	5.45 b	8.53 a
Trunks												
Semi-arid	11.47 a	8.95 b	3.06 fg	3.25 fg	3.70 f	1.90 g	3.36 f	5.49 d	4.05 ef	5.70 d	5.39 de	7.34 c
Mediterranean	7.20 abc	7.61 ab	5.13 cde	-	5.52 bcd	7.08 abc	3.20 e	4.64 de	6.46 abcd	8.01 a	5.73 bcd	8.03 a
Canes												
Semi-arid	6.61 bc	7.40 ab	3.21 d	0.66 fg	1.38 ef	1.25 fg	2.29 de	0.41 fg	0.32 g	6.79 abc	7.74 a	6.34 c
Mediterranean	7.25 b	3.99 c	2.38 de	1.48 e	3.55 cd	1.47 e	1.87 e	-	-	6.37 b	6.97 b	10.70 a
Shoots												
Semi-arid	-	-	3.70 de	2.72 ef	0.95 g	4.59 cd	3.13 de	1.59 fg	4.47 cd	6.19 b	8.11 a	5.45 bc
Mediterranean	-	-	4.67 c	2.60 ef	3.41 de	2.12 f	2.58 ef	4.00 cd	2.54 ef	6.16 b	7.00 b	10.22 a
Starch (g/g %)*												
Roots												
Semi-arid	1.67 f	8.58 d	5.87 e	2.67 f	2.35 f	1.79 f	3.28 f	8.01 d	9.66 cd	10.69 c	15.49 b	18.31 a
Mediterranean	1.59 g	6.01 f	6.15 f	6.42 ef	1.18 g	2.29 g	10.95 d	8.42 e	18.78 ab	20.01 a	15.80 c	17.70 bc
Trunks												
Semi-arid	1.50 f	4.56 e	8.97 b	6.97 cd	3.79 e	6.14 d	8.98 b	10.25 a	7.29 c	7.99 bc	10.45 a	10.34 a
Mediterranean	1.69 f	5.50 cde	9.37 a	.	4.66 de	4.31 e	4.33 e	5.08 cde	6.09 cde	6.37 bcd	8.00 ab	6.58 bc
Canes												
Semi-arid	1.53 efgh	4.39 d	-	1.68 efg	0.92 gh	1.42 fgh	2.53 e	2.12 ef	0.53 h	8.48 c	20.33 b	21.55 a
Mediterranean	1.62 fg	7.17 d	-	2.77 f	1.71 fg	1.12 g	3.97 e	-	-	17.41 b	21.41 a	11.71 c
Shoots												
Semi-arid	-	-	5.73 de	5.09 e	0.39 g	0.68 g	3.39 f	2.73 f	8.26 c	6.51 d	9.74 b	12.80 a
Mediterranean	-	-	3.94 d	0.58 f	0.48 f	0.98 f	2.35 e	4.20 d	7.53 c	10.58 a	11.02 a	9.08 b
Total non-structural carbohydrates (g/g %)*												
Roots												
Semi-arid	6.29 ef	12.19 c	9.15 d	5.67 ef	4.73 fg	2.55 g	7.32 de	12.59 c	13.25 c	19.09 b	19.72 b	24.93 a
Mediterranean	11.11 de	9.90 de	10.73 de	9.38 e	3.54 f	6.04 f	15.49 c	12.47 d	25.47 a	25.18 a	21.24 b	26.61 a
Trunks												
Semi-arid	12.88 de	13.68 bcd	13.24 cde	10.21 f	7.49 g	7.52 g	12.34 def	15.30 bc	11.18 ef	13.78 bcd	15.84 b	18.39 a
Mediterranean	8.73 ef	12.87 ab	14.43 ab	-	10.18 de	12.29 bcd	7.53 f	10.48 cde	12.62 abc	14.38 ab	13.73 ab	14.61 a
Canes												
Semi-arid	8.14 c	11.75 b	-	2.70 e	2.30 ef	2.67 e	4.67 d	2.78 e	0.86 f	13.23 b	27.30 a	28.80 a
Mediterranean	9.38 c	11.16 c	-	4.57 de	5.24 d	2.59 e	5.84 d	-	-	24.68 b	28.10 a	22.41 b
Shoots												
Semi-arid	-	-	10.57 b	7.09 c	1.34 d	5.42 c	6.53 c	4.32 c	12.73 b	12.78 b	17.85 a	19.22 a
Mediterranean	-	-	8.76 d	3.18 f	3.89 f	3.10 f	5.35 e	8.62 d	11.03 c	16.73 b	18.02 a	18.85 a

*Comparing tissue type per region and month. means with the same lower-case letter (of the range a. b. c) within the rows and columns of each data set. do not differ significantly ($p < 0.05$).

Appendix 4B

Table 4: Non-structural carbohydrate reserve concentrations at different sampling dates in the roots, trunks, canes and shoots of the semi-arid Lower Orange River and Mediterranean Hex River regions in the 2018/2019 season.

Region	July	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun
Sucrose (g/g %)												
Roots												
Semi-arid	2.71 abcde*	2.22 cdefgh	2.34 bcdefg	2.48 abcdef	1.08 ij	0.30 j	1.37 hi	1.89 efghi	2.33 bcdefg	2.31 bcdefg	2.85 abcd	3.25 a
Mediterranean	3.25 a	1.97 defghi	3.13 abc	2.32 bcdefg	1.07 ij	1.85 efghi	1.39 hi	1.80 fghi	2.45 abcdef	3.06 abc	3.16 ab	1.51 ghi
Trunks												
Semi-arid	2.89 a	3.00 a	1.26 c	0.95 cde	0.21 hijk	0.00 k	0.12 jk	0.36 ghijk	0.29 ghijk	1.04 cd	0.62 defgh	0.73 defg
Mediterranean	2.73 a	2.01 b	0.53 efghij	-	0.84 cdef	0.54 efghij	0.21 hijk	0.38 fghijk	0.60 defghi	0.13 ijk	0.48 efghij	0.72 defg
Canes												
Semi-arid	0.56 de	0.70 cd	0.91 bc	0.13 ghi	0.00 i	0.02 hi	0.27 fgh	0.09 ghi	0.11 ghi	0.45 def	0.57 de	1.49 a
Mediterranean	0.50 def	0.58 de	0.67 cd	0.33 efg	0.11 ghi	0.00 i	0.40 ef	-	-	0.84 bc	1.01 b	0.45 def
Shoots												
Semi-arid	-	-	-	0.41 def	0.09 ghi	0.14 ghi	0.06 hi	0.62 bcd	0.30 fgh	0.24 fghi	0.56 cde	1.44 a
Mediterranean	-	-	-	0.05 i	0.12 ghi	0.00 i	0.25 fghi	0.31 efg	0.73 bc	1.21 a	0.82 b	0.40 def
D-fructose (g/g %)												
Roots												
Semi-arid	0.23 fghi	0.17 ij	0.18 hij	0.17 ij	0.22 fghi	0.11 j	0.21 ghi	0.41 a	0.26 efgh	0.32 bcde	0.30 cdef	0.21 ghi
Mediterranean	0.41 ab	0.20 ghi	0.21 ghi	0.41 a	0.14 ij	0.32 cde	0.15 ij	0.40 ab	0.28 defg	0.36 abcd	0.38 abc	0.20 ghi
Trunks												
Semi-arid	1.01 bc	1.12 b	1.66 a	0.59 fgh	0.66 efg	0.41 hij	0.34 ij	0.56 fghi	0.74 defg	0.53 ghi	0.67 defg	0.88 bcde
Mediterranean	0.80 cdef	0.68 defg	1.61 a	-	0.33 ij	0.20 j	0.28 j	0.28 j	0.40 hij	0.61 fgh	0.91 bcd	0.88 bcde
Canes												
Semi-arid	1.93 a	1.98 a	0.82 c	0.51 ef	0.35 gh	0.16 i	0.35 gh	0.29 hi	0.29 hi	0.77 cd	0.70 cd	0.80 cd
Mediterranean	1.82 a	1.32 b	0.69 cd	0.78 cd	0.35 gh	0.47 fg	0.37 gh	-	-	0.66 de	0.69 cd	0.71 cd
Shoots												
Semi-arid	-	-	-	0.86 abc	0.31 fgh	0.19 h	0.27 gh	0.51 efg	0.50 efg	0.89 abc	0.89 abc	0.95 ab
Mediterranean	-	-	-	1.03 a	0.18 h	0.52 defg	0.57 def	0.54 defg	0.66 cde	0.79 abcd	0.96 ab	0.69 bcde
D-glucose (g/g %)												
Roots												
Semi-arid	0.09 defg	0.06 efg	0.00 g	0.01 g	0.07 defg	0.00 g	0.07 efg	0.22 abc	0.04 fg	0.19 abcd	0.18 abcde	0.11 cdefg
Mediterranean	0.18 abcde	0.09 defg	0.09 defg	0.29 a	0.04 fg	0.16 bcdef	0.04 fg	0.24 ab	0.15 bcdef	0.11 cdefg	0.15 bcdef	0.00 g
Trunks												
Semi-arid	0.98 c	1.30 b	1.95 a	0.48 ghij	0.51 fghi	0.40 hijkl	0.30 ijkl	0.45 ghijk	0.62 defgh	0.45 ghijk	0.54 efghi	0.76 cde
Mediterranean	0.75 cdef	0.66 defg	1.50 b	-	0.23 jkl	0.19 l	0.21 kl	0.21 kl	0.38 hijkl	0.49 ghi	0.83 cd	0.78 cde
Canes												
Semi-arid	1.47 ab	1.58 a	0.65 de	0.46 fgh	0.39 ghij	0.19 k	0.43 fghi	0.21 k	0.25 jk	0.56 defg	0.43 fghij	0.60 def
Mediterranean	1.33 b	0.83 c	0.50 efgh	0.72 cd	0.33 hijk	0.51 efg	0.27 ijk	-	-	0.51 efgh	0.46 fgh	0.43 fghij
Shoots												
Semi-arid	-	-	-	0.91 a	0.35 fgh	0.24 gh	0.28 gh	0.40 defgh	0.38 efgh	0.70 abcd	0.63 abcdef	0.69 abcde
Mediterranean	-	-	-	0.84 ab	0.15 h	0.72 abc	0.62 abcdef	0.55 bcdefg	0.78 ab	0.71 abcd	0.64 abcdef	0.43 cdefgh

*Comparing tissue type per region and month. means with the same lower-case letter (of the range a. b. c) within the rows and columns of each data set. do not differ significantly ($p < 0.05$).

Appendix 5

Table 5: Soil analysis of the semi-arid Lower Orange River experimental block in the 2018/2019 season.

Block	Depth	Sand	Silt	Clay	P	Ca	Mg	K	Na	pH_{KCl}
	(cm)	%	%	%	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)	
1-4	0-30	68	10	22	0	4510	223	177	23.3	7.85
	30-60	76	8	16	0	4420	176	128	19	7.74
5	0-30	74	8	18	73.1	4190	309	151	38.6	7.79
	30-60	76	8	16	0	4700	267	143	63.2	7.84
6	0-30	78	6	16	93.1	4570	305	209	27.8	7.68
	30-60	78	8	14	65.15	4220	254	175	25.2	7.81

Appendix 6

Table 6: Effect of post-harvest summer pruning on PAR and transmittance through Sultanina H5 canopies in the 2018/2019 season.

Treatment	Time									
	10:00		12:00		14:00		16:00		18:00	
	Photosynthetic rate (µmol CO_2 m^{-2} s^{-1})									
Ctr	7.72	cd*	6.05	def	8.69	bcd	11.60	a	3.54	fg
33_1dAH	4.47	efg	6.97	de	8.67	bcd	10.38	abc	2.43	g
66_1dAH	6.21	def	3.60	fg	6.68	de	11.52	ab	3.67	fg
Mean	**6.13**	**y**[#]	**5.54**	**y**	**8.01**	**x**	**11.17**	**w**	**3.21**	**z**
	Stomatal conductance (mol H_2O m^{-2} s^{-1})									
Ctr	0.14	cd	0.36	a	0.17	bc	0.11	de	0.20	b
33_1dAH	0.10	def	0.06	fgh	0.08	efg	0.07	efg	0.04	gh
66_1dAH	0.17	bc	0.06	fgh	0.02	h	0.05	gh	0.04	gh
Mean	**0.13**	**w**	**0.15**	**w**	**0.09**	**x**	**0.08**	**x**	**0.09**	**x**
	Intercellular CO_2 (µmol CO_2 mol^{-1})									
Ctr	319.20	a	317.80	a	294.80	ab	298.00	ab	281.00	abc
33_1dAH	230.00	abcde	113.50	fg	149.60	ef	173.20	def	-5.75	h
66_1dAH	239.50	abcd	52.80	gh	223.60	bcde	238.00	abcde	198.00	cdef
Mean	**264.57**	**w**	**164.79**	**x**	**222.67**	**w**	**236.40**	**w**	**169.43**	**x**
	Transpiration rate (mmol H_2O m^{-2} s^{-1})									
Ctr	6.49	de	11.18	a	8.22	bc	6.41	de	8.96	b
33_1dAH	6.09	def	3.67	gh	4.81	efg	4.55	fg	2.61	hi
66_1dAH	7.24	cd	3.48	gh	1.15	i	2.01	hi	1.25	i
Mean	**6.56**	**w**	**5.75**	**w**	**4.73**	**x**	**4.18**	**x**	**3.94**	**x**

*Comparing the effect of pruning treatments at different time frames. Means with the same lower-case letter (of the range a, b, c) within columns for each parameter do not differ significantly ($p < 0.05$).
[#]Comparing means at different time frames. Means with the same lower-case letter (of the range w, x, y, z) within rows for each parameter do not differ significantly ($p < 0.05$).