A case study of source-sink relationships using shoot girdling and berry classification (*Vitis vinifera* L. cv. Cabernet Sauvignon)

by

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Declaration

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Summary

The relationships between leaf and fruit represent a fundamental concept in perennial plants. This concept allows to understand and to manage, with regard to farming, the balance of a vine, which is important in terms of fruit quality (i.e. fruit composition), mainly when it comes to producing wines of different categories and styles. The understanding of vine structure, physiology and vine functioning ultimately allows for appropriate recommendations to be given with regard to farming procedures. These include the adaptation of the canopy architecture to achieve a certain yield per vine, the determination of an appropriate fruit microclimate as well as the prediction of harvest dates. One of the central notions of vine balance involves the relationship between the source and the sink organs. The definition of source-sink relationships incorporates several concepts, including the ability of a source tissue to produce carbohydrates through photosynthesis, the transport of these carbohydrates to various plant organs-tissues via appropriate transport channels, and the assimilation and storage of the carbohydrates in the sink organs. In past years, a number of simple ratios have been created to incorporate the relationship between source and sink organs and thereby define vine balance in order to aid in practical management decisions (choice of a training system, irrigation, canopy manipulation etc.). However, vine functioning is very complex and cannot be defined accurately by simple, static ratios. More integrated and dynamic physiological indicators of vine balance and functioning are needed in order to understand the complex communication between organs and ultimately improve on farming practices. In order to achieve this, a better grasp of source-sink relationships, including the signalisation between organs and the functioning of the transport tissues is required.

A two year experiment was proposed to study the interaction between source and sink organs using a combination of both primary shoot girdling methods and berry classification according to size. Girdling removes the bark and phloem tissue, thereby interrupting carbon import as well as water flow to the bunch to a certain degree. The aim of the study was to demonstrate the complexity of vine functioning by investigating the dynamics of berry sugar and water accumulation (used as physiological indicators) and the influence thereof on berry fresh mass evolution. Furthermore, the use of berry sugar loading was proposed as an improved physiological indicator of vine balance as it is directly linked to source and sink functioning. Sugar production and the dynamics of berry sugar accumulation rely on photosynthesis which in turn is dependent on stomatal conductance and therefore also incorporates the effects of external abiotic factors (temperature, light and water). It furthermore gives a direct indication of sink functioning as it shows the progressive accumulation of sugar throughout the ripening period and the possible consequences on berry volume evolution.

A primary shoot which bore two bunches was used to represent a biological replicate. The lower bunches were girdled above and below in order to completely isolate them from any carbohydrate import. These bunches, along with the upper ungirdled bunches and two control bunches from another shoot were sampled. The berries from these bunches were classified according to diameter, thereby providing the unique opportunity to study berries of the same volume/size. Measurements were done to determine the fresh and dry masses of the sampled berries, as well as to analyse the concomitant sugar concentrations.

It was found that girdling clearly had an effect on berry sugar dynamics and the method was improved in the second year of the trial. Girdling in interaction with berry classification according to diameter demonstrated that berries from the same size could have different sugar concentrations. It further showed that, to a certain degree, a relationship exists between the first rapid phase of sugar accumulation and the post véraison increase in berry fresh mass, until the plateau of fruit sugar accumulation, which generally occurs around a sugar concentration of 20 Brix. Additionally, and more importantly, it was found that vine functioning and the balance between the source and the sink organs may be controlled to a certain degree. There is a strong degree of compensation within a vine which results from signalling between and within organs. When taking the results of this study into consideration, it becomes clear that the classical ratios used to quantify the complex relationships between the fruit and the leaves may not be completely adequate to do so. The current way of looking at source-sink relationships and thereby determining whether a vine is balanced or not is over-simplified and there are numerous limitations involved in this approach. The vine is far more complex and various aspects must be taken into consideration before any claims can be made concerning source-sink relationships and consequently leaf to fruit balance.

Opsomming

Die verhoudings tussen blaar en vrug verteenwoordig ‘n fundamentele konsep in meerjarige plante. Begrip van hierdie konsep maak dit moontlik om in boerdery die balans van ‘n wingerdstok te verstaan en bestuur. Hierdie wingerdbalans is belangrik in terme van vrugkwaliteit (d.w.s. vrugsamestelling), hoofsaaklik met betrekking tot die produksie van wyne van verskillende kategorieë en style. Begrip van die wingerdstok se struktuur, fisiologie en funksionering maak dit moontlik om gepaste aanbevelings te maak rakende boerdery prosedures. Dit sluit in die aanpassing van die lower argitektuur om ‘n sekere opbrengs per wingerdstok te verkry, die vasstel van ‘n geskikte vrug mikroklimaat asook die voorspelling van oesdatums. Een van die sentrale denkwyses rondom wingerdstok funksionering behels die die bron-vragpunt verhouding. Die definisie van bron-vragpunt verhoudings inkorporeer verskeie konsepte, insluitende die vermoë van ‘n bronweefsel om koolhidrate te produseer deur fotosintese, die vervoer van hierdie koolhidrate na verskeie plantorgaan weefsels via die gepaste vervoerkanale asook die opname en berging van hierdie koolhidrate in die vragpunt organe. In die verlede is ‘n aantal eenvoudige verhoudings geskep om die verband tussen die bron en vragpunt organe te beskryf en sodoende die wingerdstokbalans te definieer met die doel om ondersteuning te bied in praktiese bestuursbesluite (die keuse van opleistelsel, besproeiing, lowermanipulasie, ens.). Wingerdstok funksionering is egter baie kompleks en kan nie akkuraat gedefinieer word deur eenvoudige, statiese verhoudings nie. Meer geïntegreerde en dinamiese fisiologiese aanwysers van wingerdstokbalans en funksionering is nodig om die komplekse kommunikasie tussen organe te verstaan en uiteindelik boerdery praktyke te verbeter. Om dit te bereik is ‘n beter begrip van bron-vragpunt verhoudings asook die seinoordrag tussen organe en die werking van die vervoerweefsels nodig.

‘n Twee jaar lange eksperiment is voorgestel om die interaksie tussen bron- en benuttingsorgane te ondersoek deur gebruik te maak van beide die primêre loot ringelering metode en korrel klassifikasie volgens grootte. Ringelering verwyder die bas en floëem weefsel en onderbreek sodoende koolstof invoer sowel as watertoevoer na die tros tot ‘n sekere mate. Die doel van die studie was om die kompleksiteit van wingerdstok funksionering aan te toon deur die dinamika van suiker en water akkumulasie in die korrel te ondersoek asook die invloed daarvan op korrel vars massa ontwikkeling. Verder is die gebruik van korrel suikerlading voorgestel as ‘n beter fisiologiese aanduiding van wingerdstok funksionering aangesien dit direk geassosieer is met bron-vragpunt funksionering. Suikerproduksie en die dinamika van suiker akkumulasie in die korrel berus op fotosintese wat weer afhanklik is van stomatale geleiding en daarom ook die effek van eksterne abiotiese faktore (temperatuur, lig en water) inkorporeer. Dit gee verder ‘n direkte aanduiding van die funksionering van die vragpunt organe omdat dit die

progressiewe akkumulasie van suiker gedurende die rypwordingsperiode aantoon, asook die moontlike gevolge op korrelvolume ontwikkeling.

‘n Primêre loot wat twee trosse dra is gebruik om ‘n biologiese herhaling te verteenwoordig. Die laer trosse is bo en onder geringeleer om hulle heeltemal te isoleer van enige koolhidraat invoer. Hierdie trosse, tesame met boonste ongeringeleerde trosse en twee kontrole trosse vanaf ‘n ander loot is gemonster. Die korrels van hierdie trosse is geklassifiseer volgens hulle deursnee, om sodoende die unieke moontlikheid daar te stel om korrels van dieselfde volume/ grootte te bestudeer. Metings is gedoen om die vars en droë massas van die gemonsterde korrels te bepaal, asook om die gepaardgaande suikerkonsentrasies te analiseer.

Daar is gevind dat ringelering duidelik ‘n effek gehad het op korrelsuiker dinamika en die metode is verbeter in die tweede jaar van die proef. Ringelering in wisselwerking met korrel klassifikasie volgens korrel deursnee het aangetoon dat korrels met dieselfde grootte verskillende suikerkonsentrasies kon hê. Dit het verder aangedui dat daar, tot ‘n sekere mate, ‘n verhouding bestaan tussen die vinnige fase van suiker akkumulasie en die na-véraison toename in korrel vars massa, totdat die plato in suiker akkumulasie bereik word, gewoonlik rondom ‘n suikerkonsentrasie van 20 Brix. Daarbenewens, en van groter belang, is gevind dat wingerdstok funksionering en die balans tussen die bron en vragpunt organe onder ‘n mate van beheer is. Daar is ‘n sterk mate van kompensasie binne ‘n wingerdstok wat die gevolg is van seinoordrag tussen en binne organe in die wingerdstok. Wanneer die resultate van hierdie studie in aanmerking geneem word, word dit duidelik dat die klassieke verhoudings, wat gebruik word om wingerdstok funksionering en balans mee te bepaal, moontlik nie beduidend betekenisvol is nie. Die wyse waarop bron-vragpunt verhoudings tans beskou word is, tot ‘n mate, ‘n oorvereenvoudiging en daar is heelwat beperkinge betrokke by hierdie benadering. Die wingerd is baie meer kompleks en verskeie aspekte moet in aanmerking geneem word voordat enige bewering gemaak kan word rakende bron-vragpunt verhoudings.

This thesis is dedicated to my parents for their love, encouragement and support.

Biographical sketch

Chandré Joubert was born in Kimberley on the 6 May 1988. In 2006, she matriculated from Kimberley Girls' High School after which she enrolled for a BScAgric in Viticulture and Oenology at the University of Stellenbosch. She obtained her degree cum laude in December of 2010 and enrolled for an MSc in Viticulture in 2011, also at Stellenbosch University.

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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 Viticulture and Oenology.

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Chapter 1

Introduction and project aims

INTRODUCTION AND PROJECT AIMS

1.1 Introduction

Source sink relationships can be defined as the ability of a plant to undergo photosynthesis, thereby fixing CO_2 in the source organs, and to transport this fixed carbon to various sink tissues or organs. It also defines the ability of the sink organs to assimilate or store the fixed carbon structures such as glucose and fructose.

The source sink concept typically refers to the ratio between the leaves and the fruit. In literature about the grapevine, it is common to state that to ripen 1 g of grapes, a leaf area of 8 – 10 cm^2 is necessary. (Nuzzo & Matthews, 2006; Conde *et al.*, 2007). This however is very misleading as the nature of the leaf area is not fully defined. It is unclear whether total leaf area or exposed leaf area is being referred to and also whether primary shoots or lateral shoots are exclusively used or if both are considered when referring to this relationship. Additionally, there is a vast difference between 8 cm^2 and 10 cm^2 when it comes to the real size of a vine's architecture. Furthermore, there is a degree of vagueness with regard to what berry ripening means exactly, whether it refers to sugar accumulation, phenolic ripeness, or flavour development. The concept of source sink relationships needs to be reassessed and approached from a different angle using unique ideas and various scientific procedures. Areas which may be looked into can include signalling between organs, using biological tracers to follow the movement of various compounds (sugar, amino acids, hormones), through the vine, or plant signals involving compounds such as carbohydrates, jasmonic acid or calcium to understand the communication between organs.

The current way of looking at source-sink relationships is over-simplified and there are numerous limitations involved in this approach. The vine is far more complex and various aspects must be taken into consideration before any claims can be made concerning source-sink relationships. The concept of source-sink relationships cannot be described using only simple ratios such as the ratio between exposed leaf area and yield or the ratio between pruning mass and yield. This is due mainly to the nature of the leaf, particularly taking into account stomatal size and density. This in is turn linked to stomatal regulation and conductance, which is directly connected to photosynthesis and is therefore able to exercise an influence upon sugar production and accumulation as well as reserve carbohydrate production.

1.2 Project aims

The aim of this project was to investigate the dynamics of berry sugar and water accumulation under a particular circumstance, thereby providing further insights into the complex relationship between the leaves and fruit, and to address the use of sugar accumulation as a potential physiological indicator of vine functioning and berry ripeness. Currently berries are mainly harvested according to a particular sugar concentration. However, other ripening characters important to winemaking, such as aromas, acids and colour, can vary widely at any particular sugar concentration. Therefore a new indicator should be considered. In order to address this issue, the following subjects were investigated:

- The source-sink interactive relationship between leaf and grape berry (post véraison) on a primary shoot,
- Dynamic of berry sugar and water accumulation during berry ripening (post véraison)
- Relation between the volume of a berry and its sugar content
- The effect of the isolation of one sink from carbohydrate import using girdling on the remaining sink.

Chapter 2

Literature review

LITERATURE REVIEW

2.1 GRAPE BERRY DEVELOPMENT

Grapes are non-climacteric fruits that exhibit a double-sigmoid pattern of development, with two distinct phases of growth separated by a lag phase which precedes véraison and during which the berry cells are re-engineered at the molecular level to prepare for the ripening process (Figure 2.1) (Coombe, 1992; Ollat *et al.*, 2002; Conde *et al.*, 2007). The berry green growth stage is characterised by a short period of berry cell multiplication 8-10 days after flowering (Fougere Rifot *et al.*, 1996; Ojeda *et al.*, 1999), followed by a period of cell enlargement which is mainly dependent on the vine water and nitrogen status. Véraison is the start of ripening and is characterised by berry softening, the beginning of berry sugar accumulation and the biosynthesis of anthocyanins at the skin level for red cultivars. The second period of berry growth, referred to as maturation, is only due to berry cell enlargement, during which the berries accumulate mainly water, sugar, potassium, nitrogen and amino acids, all of which come from the vine.

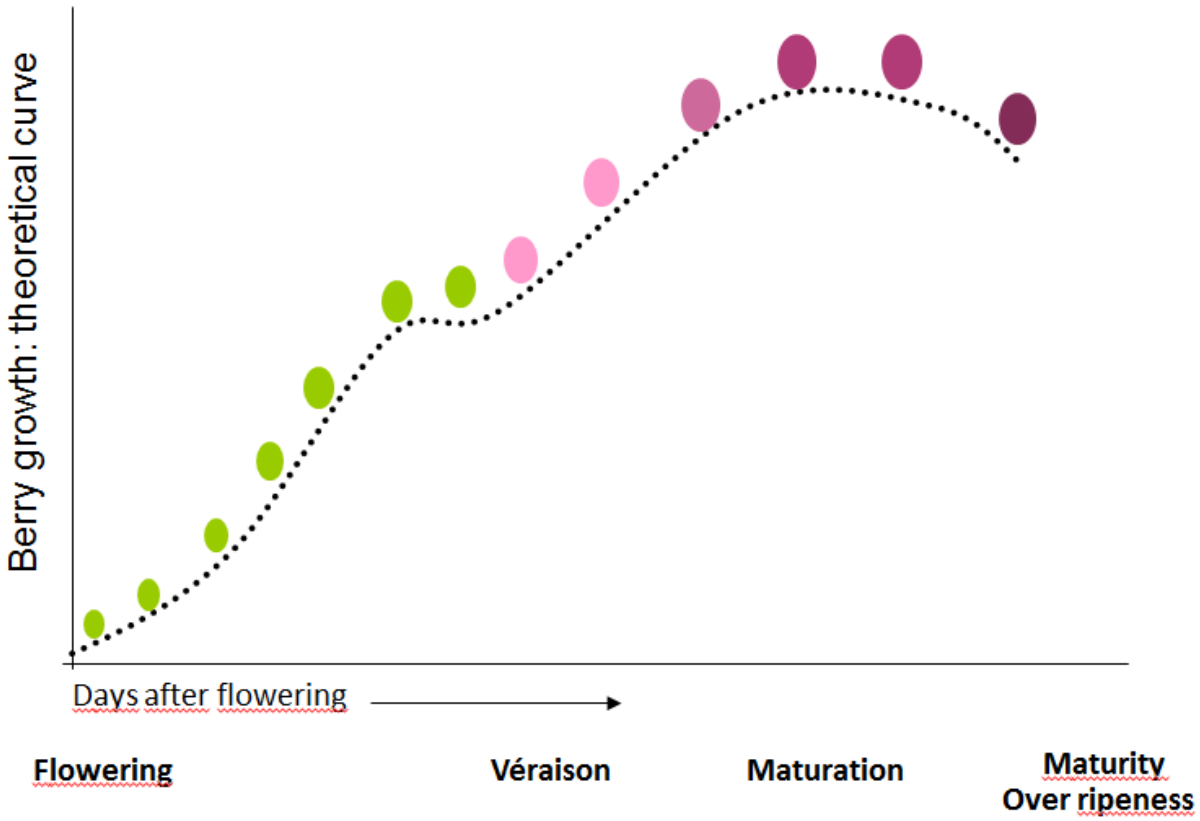

Figure 2.1 Theoretical curve showing the development of grape berry growth (Deloire).

2.2 SOURCES AND SINKS

2.2.1 The relationship between source and sink organs

Source sink relationships can be defined as the ability of a plant to undergo photosynthesis, thereby fixing CO_2 in the source organs, and to transport this fixed carbon to various sink tissues. It also defines the ability of the sink organs to assimilate the fixed carbon structures. The ability of a vine to perform these functions is based on its genotype, the environment in which it is situated (abiotic factors), and the viticultural management practices exercised upon it.

Plant function relies on a source of carbon, and transport and assimilation thereof is vital. The movement of water and solutes occurs via the xylem and phloem tissues. Certain solutes are tissue specific such as calcium which is exclusively xylem mobile, or sugar, which is conducted via the phloem. Source tissues encompass those organs which are capable of exporting solutes. These organs are where compounds are produced or stored and from which these compounds are sent. Sink organs indicate those tissue to which solutes are sent and used for metabolic processes and growth (Iland *et al.*, 2011). There are four major sink tissues included in the morphology of the grapevine, namely the shoot including leaves, petioles and stems, the woody trunk, roots and developing fruit (Vivin *et al.*, 2001). It is the fruit which can be considered as the most important sink from véraison; however, other vine organs do compete with the berries for carbohydrate resources, such as the roots which are the larger user of fixed carbon (up to 75%) (Escalona *et al.*, 2012).

Ollat & Gaudillère (1997) conducted an experiment on Cabernet Sauvignon in order to better understand carbon balances within the vine. They established that all imported carbon is mostly divided equally between the pericarp tissue and seed growth, and respiration between anthesis and véraison. A significant portion (43%) of carbon lost due to respiration is recycled by photosynthesis. During the first growth stage, the berries can be described as utilisation sinks due to the fact that carbon demand for respiration and therefore growth is high. A sharp increase in carbon import occurs at véraison and the berry becomes a storage sink. Carbon is allocated to the pericarp tissue and is stored as hexoses. Respiration demands decrease significantly from véraison, suggesting that the energy requirements for both the carbon import and storage mechanisms are lower than for the metabolic processes which occur before véraison. The berry is capable of accumulating 12 mmoles of carbon during the growth period, with respiration using 18% of imported carbon and photosynthesis restoring 10% of the carbon needed for the development of the grape berries (Ollat & Gaudillère, 1997).

The double sigmoidal growth pattern of berry development provides strong evidence demonstrating the strength of the post véraison berry as a sink organ. During this period the

berry is capable of increasing its dry matter four-fold as opposed to the comparatively small variation in other vine organs (Coombe, 1989). In a situation where leaf area is severely limited, post-véraison berries are able to procure the necessary carbohydrates from the storage organs of the vine. It was shown that when vines were completely defoliated at véraison, the bunches were able to reach 14.4°B at standard ripening dates (Kliewer & Antcliff, 1970). This demonstrated that the woody parts of the vine were able to supply the ripening fruit to a certain extent (Rebucci *et al.*, 2008).

2.2.2 The interaction between source and sink organs

The activities of both sink and source organs seem to be closely co-ordinated in order to achieve balance between the supply and demand of carbohydrates in a number of plant (Wardlaw, 1990; Ho, 1992). For example, it was found that the partial defoliation of a vine led to a lower grape growth and therefore a lower yield (Candolfi-Vasconcelos & Koblet, 1990). Furthermore, it was observed that a reduction in berry number lead to lowered rates of photosynthesis. It is clear that a compensation effect is present in a vine and that there is communication between sink and source organs. In an experimental and modelling trial conducted on Cabernet Sauvignon, Quereix *et al* (2001) suggested the existence of a sink feedback mechanism. The trial was conducted under non-limiting conditions, yet photosynthesis and stomatal conductance were found to decrease continually during the given photoperiod. This suggested the stomatal regulation was mediated at an internal level, possibly by the sink. The model which was created agreed with existence of a phloem feedback signal (Quereix *et al.*, 2001).

2.3 CARBON COSTS OF THE GRAPEVINE

2.3.1 Root energy requirements

It is accurate to state that from véraison onwards the grape berry is a strong sink; however, it should not be assumed that it is the only sink. In order for the roots to survive, a certain percentage of carbohydrates must be allocated to them for various applications. A portion of the allotted carbon is necessary for respiration for the maintenance of the existing biomass. A further fraction will be utilised for growth respiration, thereby allowing for the development of the root system and the replacement of damaged or dead parts. Respiration will also be required for ion uptake; however, this will be dependent on the nutrient requirements of the entire plant. Lastly, carbon may be lost due to leakages, stress conditions and root associations such as with mycorrhizal fungi (Buwalda, 1993). Escalona *et al* (2012) found that under irrigation, i.e., in a

non-stressed environment, the estimated carbon losses due to respiration amounted to 47 g to 65 g per plant. This equated to 30% to 50% of the total estimated gains due to photosynthesis. Furthermore, respiration by the root system represented a percentage of 70 to 80 of the total carbon losses, illustrating the large requirements of this organ. The remaining percentage consisted of both leaf and stem respiration (Escalona *et al.*, 2012).

2.3.2 Leaf energy requirements

The energy required by the leaf in the form of ATP and NAD(P)H can be directly drawn from the light reaction of photosynthesis. This process occurs in the chloroplasts during the day when there is a superfluous production of ATP. This is able to partially supply the necessary energy required for growth, leaf maintenance, protein turnover and phloem loading without using any carbon substrates such as glucose. The excess energy in the chloroplasts can therefore in effect be directly used for respiration, without any need for sugar synthesis. Furthermore, during the night, photosynthetic proteins are not activated, thereby resulting in a lower need for ATP. The carbon consumption may therefore be less than expected (Cannell & Thornley, 2000).

2.3.3 Other energy requirements

Along with the carbon which is essential for root respiration and the possibly low, yet necessary requirements of the leaves, numerous other processed require energy to function. The maintenance and growth (increase in biomass) of the entire plant require a portion of manufactured carbon to be continued. Phloem loading and unloading require a certain amount of energy. Other processes which require energy include nitrogen uptake, uptake of other ions, the preservation of cell ion concentrations and gradients and the maintenance of alternative respiration pathways and futile cycles (Cannell & Thornley, 2000).

2.4 TRANSLOCATION PATTERNS

2.4.1 Budburst to berry set

During this period, carbohydrates are moved from the storage sites in the roots and the permanent woody structures to the growing shoot, providing new leaves and other organs with essential reserves needed for growth. Young leaves utilize these sugars for growth and metabolism until they reach approximately a third of their final size (Hale & Weaver, 1962). At this point they become net exporters. Photosynthates produced in the leaves are initially allocated to the growing shoot tip and then bi-directionally to the base of the shoot. During this

period, the shoot tip is one of the strongest sink organs. The inflorescences are initially weak sinks, but become stronger as fruit set is approached (Iland *et al.*, 2011).

2.4.2 Berry set to véraison

During this time, photosynthates translocated to the shoot tip mainly emanate from the apical leaves. The middle and basal leaves primarily provide the lower older leaves, bunches and trunk with manufactured photosynthates (Illand, Dry *et al.*2011).

2.4.3 Véraison to harvest

From véraison, the majority of photosynthates are translocated to the grape berries, mainly from middle and basal leaves. If shoot growth has not stopped by véraison, the apical leaves will continue to provide the shoot tip with carbohydrates. In the instance that shoot growth has ceased at véraison, the apical leaves will direct their photosynthates to the grape berries. After harvest, all carbohydrates will be conducted to the trunk and roots where they will be stored for utilisation in the following season (Iland *et al.*, 2011).

2.5 THE CONCEPT OF SUGAR LOADING AND BERRY AROMATIC SEQUENCE

2.5.1 Sugar loading

Sugar loading can be defined as the changes in the quantity of sugar per berry, expressed as mg per berry, from véraison onwards. Véraison corresponds with the onset of fruit maturation. In the grapevine, this fruit maturation starts with an abrupt softening of the berry (within 24 hours). This softening goes hand-in-hand with sugars being actively introduced into the berry (sucrose rapidly hydrolysed into hexoses: glucose and fructose). In red and black cultivars, véraison is characterised, after softening, by skin colouring as a result of the biosynthesis of anthocyanins. The accumulation of sugars in grape berries gives an indication of the ripening process from a new perspective and is a novel approach to identifying practical indicators for obtaining particular styles of grapes and wine (Deloire, 2008, 2011). Sugar loading may also provide information on ripening kinetics and enables the identification of the principal phases of ripening (McCarthy & Coombe, 1999). Furthermore, this information provides a greater understanding of how grape quality develops in the vineyard. Phloem sugar transport, principally to the flesh cells, has been characterised in studies on plant-to-berry sugar loading, and phloem sugar unloading, notably by the peripheral vascular system of the berry (Ollat & Gaudillère, 1997;

Wang *et al.*, 2003). Phloem sugar unloading into cell vacuoles occurs mainly via an apoplastic mechanism, which requires the intervention of hexose transporters (Terrier *et al.*, 2005). From the above-mentioned studies, it can be concluded that sugar loading into the berry, coupled with the dynamics of sugar concentration changes, may be considered a useful indicator of grape quality. It takes into account the changes in the sugar level per berry (mg per berry) and therefore enables the kinetics of sugar concentration changes to be monitored. Kinetic monitoring of the quantity of sugar per berry may be considered as a method of measuring the plant's physiological functioning (Ojeda *et al.*, 2001; Wang *et al.*, 2003). Active sugar loading is calculated on the basis of berry volume (or berry fresh mass) and sugar concentration (McCarthy & Coombe, 1999).

2.5.2 Profiles of sugar loading

It is possible to distinguish three principal sugar loading profiles (Figure 2.2):

1. Continual and rapid loading

This type of sugar loading occurs from véraison and is related to the active functioning of carbon production sources (leaves) which supply plant sinks (berries, secondary shoots etc.) during their growth phases. It is therefore often associated with significant vegetative growth and greater berry volume. Phenolic maturity is not affected. This type of loading is often considered beneficial for the production of rosé, fresh fruit red wines, or pleasant aromatic white wines.

2. Slow sugar loading – inhibition of ripening

Low sugar content per berry, associated with a slow loading rate, can be considered to "block" ripening and this could be indicative of an imbalance in the vine. If major physiological problems, such as mineral deficiencies, viral diseases etc., are excluded, blocked ripening can often be related to excessive water deficit, or to an excessive crop load in relation to the exposed leaf surface (Ojeda *et al.*, 2001).

3. Sugar loading presenting a plateau phase

Vines showing this tendency present a phase of active sugar loading in the berry (ripening), followed by a plateau representing a cessation of sugar loading (or a slowing-down of sugar accumulation) and corresponding to maturity (Deloire *et al.*, 2008).

In some cases, there is a fourth phase corresponding to a possible decrease of the quantity of sugar per berry (over ripening). To date a probable explanation for the occurrence of this phase has not been confirmed. It might occur due to the consumption of sugars by microflora after

exudation onto the berry skin late in the ripening phase. A further hypothesis is the possibility of sugar backflow. Further research will however be needed to confirm all hypotheses.

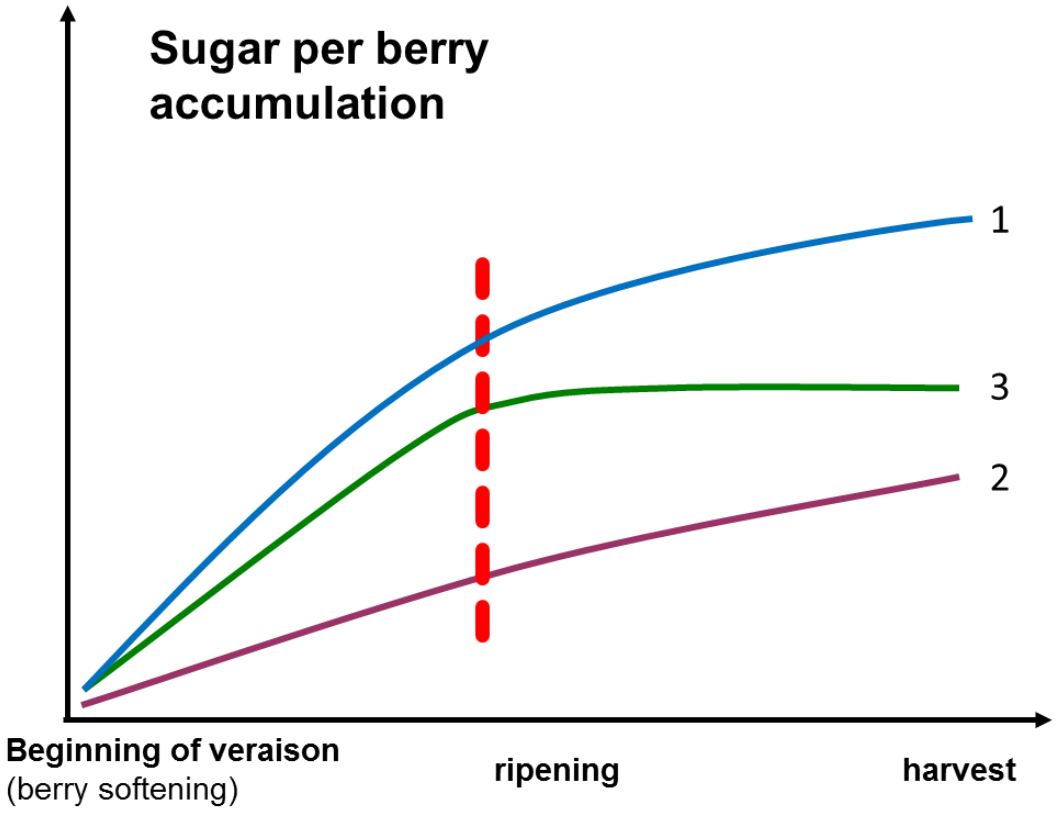

Figure 2.2 Theoretical sugar loading curves for the ripening season. Curve 1 represents continual sugar accumulation; curve 2 depicts a slow sugar loading and curve 3 shows sugar loading which reaches a plateau**.**

A theoretical berry sugar loading curve (evolution of berry sugar content over time) is presented in figure 2.3. This curve is based on data obtained over five years using at least 20 different grape varieties in mainly France, Spain, Argentina, Chile, and in South Africa. The implications of this curve in terms of defining the finished wine are important: depending on whether grapes are harvested in the early, mid or later stages of the plateau phase, the wine will be characterized by fresh fruit, neutral-spicy (or pre ripe) or mature fruit flavours, respectively (Figure 2.4).

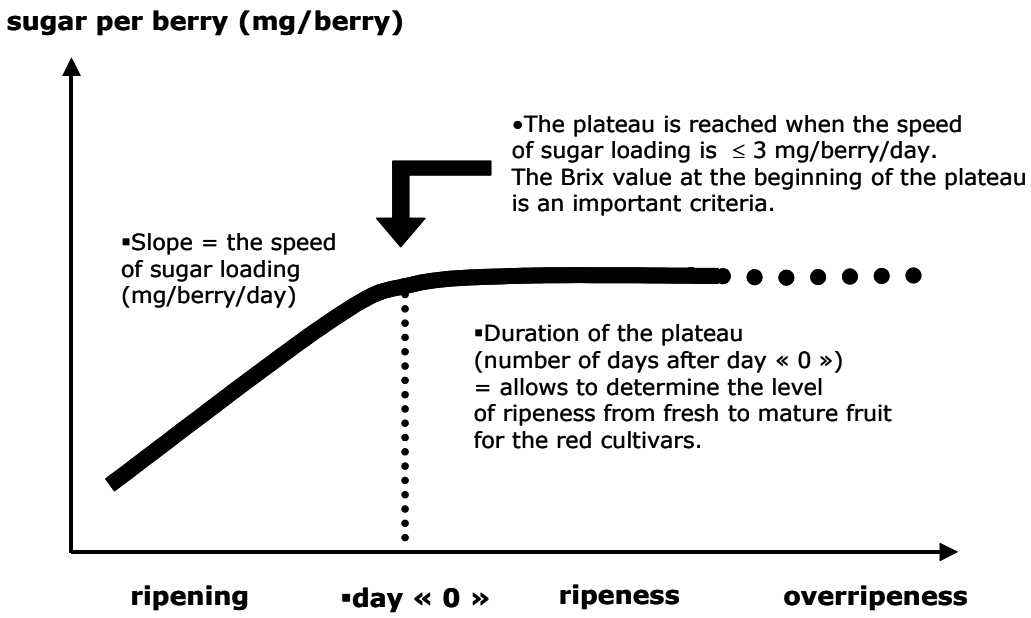

Figure 2.3 Berry sugar loading concept. The theoretical curve of sugar loading established over five years using at least 20 grapevine varieties from various regions. (Deloire 2011)

Photosynthesis does not cease or slow down once sugar accumulation has slowed down as the leaves are still able to function. It therefore stands to reason that the carbon outputs from photosynthesising leaves are translocated to the old wood and roots before harvest. These carbohydrates may be used for respiration, particularly of the root system, or for storage

2.5.3 Berry aromatic sequence

The curve demonstrates that selecting a harvesting date according to the quantity of sugar per berry in conjunction with other indicators (titratable acidity, malic and tartaric acids, pH, berry volume, berry tasting, tannins, anthocyanins, etc.) enables different styles of wine to be produced. Hence, for a balanced red wine, complete ripeness will be achieved between one and five weeks after the cessation of sugar loading, depending on the cultivar.

Once the plateau phase of berry sugar loading has been reached, ripening will depend on other factors such as cultivar, bunch microclimate, the leaf/fruit balance, the vine water status, and the climate mainly during berry ripening (maximum temperature, night-time coolness, sea-breeze, wind-speed, late season rains, and various factors which are quantifiable) (Wang *et al.*, 2003).

It should be noted that the plateau phase in sugar loading may be reached at different sugar concentrations (brix), depending on the cultivar and environmental conditions. A red cultivar, with a very high sugar concentration (brix) when the maturity plateau is reached, will not always be desirable for the production of certain types and/or wine styles. (McCarthy & Coombe, 1999).

Monitoring ripening with various indicators, coupled with appropriate analytical data measurements such as berry fresh mass or volume, brix, sugar loading, titratable acidity, malic acid tartaric acid, pH, colour development, anthocyanins, tannins, berry tasting, etc. will enable decision-makers to determine the optimum harvesting date, a major consideration in determining grape quality. Such monitoring provides a greater understanding of vine morphological and physiological parameters during ripening and therefore vineyard practices can be adapted to production objectives (yield/vine and berry quality/composition in relation with wine style). There are, in most vineyards, several potential optimal harvesting dates and optimal ripening levels according to the desired style of wine. The wine can therefore be said to be created in the vineyard (Figure 2.4).

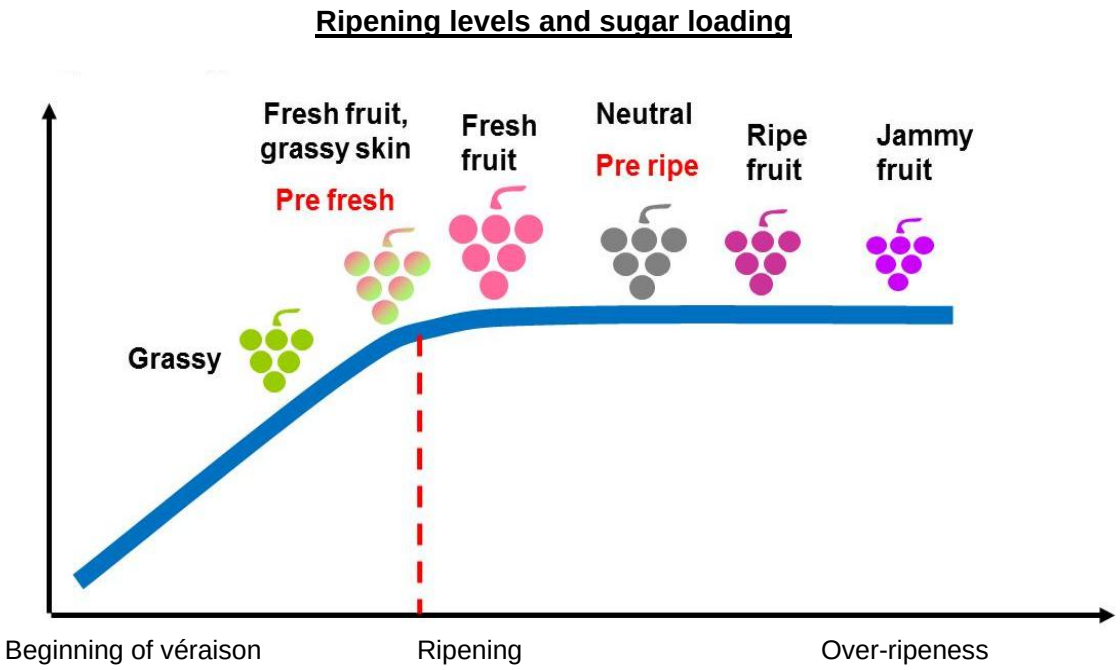

Figure 2.4 Berry ripening according to a physiological clock and the style of wine. (Vivelys, Deloire 2008)

2.6 CELL WALLS OF THE GRAPEVINE

The cells of a plant exhibit a polysaccharide rich wall which serves to enclose the cell while allowing for the transfer of certain signalling molecules and solutes between adjacent cells. This occurs via certain structures situated within the cell wall itself and includes both plasmodesmata and pore structures. Furthermore, the cell wall is responsible for maintaining the general plant form as well as for providing strength and stability to the plant structure. It serves a key role in the growth and development of plant tissue. The walls are composed mainly of an intricate arrangement of polysaccharides and include a small percentage of protein molecules. These

components are strongly connected with each other, either through covalent or non-covalent bonding. The cell walls can be described as both diverse and complex with the ability to alter during various stages of development including cell division, enlargement and differentiation. (Doblin *et al.*, 2010).

Three layers are typically identifiable in the structure of cell walls, namely, the intercellular space or middle lamella, the primary wall and the secondary wall (Figure 2.5). The intercellular substance is found between two adjoining primary cell walls, with the secondary wall laid over the primary wall, bordering the cell lumen. The primary cell wall is the first to be formed during cell development and in some cell types, is the only cell wall which is established. These cell walls are typically associated with living protoplasts and any alterations made to them are therefore reversible. Secondary cell walls are laid down after the formation of primary cell walls. They are composed mainly of cellulose, or a combination of cellulose and hemicellulose. Modifications to the cell wall through the deposition of lignin or other substances may occur. These cell walls can be very complex and typically display a lack of homogeneity. Tracheary cells and fibres typically display a three-layered secondary cell wall, each of which is chemically and physically distinct from the other. However, the number of layers can differ depending on the cell type. Secondary cell walls can be considered to be a supplementary structure which predominantly serves a mechanical function. They are commonly devoid of protoplasts at maturity and any changes which occur during development are mostly irreversible. The middle lamella is amorphous and is mainly comprised of a pectic substance. In woody structures, it is commonly impregnated with lignin, further aiding in the mechanical support of the plant (Esau, 1953).

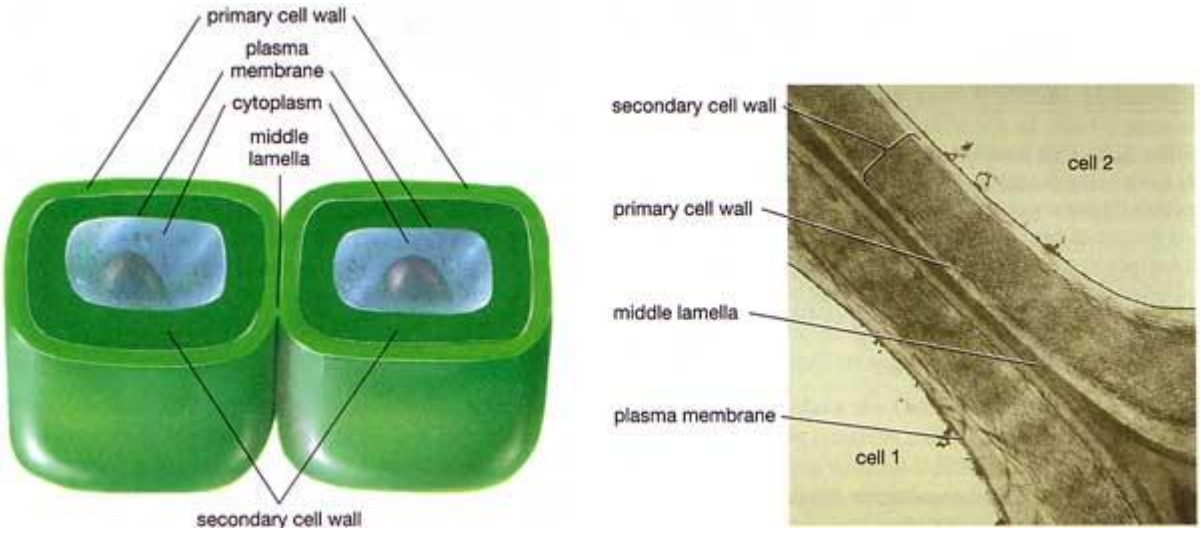

Figure 2.5 Plant cell depicting the primary and secondary cell wall, middle lamella and plasma membrane relative to the cytoplasm (Audesirk & Audesirk, 1999).

The ripening stage of grape berry development is signified by the onset of véraison. This is characterised by berry softening, indicating a change in the cell wall structure. It has been observed that during fruit softening more subtle modifications to the structure of integral polysaccharides are likely to occur as opposed to large alterations (Brady, 1987). The solubility, molecular mass, substitution and branching within a polysaccharide may change without greatly altering the amount of that polysaccharide. Non-covalent alterations may be incurred in the cell wall by changes in the pH or ion concentration (Carpita & Gibeaut, 1993). Enzymatic processes are largely responsible for covalent changes which occur in cell wall polysaccharides. During fruit softening, components within the polysaccharide cell wall are broken down or modified. In conjunction with this, newly synthesised constituents are incorporated into the cell wall (Gibeaut & Carpita, 1994). The synthesis of these components is thought to be an on-going process for the entire duration of the ripening stage. Any change in the turnover rate of a particular compound may lead to modifications within the cell wall structure. It is expected that these processes also occur in ripening grape berries, however, the knowledge of the cell wall composition as well as the mechanisms involved in berry softening are deficient. Certain features have however been reported, including the composition of monosaccharides and the structure of particular pectic polysaccharide fractions. Changes in the solubility of pectins have also been monitored during berry ripening, however in depth analysis has not been conducted on the cell walls of a berry during the ripening process (Nunan *et al.*, 1998).

2.7 PLANT TISSUES

2.7.1 Xylem tissue

2.7.1.1 Structure

Most plants, including grapevines, exhibit a primary cell wall. The cells wall of xylem is composed of an arrangement of cellulose fibrils. This allows for stretching and expansion of the cell wall during plant growth. The secondary cell wall in laid down on the inside of the primary cell wall during and after elongation and expansion of the plant cells. The cellulose fibrils of this wall are arranged in an ordered manner with the alternating layers being formed at fixed angles to the main axis of the cell. This structural arrangement of the secondary cell wall ensures the rigidity of the cell while maintaining the flexibility of the primary cell wall (Myburg & Sederoff, 2007).

Xylem consists of both parenchyma cells and sclerenchyma cells. The parenchyma cells serve a storage function and are able to store water, mineral nutrients and carbohydrates. In woody structures, these cells usually have a lignified secondary cell wall. In other plant tissues, a thin primary wall is present in which areas of plasmodesmata can be found. These areas allow for cell to cell transport of certain substance including water and nutrients.

The sclerenchyma cells are responsible for the mechanical support, defence and transportation of substances including water. The conducting cells or tracheary elements include xylem tracheids and vessels. The vessel elements are connected in such a manner so as to form a tube-like structure. The end walls of vessels are perforated to allow movement of substances from one vessel to the next. The tracheids are connected via circular bordered pits located primarily in the tapered ends of the cells. Mature tracheary elements contain no cellular content and are principally comprised of thickened secondary walls. These cells are therefore considered to be dead cells (Myburg & Sederoff, 2007).

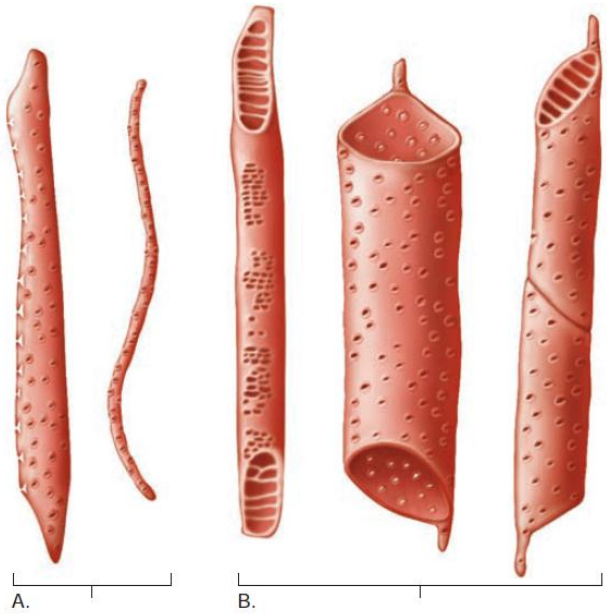

Figure 2.6 The structure of xylem tracheids (A) and xylem vessels (B) (Stern *et al.*, 2008).

2.7.1.2 Forces of cohesion and adhesion

The movement of water upwards in a plant is dependant of two forces, namely cohesive and adhesive forces. Cohesion involves the attraction of water molecules to each other through weak hydrogen bonds. This ensures that a continuous water stream is maintained allowing for the upward movement of water. Adhesion involves the attraction of water molecules to the cell walls of xylem tissue (Iland *et al.*, 2011). The movement of water within tracheary elements will cause tension in the water column. An increased transpiration rate may lead to very high negative pressures within the tissues which could cause the cells to collapse inwards. The

secondary thickening of xylem elements provides rigidity and strength to ensure that the structure of these tissues is maintained, even under extreme force (Myburg & Sederoff, 2007).

2.7.1.3 Cavitation and embolisms

Within the xylem tracheids and vessels, water is present under tension due to the forces of cohesion. This stable state can be interrupted by the formation of a small air bubble which may cause the tension within the xylem tissue to collapse and form a vacuum. This phenomenon is known as cavitation. Once this has occurred, an increase in pressure due to the admission of water vapour and air into the cavitated area will lead to the formation of an embolism (Iland *et al.*, 2011). This phenomenon is not of great concern in the tracheids which possess a small diameter; however it may be a problem in the xylem vessels. In these tissues, the embolisms may spread from one element to the other through the pitted end walls, effectively rendering water transport dysfunctional (Myburg & Sederoff, 2007).

Cavitation and the consequent embolisms are typically caused by freezing and thawing of plant tissue or by pathogens capable of moving into the xylem. It may also occur under conditions of water stress due to the sucking up of air into the vessels through the pit membrane (Iland *et al.*, 2011).

2.7.2 Phloem tissue

2.7.2.1 Phloem development

Protophloem sieve elements are the first vascular bundle cells which develop and become functional in young plant organs. Differentiation of these elements occurs within 1 mm of the apical meristem, demonstrating that assimilates unloaded from the phloem tissue nourish cell division and growth. From the onset of plant development, the differentiation of phloem tissues occurs in step with the growth of the stem and organs. The continuation of the phloem to the farthest ends is ensured by the orientation of the procambial strands which guide the development of the phloem tissue. Radial growth of the phloem involves the addition of parallel sieve element strands, which consequently increases the cross-sectional area of conducting tissue.

During primary development of the shoot organs, including shoot axis, petioles and the main leaf axis, the phloem is typically laid down parallel to the xylem tissue. These two conducting tissues are organised into vascular bundles which occur in varying numbers within the plant organs. In roots, the xylem and phloem are combined in a singular central cylindrical stele.

During secondary growth, phloem elements are continuously added to the outside, whereas xylem tissue is added to the inside (Schulz & Thompson, 2007).

Phloem tissue is responsible for the transport of photoassimilates to various heterotrophic sinks. It furthermore contributes to the water balance of the plant. As opposed to xylem transport which is driven by water potential gradients, the transport of phloem sap is powered by hydrostatic pressure which is initiated by the pumping of solutes into the phloem tissue.

2.7.2.2 Sieve areas

Phloem tissue consists of sieve elements, various parenchyma tissues, fibres and sclereids. The sieve elements are responsible for conduction and may be separated into two distinct tissue types, namely, the less specialised sieve cells and the more specialised sieve tube elements. In both classifications, the elements are distinguishable by the characteristics of the wall structures. These include the sieve areas and sieve plates. The sieve areas are wall areas pitted with numerous pores through which adjacent sieve elements are connected via strand-like extensions of their protoplasts. These pores occur in various sizes. Sieve areas with bigger pores most often occur on the end walls of sieve tubes, thereby forming the sieve plates. Simple sieve plates are characterised by a single sieve area, whereas compound plates display multiple sieve areas arranged in various manners (Esau, 1953).

2.7.2.3 Sieve cells and sieve tube members

Sieve cells are elements with relatively unspecialised sieve areas. They display a lack of differentiation and consequently have no wall parts which can specifically be identified as sieve plates. These cells are typically long, thin and taper towards the ends, or have steeply inclined end walls. They tend to overlap each other at the ends and exhibit numerous sieve areas in these regions. Sieve tube elements have more specialised sieve areas which are localised as sieve plates, mainly on the end walls. These elements are typically connected end to end to form a long series with the sieve plates serving as the common wall parts. These structures are known as sieve tubes (Esau, 1953).

2.7.2.4 Companion cells

Closely associated with the sieve tube elements are specialised parenchyma cells known as companion cells. These cells are formed from the same meristematic cell as the associated sieve-tube member and are therefore ontogenetically closely related (Esau, 1953). These cells are thought to be involved in certain metabolic processes necessary to maintain the sieve tube member (Raven *et al.*, 2005).

2.7.2.5 Supportive cells

Phloem tissue also includes supportive cells which can generally be classified into two groups; namely fibres and sclereids. Both cell types exhibit a secondary cell wall making them exceptionally rigid and impervious to damage. Fibres typically have a long thin shape and are grouped together in strands. They provide support and rigidity without adversely affecting flexibility. Sclereids are variably shaped cells which contribute to compression strength but lessen flexibility to a certain degree. (Evert & Eichhorn, 2004). In combination, these tissues provide support and structure while maintaining a degree of flexibility in the phloem tissue.

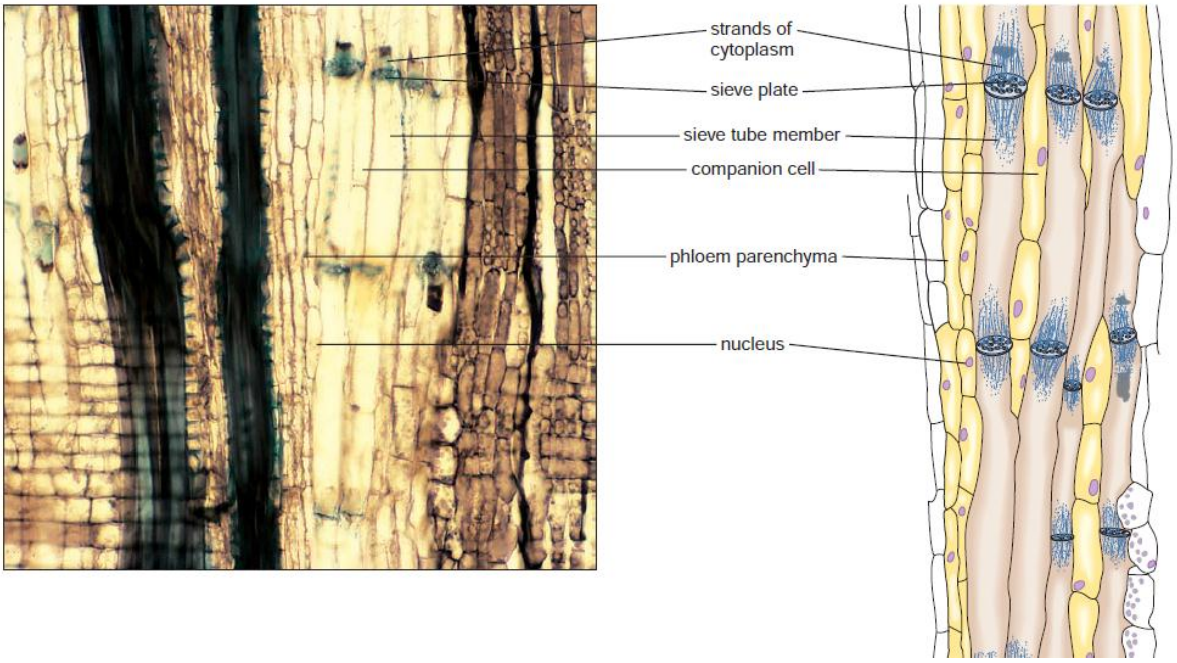

Figure 2.7 A longitudinal view of part of the phloem tissue (Stern *et al.*, 2008).

2.8 GRAPEVINE ORGANS

2.8.1 Root anatomy

The root is comprised of a number of anatomically and functionally distinct regions which exist in relation to the root tip as they are transitional phases to maturity. The root tip includes the apical meristem and in protected by the root cap. The cells of this cap are continuously sloughed off and replaced via cell division as the root moves through the soil. Behind this region is the zone of elongation, followed by the zone of maturation. Numerous root hairs are found in the region which greatly increases the absorptive surface area of the root.

The root is made up of various tissues. The first layers include the epidermis, exodermis and cortex. The inner boundary to these layers is the endodermis. The primary cell walls of the endodermis are impregnated with suberin, a fatty substance which is impermeable to water. The suberin is laid down in bands which surround each endodermal cell wall perpendicular to root surface. These bands are known as Casparian strips and serve to block transport to certain extent, thereby offering a degree of selectivity and control. All tissue surrounded by the endodermis are collectively known as the stele. The vascular bundles comprising the xylem and phloem are found in this region (Stern *et al.,* 2008; Iland *et al.,* 2011).

2.8.2 Leaf anatomy and stomata

2.8.2.1 Anatomy

The internal leaf structure typically consists of three main regions including the epidermis, mesophyll and vascular bundles. The epidermis is made up of a single layer of cells which cover the leaf surface. Stomata will be found in this epidermal cell layer. The leaves very often also exhibit a waxy coating known as the cuticle, which is responsible for the prevention of excessive water loss and in many cases, for protection. Photosynthesis occurs in the mesophyll cells as these cells house numerous chloroplasts. Mesophyll can be classified into two different layers, the palisade mesophyll and the spongy mesophyll. The vascular bundles or veins contain the xylem and phloem tissues and are spread throughout the mesophyll. These vascular bundles are encased by protective, thick-walled parenchyma cells which make up the bundle sheath. Xylem and phloem are responsible for supplying the leaves with water and needed carbohydrates, as well as for moving manufactured photosynthates from the leaves to the rest of the plant.

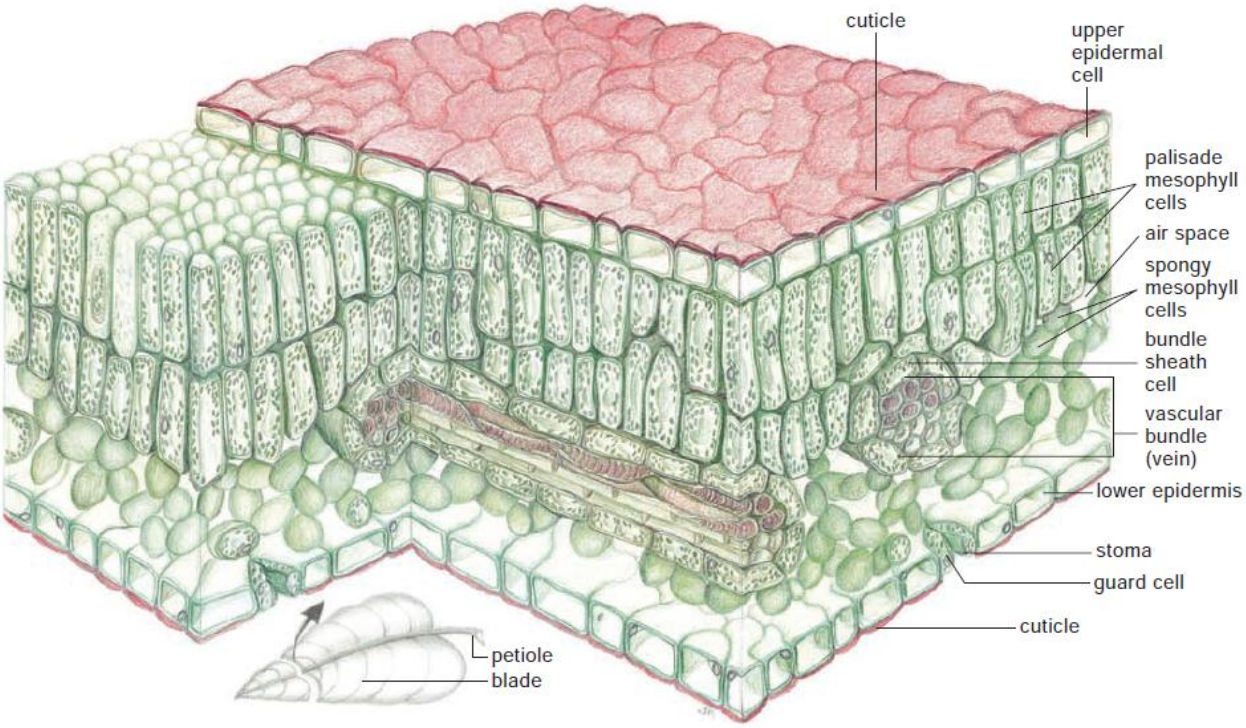

Figure 2.8 A stereoscopic view of a portion of a typical leaf (Stern *et al.*, 2008).

2.8.2.2 Stomata

The stomata can be defined as pores found in the epidermal layer of the leaves through which transpiration and gas exchanges occurs. The stomata are bordered by two bean-shaped cells known as guard cells (Figure 2.9). These structures are responsible for the regulation of the aperture of the stomata and therefore control both gas exchange between the interior and exterior of the plant, as well as the evaporation of water from the leaves. They are therefore directly involved in photosynthesis. Stomatal conductance will be determined by stomatal size and density (Franks & Beerling, 2009). This in combination with mesophyll conductance of CO_2 will exercise a large influence on the photosynthetic capacity (Flexas *et al.*, 2007). The formation of the stomata will occur during the development of the leaves. Stomatal density will depend on both the genotype of the plant, (Nadeau & Sack, 2002) as well as the influence of certain environmental parameters including CO_2 concentration, soil temperature, light intensity, air temperature and photoperiod. (Woodward & Kelly, 1995; Rogiers *et al.*, 2011). Regulation of stomatal conductance will occur after leaf size had been set. Factors such as humidity, water constraints and CO_2 concentration in combination with plant genotype will control the degree of stomatal regulation.

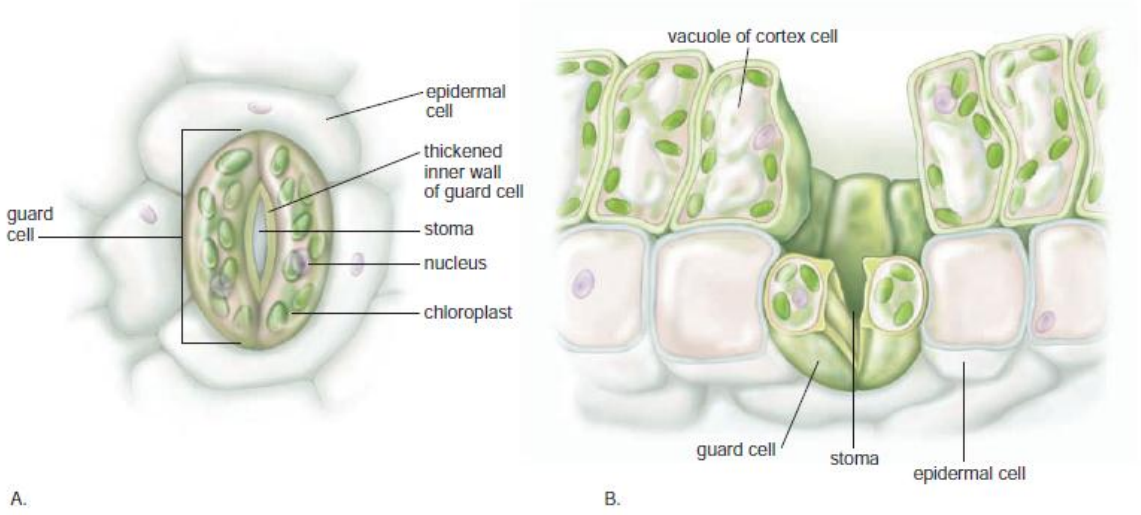

Figure 2.9 The stomata of a leaf: Surface view (A). Transverse view (B) (Stern *et al.*, 2008)

2.8.3 Berry anatomy

The grape berry is comprised of the skin, flesh and seeds. The pericarp, which develops from the ovary wall, encompasses both the exocarp, or skin and the mesocarp, or flesh. The skin is made up of the cuticle, the outer epidermis and the inner hypodermis (Figure 2.10).

The pedicel of a developing flower contains five to six vascular bundles which diverge in the receptacle to serve both the ovary and flower parts individually. The bundles present in the ovary develop into a complex vascular system consisting of three components within the berry. The vascular bundles which supply the seeds and the placenta respectively make up two of the components. These tissues and their associated parenchyma tissue constitute what is colloquially known as “the brush”. The third component of the vascular system includes the vastly branched peripheral vascular bundles which are located where the epicarp and mesocarp join (Mullins *et al.*, 1992).

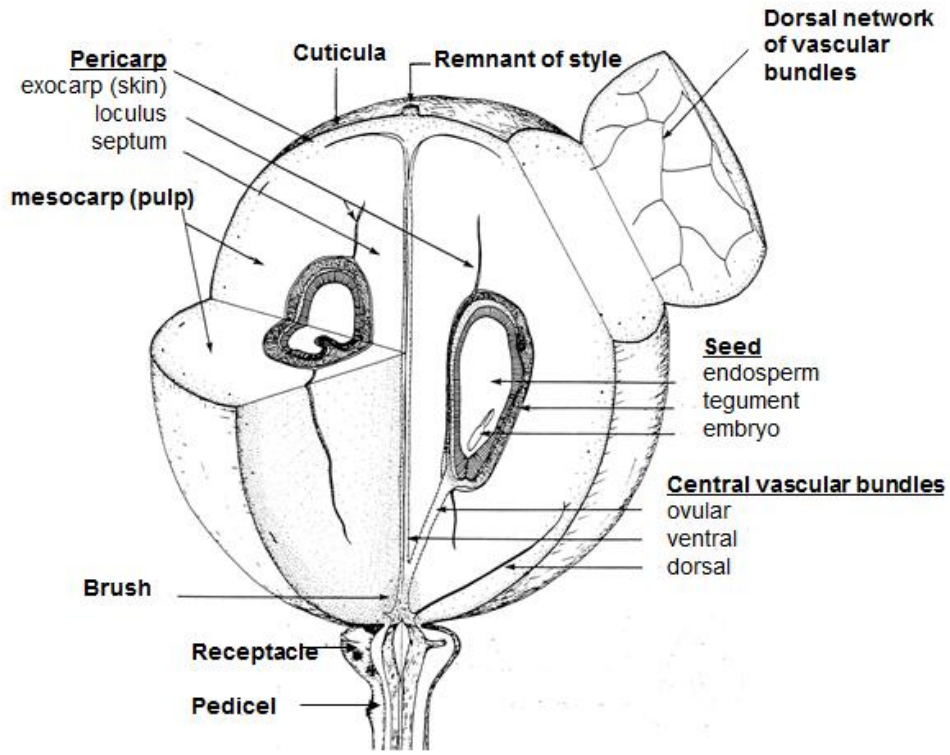

Figure 2.10 The anatomy of a grape berry (Coombe 1987).

2.9 MECHANISMS OF WATER TRANSPORT

2.9.1 Apoplastic and symplastic pathways

The term apoplast refers to the free diffusional space outside the plasma membrane. In terms of its structure, the apoplast is formed by the continuum of cells walls of contiguous cells including their intercellular spaces. This results in the establishment of a tissue level compartment which is analogous to the symplast. The apoplastic route facilitates the movement of water and solutes across a tissue or organ. This process is referred to as apoplastic transport (Figure 2.11).

The symplast refers to the living protoplast within a plant body where water and certain solutes may freely diffuse. Adjacent cells are joined via plasmodesmata or sieve pores. These structures allow for the direct flow of small molecules, including sugars, amino acids and ion, between cells. This in turn facilitates the uninterrupted cytoplasm to cytoplasm flow of water and other nutrients along their concentration gradients (Figure 2.11) (Campbell & Reece, 2002).

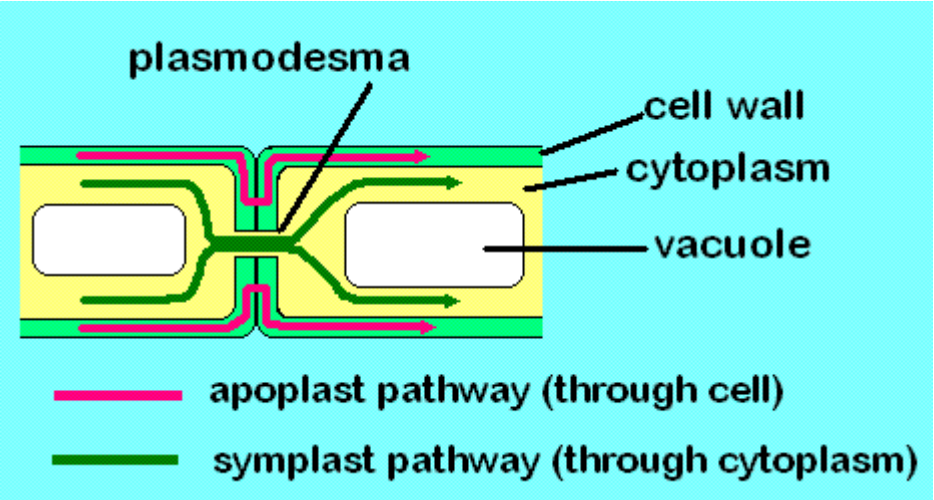

Figure 2.11 Symplastic and apoplastic pathways through adjacent cells.

2.9.2 Water potential

Within plant systems, the total water potential encompasses four components, namely; osmotic potential, hydrostatic potential, matrix potential and gravitational potential. The two most influential water potential constituents present in the grapevine include osmotic potential and hydrostatic potential. The composition of cells dictates the osmotic pressure present as most living cells contain high levels of dissolved solutes resulting in a higher negative osmotic potential. The dead cells of the xylem tissue have a lower negative osmotic potential when compared to living cells. Furthermore, the hydrostatic potential of living cells will be positive, whereas the dead xylem tissue cells will have a negative hydrostatic potential. The combination of the two potentials at any point will determine the overall water potential and therefore the direction of water flow.

Water typically moves from a point of high water potential to a point of low water potential without the input of energy. In order for water to move through the vine from the roots, up the trunk and eventually through the leaves and fruit, a potential gradient is required. The continuous flow of water through the vine is known as the transpiration stream. The driving force behind this mechanism is the existence of a very low water potential in the air surrounding the plant. Simplistically put, water will move from a high water potential in the roots to a low water potential in the leaves and fruit from which transpiration will occur through stomata and across the cuticle (Iland *et al.*, 2011) The negative pressure created by transpiration can therefore be seen as the main driving force for the upward movement of water in the xylem tissue. During the night when transpiration is low or inactive, the formation of the necessary negative pressure component is low or absent. However, ions are still actively pumped into the root tissue. This leads to a higher concentration of ions within the root hair cells, which consequently leads to the

uptake of water due to osmosis. Water movement into the xylem and throughout the plant therefore continues despite the lack of transpiration. This process is known as root pressure (Raven *et al.*, 2005).

2.9.3 Influence of the grapevine rootstock

The majority of vines planted in all viticultural regions are grated onto rootstocks, most of which were developed before 1930 from American *Vitis* species which displayed resistance to phylloxera (Granett *et al.*, 2001). Rootstocks are chosen for several reasons other than their resistance to phylloxera. These may include their affinity for grafting, rooting and propagation, as well as their tolerance to salinity, lime, high soil water contents and drought. As an example, Candolfi-Vasconcelas *et al* (1994) showed that vines grafted onto 101-14 Mgt had higher levels of CO^2 assimilation, higher transpiration rates, and a better water use efficiency than plants grafted onto 3309C. It is clear that the rootstock will influence vine growth and functioning and it should not be overlooked.

2.9.4 Water and sugar flows within the vine

As with most fleshy fruits, the water and carbon flows into and out of the berries are essential for volumetric growth and accumulation of primary compounds which determine the final fruit composition and quality (Conde *et al*., 2007; Coombe & McCarthy, 2000).These flows vary with fruit developmental stage (green growth stage versus ripening) and abiotic factors.

During the first growth period, carbon is imported at a rate equal to one-third of that required during the second growth period (Ollat and Gaudillere, 1996. This is in part due to a shift at véraison from the symplastic (plasmodesmata) to the apoplastic (cell wall) pathway of phloem sugar unloading. This allows high levels of solute hexoses to accumulate (Zhang *et al.*, 2006). Concurrently, the water budget of the berry shifts from a combination of xylem and phloem water supply to predominantly phloem (Greenspan *et al.*, 1994; Bondada *et al.*, 2005). This alteration is associated with an apparent uncoupling of fruit water status from plant water status and could possibly be involved in the ability of grapes to continue to accumulate large amounts of solutes under limited soil water availability (Wang *et al*., 2003; Keller *et al*., 2006). Abiotic factors (temperature and water) and source-sink manipulations at the vine level (leaf or lateral shoot removal) are important in the control of water and solute transport and accumulation in the berry. The berry water budget incorporates berry water input, berry transpiration or water loss through the cuticle, and xylem back flow (Lang & Thorpe, 1989; Keller *et al.*, 2006).

2.9.5 Water and mineral absorption from the soil

Water moves radially from the soil and through the roots. Various tissue layers are crossed during water movement. These include the epidermis, exodermis, cortex, endodermis and stele parenchyma before water moves into the xylem tissue. This may occur via an apolastic cell wall pathway, symplastically through the cells cytoplasm via plasmodesmata or across cell membranes via the transcellular flow path (Figure 2.12). The regulation of water movement via the transcellular pathway may be regulated by aquaporins. The combination of the symplastic pathway and the transcellular flow path is known as the cell-to-cell pathway (Tyerman *et al*., 1999). Water movements through the apoplast are ascribed only to hydrostatic gradients, whereas water flow via a membrane-delimited pathway occurs due to both hydrostatic and osmotic gradients. When the plant is actively transpiring, the tensions which develop in the xylem result in water movement being dominantly driven by a hydrostatic gradient. Both the apoplastic pathway and the cell-to-cell pathway are therefore involved in the flow of water through the tissue with the proportion being dictated by the relative hydraulic conductance of the two pathways. When the transpiration rate is low, such as during the night or in times of water stress, the osmotic flow may be the primary means of water movement. This may be explained by the fact that the ions in the stele are not diluted without great hydrostatic-driven water flows, thereby creating an osmotic gradient. The cultivar, in combination with the rootstock will however dictate to a large degree which pathway is used for water transport (Lovisolo *et al.*, 2008).

Minerals are actively pumped into the root since the concentration in the soil water is lower than in the root tissue. An expenditure of energy is therefore used for mineral ions to accumulate in the root. The energy required is supplied by ATP. Numerous protein transport channels are situated in the plasma membranes of the root hairs cells. Proton pumps transport specific ions through these channels against a concentration gradient. These ions may move to the xylem apoplastically; however the symplastic pathway is more often utilised. Once in the xylem they are transported throughout the plant (Raven *et al.*, 2005).

The water flow pathway and mineral uptake is dictated by the anatomy of the root. The endodermis, which is located between the root cortex and vascular cylinder, acts as a barrier to water flow, thereby helping to develop hydrostatic pressure in the vascular tissue by not allowing the leakage of solutes back into the cortex (Esau, 1953). This is possible due to deposits of lignin and suberin in the Casparian strip of the endodermis. Furthermore, this barrier ensures that certain ions such as Na^+ and Cl^- are prevented from moving unhindered into the xylem. A definite degree of control is therefore afforded by the presence of the Casparian strip (Iland *et al.*, 2011).

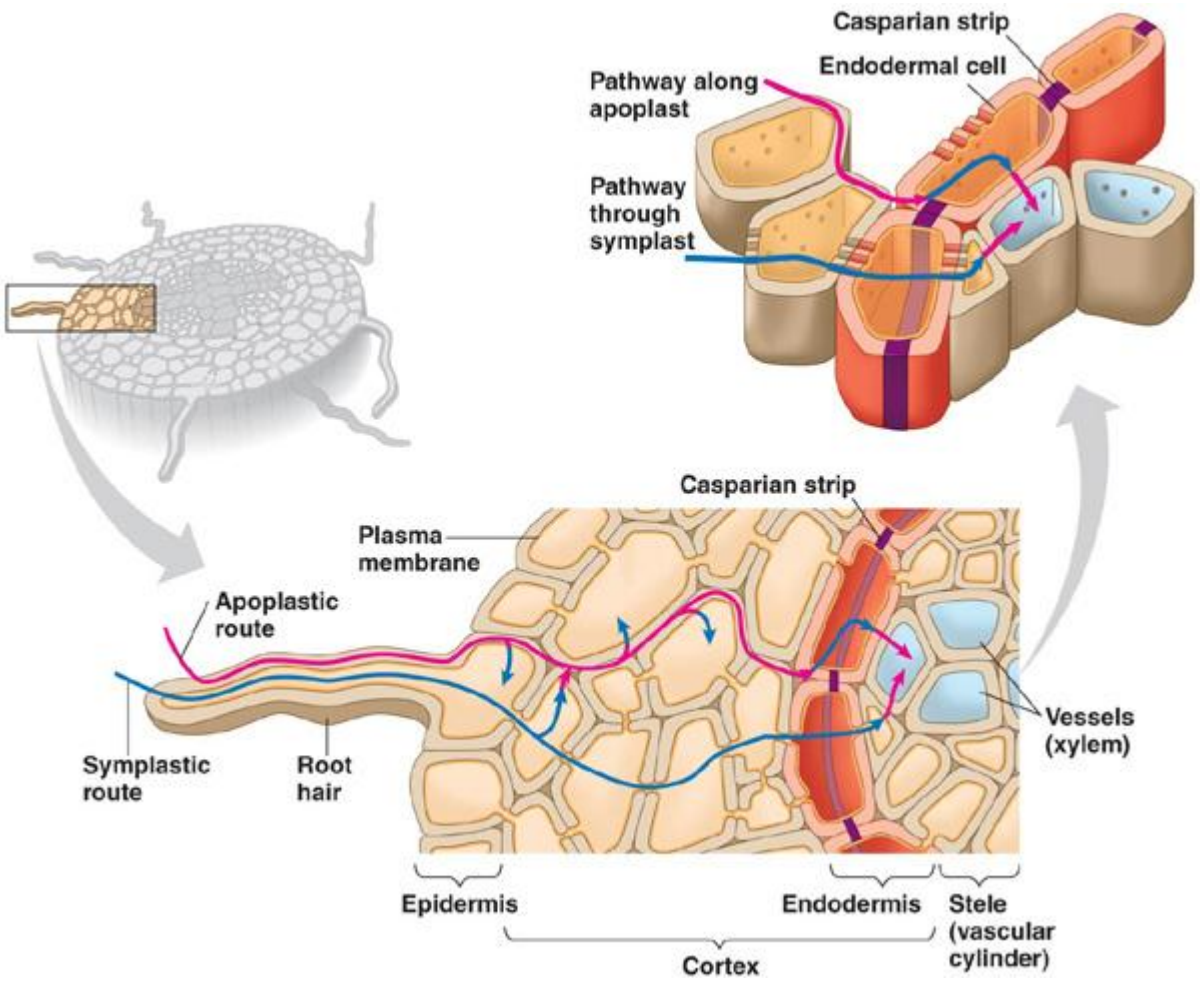

Figure 2.12 The flow of water through the root tissues (Pearson Education).

2.9.6 Water influx regulation and aquaporins

Aquaporins are water permeable protein channels which are embedded in the cellular membranes. They are responsible for the regulation of water movement across these membranes and consequently control the rate of water flow through the grapevine (Tyerman *et al.*, 2009). Aquaporins present in the vacuole tonoplast are probably responsible for the osmoregulation of the cytoplasm, which is capable of rapidly losing or taking up water (Daniels & Chrispeels, 2007).

Aquaporins form part of the major intrinsic protein (MIPs) group of protein channels. Grapevine aquaporins are not characterised as well as their counterparts in other species, however, their structure and function seems to be conserved across species. Plant aquaporins can be divided into four groups depending on their sequence homology. Plasma membrane intrinsic proteins (PIPs) and tonoplast intrinsic proteins (TIPs) are two of the groups and are named for their localisation in a specific membrane. The other two groups include NOD26-like intrinsic proteins

(NIPs) and small basic intrinsic proteins (SIPs). The PIPs group can be subdivided into two groups, namely PIP1 which show low or no water permeability and PIP2 which displays high water permeability. The SIPs group is the smallest and is localised in the endoplasmic reticulum membrane.

Regulation of aquaporin activity was initially thought to be due only to expression; however it has since been found that certain aquaporins may be directly regulated. In several cases, protein phosphorylation leads to an increase in water permeability and vice versa (Daniels & Chrispeels, 2007). Additionally, interactions between different aquaporins may also result in regulation in aquaporin activity. Furthermore, it has been shown that aquaporins may be up or down regulated in response to various environmental factors including water stress, nutrient depletion, salinity, hormones, low temperature and light and anoxia (Tyerman *et al.*, 2009).

2.9.7 Water movement through the vine

Water moves from the soil into the root and makes its way to the xylem tissue. Through a combination of various factors, the water rises in the plant after which a portion of it is lost through the stomata. Transpiration provides the pulling force which facilitates the upward movement of water through the plant. The evaporation of water from the leaves creates tension which exists in the entire water column of water, from the leaves to the roots. The flow of water is possible due to the forces of cohesion and adhesion which ensure that a stable, uninterrupted column of water is present in the xylem tissue. A pushing force due to root pressure also contributes to a certain degree. Water movement is however more complicated and is also facilitated by factors at the cellular level. Aquaporins occur in the cell membrane and enhance osmosis of water; however they do not alter the direction of water flow. These structures are important in ensuring that a water balance is maintained in the cell and that water is continuously moved into the xylem (Raven *et al.*, 2005).

2.9.8 Berry water transport mechanisms

2.9.8.1 Water flows into and from the grape berry

The balance between water influx and efflux is denoted by the berry volume. An uptake of water occurs via movement from the roots, through the stem and into the leaves (Figure 2.13). Water will also move into the berries. Water loss occurs via transpiration and water backflow from the berry.

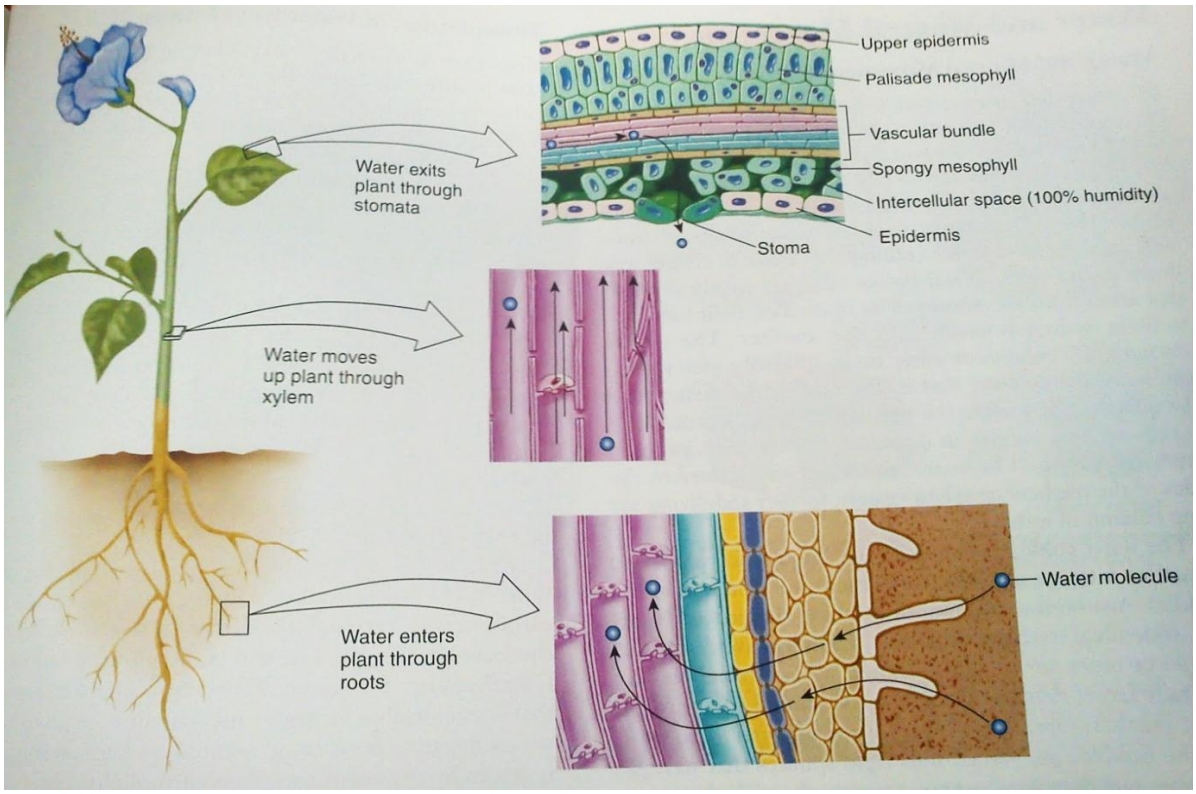

Figure 2.13 The movement of water through a plant. Water moves form the soil, through the root until it reaches the leaves where it is transpired (Raven *et al.*, 2005).

2.9.8.2 Water influx

Grape berries are typically composed of 75-85% water which serves as a solvent for various constituents including acids, sugars and phenolic compounds (Ribéreau-Gayon *et al*., 2006). Water may be transported via both the xylem and the phloem tissue into the berry (Lang & Thorpe, 1989). This however is determined by the developmental stage of the berry. Prior to véraison, water is mainly transported via the xylem tracheids, where after the phloem tissue become the dominant supply pathway (Creasy & Lombard, 1993). Many theories have been postulated as to why this happens with the most accepted being that the xylem tracheids break down due to berry expansion, leaving xylem structurally disjointed. In an experiment to demonstrate xylem malfunction in ripening berries, eosin perfusion was observed in both unripe and ripe berries. It was found that in ripe berries, movement of the eosin was obstructed beyond the pedicel. The formation of tyloses in tracheary elements was ruled out as possible cause and it was concluded that stretching of the trachieds in the dorsal vascular bundles was responsible for this blockage. This deduction was supported by visible abnormalities in the spacing of cell wall thickenings, as well as breaks in the cell membranes. The interruption of the tracheary elements occurred when the berry experienced a sudden increase in size, approximately a week after véraison (Findlay *et al.*, 1987).

Recent studies however have shown that the xylem tissue stays intact and functional throughout berry development. Creasy and Lombard (1993) conducted an experiment to show the effects of water stress and peduncle girdling on pre and post-véraison berries. A comparison was made between peduncle girdled berries, excised bunches and control berries. It was observed that the berries on the girdled bunches decreased in volume and became softer, a direct result of the reduced xylem functioning. However, excised clusters showed a progressed reduction in volume and a higher incidence of deformability suggesting that girdled bunches still maintained a degree of xylem connectivity. A similar experiment was conducted by Rogiers *et al* (2001) using pedicel girdling. Their results also showed a faster loss in volume and shape in excised berries as opposed to girdled berries. Another experiment by Rogiers *et al* (2008) involving the observation of Ca^{2+}, a phloem immobile element, showed a continued influx into the berry after véraison. These observations further substantiate the idea of continued xylem connectivity between the pedicel and pericarp, or suggest that a non-vascular route into the pedicel exists, thereby bypassing any xylem blockages. Various studies however have stated that Ca^{2+} is accumulated during the herbaceous growth period before véraison. During the ripening phase, uptake of this ion has been found to be very low or absent, depending on the plant water status as it is carried with the transpiration stream. The accumulation of Ca^{2+} can therefore be regarded as variable and this must be taken into account when conclusions are made (Etchebarne *et al.*, 2009).

In addition, it was shown by Bondada *et al* (2005) that the establishment of an artificial hydrostatic gradient in post-véraison berries enabled the flow of an apoplastic dye from the pedicel to the styler end via the peripheral and axial xylem tissue. Furthermore, when the expansion of post véraison berries and consequent potential interruption of the xylem tracheid was avoided, apoplastic dye was still unable to move into the peripheral xylem tissue without the appropriate artificial hydrostatic gradient. This led them to believe that the cessation of water influx via the xylem tissue was not due to a discontinuation, but to a reduction in the hydrostatic gradient between the pedicel and berry xylem. In addition, Tyermann *et al*. (2004) showed a significant decrease in the overall hydraulic conductance within a berry between véraison and harvest.

2.9.8.3 Water efflux

The efflux of water from the grape berry results from a combination of transpiration and berry water backflow through the xylem.

1. Transpiration

The transpiration from a berry serves a key role in maintaining a water balance within the vine, but also acts as a driving force for sugar accumulation. It has been found that the rate of transpiration decreases during berry growth and development (Greenspan *et al.,* 1994). This may be attributed to changes in the water permeability of the berry skin. Water permeability is directly reliant on the number and functionality of stomata on the berry skin (Ollat *et al*., 2002). In Cabernet Sauvignon, an average of 7 ± 2 stomata are found on the berry surface with a 1 mm to 1.2 mm diameter with other varieties exhibiting a similar number, indicating a general low number of stomata found on the berry skin (Palliotti & Cartechini, 2001). Weight loss due to transpiration may therefore not be very profound and it has been hypothesised that a reduction in phloem transport may be the main cause of a loss in berry weight (Rogiers *et al.*, 2004)

The structural arrangement of the wax which makes up the berry cuticle, in conjunction with its chemistry is able to control the movement of water from the berries (Possingham *et al.*, 1967). The structure of the cuticle changes as the fruit ages. Reynhardt and Riederer (1991) proposed a model of the physical structure of the epicuticular wax layer. It was stated that the matrix of the wax layer is composed of ordered crystalline structures as well disordered amorphous regions. The crystalline structure is practically impermeable to water due to the reduced diffusional dynamics in these regions. It therefore stands to reason that water loss occurs from the amorphous regions. The physical structure rather that the chemical composition can therefore be regarded as the main component responsible for water loss. In an experiment on Shiraz, it was found that the amount of epicuticular wax declined after véraison, but then stabilised. No structural damages were present which could be associated with an increases water loss. It was also found that the transpiration rate declined as the fruit matured, indicating that berry weight loss did not occur due to a rise in transpiration. It was therefore concluded that a loss of berry weight could not be wholly ascribed to evaporation as a result of cuticle disruption or high levels of transpiration. A possible decrease in vascular flow of water into the berry in addition to evaporation and transpiration was hypothesised to be responsible for a loss in berry weight.

Studies done on sugar loading have suggested that the driving force behind sugar loading is the osmotic gradient between the phloem and the berry flesh. In order to maintain this gradient water needs to be lost from the berry via transpiration. It has therefore been hypothesised that evapotranspiration from the berry is actually the driving force behind sugar loading. Greater evapotranspiration occurs in berries with a more favourable surface to volume ratio. Smaller berries therefore load sugar more quickly as they lose water at a higher rate than larger berries. The reason for sugar accumulation can therefore said to be due to an irreversible loss of water from the berry (Dreier, 2000).

2. Xylem backflow

Xylem back flow is a process by which water is withdrawn from the berry and re-circulated to other parts of the plant (Lang & Thorpe, 1989; Keller *et al.*, 2006). This may theoretically only occur if there is a functional xylem connection to the berry apoplast and if there is an appropriate concentration gradient to drive water from the berry back into the plant (Tyerman *et al.*, 2004). It has been demonstrated that a connection is still present between the berry and the parent vine, however the water potential of the two components as well as the hydraulic connectivity between the two will influence the pressure gradient responsible for driving water back from the berry into the vine (Tyerman *et al.*, 2004; Tilbrook & Tyerman, 2008, 2009).

It has been speculated that berry backflow serves as a means to return water to the vine in the case of water deficit and may be a direct cause for a loss in berry weight, provided that phloem transport slows down or ceases (Rogiers *et al.* 2004; Tyerman *et al.* 2004).

2.10 CARBON FLUXES THROUGHOUT THE VINE

The accumulation and concentration of sugar within a grape berry can be deemed as the most important factor contributing to “quality” along with other components such as aromatic compounds. The sugar concentration determines the final alcohol level while amino acids serve as precursors of aroma molecules responsible for the aromatic and flavour profile of the wine. In addition, sugars serve a vital function in both the signalling involved in gene expression (Conde *et al.,* 2007) as well as the production control of various secondary compounds including anthocyanins (Vitrac *et al.*, 2000).

2.10.1 Sugar composition and accumulation in the grape berry

Glucose and fructose are the two dominant sugars accrued in the berry. The final sugar composition is primarily controlled by the genotype, however, variations may occur due to berry development (Coombe, 1992), environmental conditions and management practices (Nuzzo & Matthews, 2006) Sugar concentration is theoretically determined at the berry level by the interaction between sugar loading, water budget and berry metabolism, including respiration (Coombe, 1992).

The ripening phase, which commences from véraison, is accompanied by an increased accumulation of soluble sugars in the grape berry, and by the synthesis of certain phenolic and aromatic precursors. Sugars are accumulated in the vacuoles of the berry flesh (mesocarp) cells. Berry sugar accumulation will depend on the source functioning (current photosynthesis of the leaves and sometimes wood reserves) and will be mediated at the berry level by the

expression and activity of sugar transporters and certain enzymes. The change from a symplasmic to an apoplasmic unloading pathway, which occurs from véraison onwards, is accompanied by an increase in the expression and activity of cell wall invertases (Zhang *et al.*, 2006). As a result, the sugar concentration of the apoplast, as well as the osmotic pressure increases. This could in turn lead to a rise in sugar uptake via the stimulation of the proton pumping ATPase activity (Li & Delrot, 1987). From véraison onwards the berries will accumulate approximately equal amounts of glucose and fructose, with each hexose reaching about 1M (Coombe, 1997). This implies that the sucrose molecules within the phloem tissue are hydrolysed at some stage during their transport (Agasse *et al.*, 2009). Hexose accumulation in the berries is linear with time, however, the increase in berry volume continues at a variable rate (Coombe, 1989).

2.10.2 Loading of assimilates

Translocation within the phloem tissue is generally accepted to occur due to the active accumulation of photo assimilates in the source organ against a concentration gradient. This process occurs across the cell walls of the sieve element-companion cell complex and is termed phloem loading. Prior to this, assimilates move from photosynthetically active palisade cells or spongy parenchyma, to the connecting mesophyll cells and finally into the bundle-sheath and phloem parenchyma cells of the leaf veins which are adjacent to the companion cell-sieve element complex (Schulz & Thompson, 2007). The movement of assimilates from the mesophyll to the apoplast of the phloem tissue has been theorised to occur due to facilitated transfer by ATP or a sucrose/cation symport system with K^+ based on the electrochemical potential gradient across the membrane, instead of simple diffusion. Assimilates then move into the symplast of the phloem via the companion cell-sieve element membrane through active uptake. Movement of assimilates to the phloem can occur either symplastically or apoplastically (Giaquinta, 1983).

2.10.3 Movement of assimilates to the sink organs

Passive flow via diffusion represents one of the theories postulated with regard to movement of assimilates in the phloem tissue, however, this process is too slow to account for assimilate translocation. The most accepted and studied theory of movement is that which was proposed in 1926 by Ernst Münch. The theory is demonstrated using two osmometers connected to each other with a tube (Figure 2.14). The first osmometer represents the sources, the second represents the sink and the connecting tube represents the phloem transport system. Both osmometers are placed in a solution with an arbitrary concentration. This solution represents

the dilute solution found in the cell apoplast, especially the cell wall and xylem. The source osmometer (1) contains a solution with a higher concentration than the surrounding solution, while a solution with a lower concentration than that of the solution in the source osmometer is placed in the sink osmometer (2). Due to the high concentration of the source osmometer (1), water moves in from the surrounding solution via osmosis, causing the pressure to build within. The solution consequently moves to the sink osmometer (2) through the connecting tube. Pressure is transferred from the source osmometer to the sink osmometer owing to that fact that they are connected via the tube. Over time the pressure in the sink osmometer (2) builds up resulting in more positive water potential in comparison to the surrounding solution. Water molecules will therefore move out of the sink osmometer (2) by osmosis. The solute molecules (sugar) will however be left behind since the membrane is not permeable to them. In this way, both water and solutes are moved via bulk flow from the source to the sink (Salisbury & Ross, 1978).

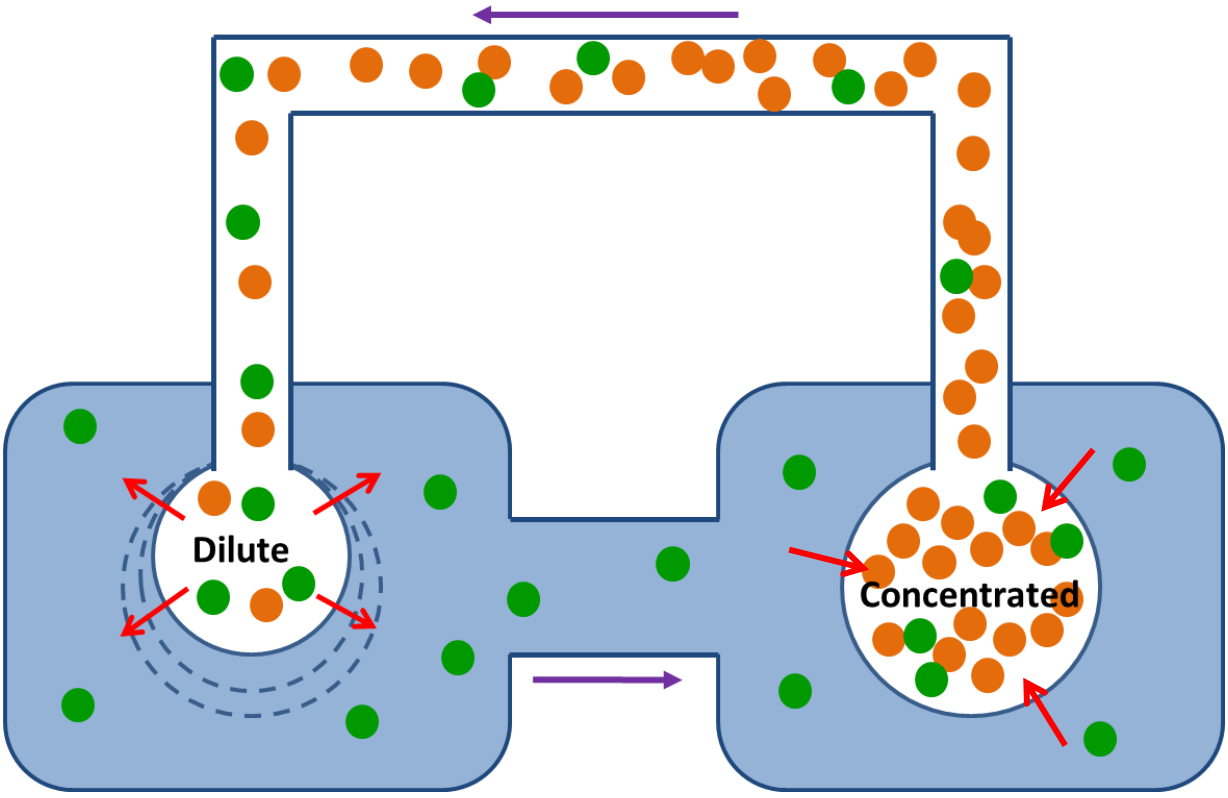

Figure 2.14 A model which illustrates the Munch theory. The concentration of the orange particles will control the rate and direction of flow, while the green particles will move along with the flow of the stream. The dashed lines in osmometer 2 indicate that flow may happen due to expansion. The purples arrows indicate the direction of flow, while the red arrows show the direction of movement of the green particles.

Bulk flow can contribute to sugar accumulation provided that there is a favourable water potential gradient between the phloem tissue and the fruit. Hexose sugars which accumulate in the grape berry are osmotically active and are therefore capable of influencing the osmotic potential of the cell and consequently, the water gradient. This in turn is responsible for sugar

import regulation. The metabolism and incorporation of assimilates ensure that a potential gradient is maintained in order to ensure the unimpeded transport of sucrose from the source organs. Active sugar loading will implicate water loading to balance the osmotic pressure, but water loading is linked to transpiration as well. This is why the osmometer theory is not entirely accurate; the berry does not function quite the same way.

2.10.4 Import of assimilates

The entire process of assimilate import includes various steps including the movement of sucrose via the phloem tissue, the transfer of sucrose to the intercellular free space of sink cells, the retrieval of the sucrose and its metabolites from the cell wall by the sink cell and finally, the compartmentation or metabolism of the sucrose in the sink cell (Ho *et al.*, 1989). Assuming the presence of a pressure flow, the rate of import of assimilates for individual sink organs can be regulated by an osmotic potential gradient between the source and sink. In order to maintain a low osmotic potential gradient, the sucrose concentration must remain low at the unloading site within the sink tissue. This may be accomplished by the chemical conversion of sucrose to hexoses, by the compartmentation of sucrose within the vacuole or by the utilisation of sucrose for respiration or growth (Ho, 1988). Should anything negatively affect these processes, the concentration of sucrose at the unloading sight will increase, thereby decreasing the osmotic potential gradient between the source and sink necessary to sustain the rate of import until the plateau is reached. This demonstrates that the process is under regulation.

Sugar is transported via the phloem tissue and moves either symplastically or apoplastically into the cytoplasm of the mesocarp cells. It then moves across the tonoplast into the vacuole where it is stored (Conde *et al*., 2007). Sucrose may be hydrolysed to form glucose and fructose in the cell wall prior to the movement thereof into the mesocarp cells, of after compartmentation in the cell vacuoles. The enzymes involved include invertases and sucrose synthases. Furthermore, since these enzymes modulate the pool of available sugars, they may also be involved in sugar signalling (Agasse *et al.*, 2009). Sucrose and hexose transporters are also involved in the movement of sugar into the berry (Figure 2.15).

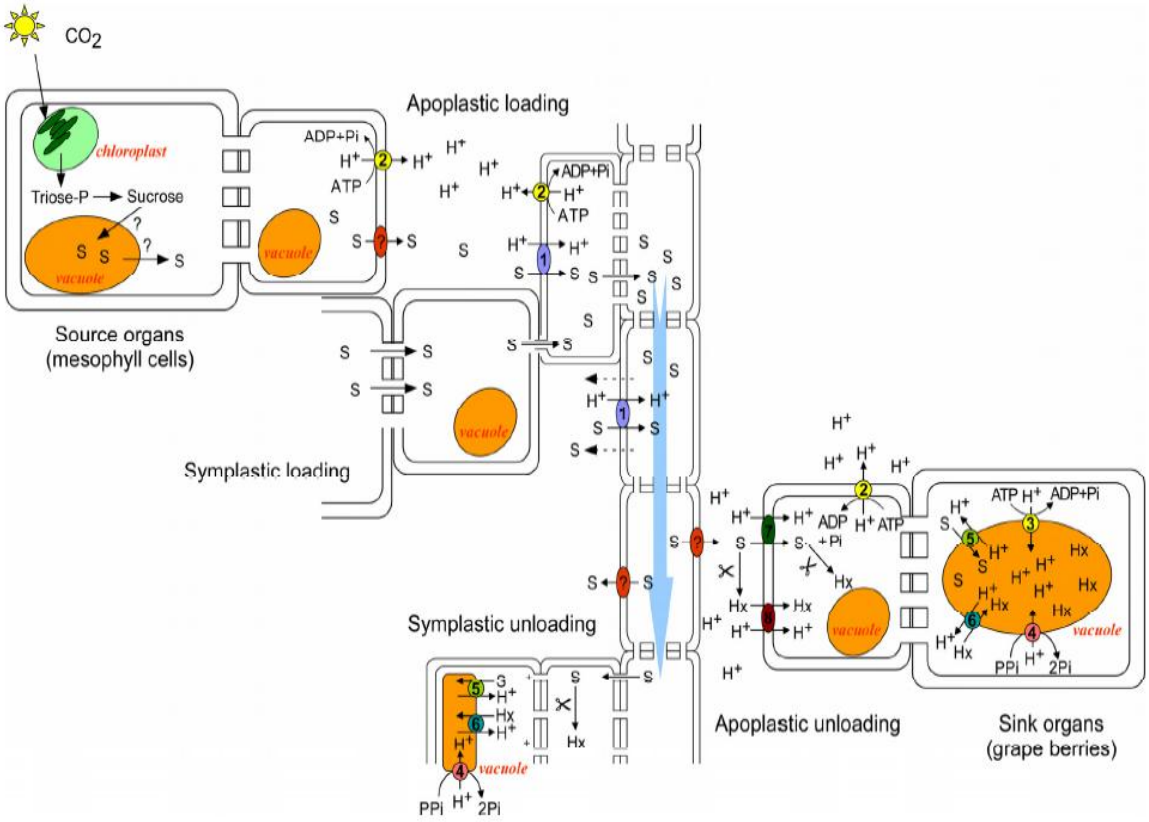

Figure 2.15 The long distance sugar transport through the phloem tissue. From the mesophyll in the leaves, sucrose may be loaded into the sieve element/companion cell complex either symplastically or apoplastically. The phloem tissue transports the sucrose to the sink organs where it is unloaded. Apoplastic unloading implies the existence of a sucrose exporter at the sink tissue. The import of sucrose into the sink tissue can occur through plasmodesmata or by sucrose transporters (Goren *et al.*,

2.10.5 Grape invertases

The sugar which is imported into the berry up until véraison is mostly metabolised with very little of it being stored. From véraison, the accumulation of sugar greatly increases in the pericarp cells. High levels of fructose and glucose are attained in the berry at maturity. The accumulation of hexoses from sucrose import implies a mechanism whereby sucrose is cleaved into its constituent hexoses. The activity of an enzyme is clearly present between the point of phloem unloading and pericarp cell vacuoles (Ollat *et al.*, 2002). The enzymes involved in these processes include invertases and sucrose synthases. Invertases are hydrolases responsible for the cleaving of sucrose molecules into fructose and glucose. Acidic invertases occur in the vacuole and cell wall, whereas neutral invertases are found in the cell cytoplasm (Agasse *et al.*, 2009). Sucrose synthase occurs in the cytoplasm and catalyses the reverse hydrolysis of sucrose into fructose and UDP-glucose (Boss & Davies, 2001).

Invertases are responsible for carbon partitioning with the plant, regulating the composition of stored sugars and provision of hexoses for metabolic processes. Additionally, since these enzymes modulate the pool of available sugars, they may also be involved in sugar signalling

(Agasse *et al.*, 2009). Furthermore the sucrose gradient necessary to sustain mass flow of the phloem sap is maintained by the participation of sugar transporters and sucrose metabolic enzymes.

Two cDNAs for genes encoding invertases in the grape berry have been isolated and characterised (Boss *et al.*, 1996). It was found that the expression of these two genes was consistent with invertase activity. Invertase activity per unit weight was shown to be generally low during the first growth phase. It reached a maximum at véraison and then decreased again during hexose accumulation, probably due to a rapid increase in berry volume (Hawker, 1969). On a per berry basis, the invertase activity stayed high and stable during the ripening process. The direct parallel between invertase activity and the invertase gene expression insinuates that activity is mostly regulated at the transcriptional level in grapes (Boss & Davies, 2001).

2.10.6 Hexose and sucrose transporters

Sucrose transport from the phloem to the point in the pericarp cells where hydrolysis occurs, infers that sucrose transporters are present on the plasma membrane, the tonoplast or both. Genes for putative sucrose transporters have been isolated from Shiraz berries and partially from Ugni Blanc berries (Davies *et al.*, 1999; Manning *et al.*, 2001) The expression of a few of these genes, including vvsuc11 and vvsuc12 has been found to be up-regulated at véraison. This implies that these transporters play a role in the movement of sucrose into the ripening grape berry (Davies *et al.,* 1999). Furthermore, a number of hexose transporters have also been identified in the grape berry (Fillion *et al.*, 1999). The expression of these transporters are typically associated with sink tissue as that is where sugars are unloaded from the apoplast and imported. The most highly occurring transporters include vvht1, vvht2 and particularly vvht3 (Agasse *et al.*, 2009).

In figure 2.16, two possible pathways of sugar uptake are depicted. One pathway involves the transport of sucrose into the vacuole where it is then cleaved into glucose and fructose. The second pathway involves the inversion of sucrose by an extracellular invertase, followed by the transport of glucose and fructose into the vacuole by hexose transporters (Robinson & Davies, 2008)

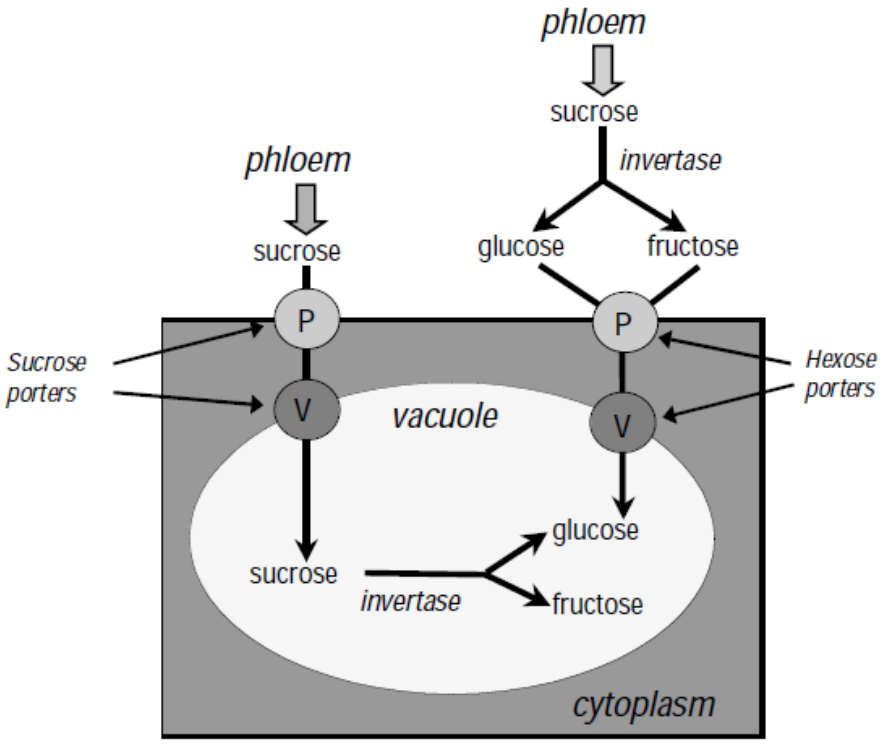

Figure 2.16 Sugar uptake and metabolism in ripening grapes (Robinson & Davies, 2008)

The functionality and the precise location of both sucrose and hexose transporters is yet to be clarified. However, it is assumed that there are several mechanisms involved in the accumulation of sugar during berry ripening since both sucrose and hexose transporters are present during this time (Ollat *et al.*, 2002).

2.10.7 Sugar signaling

The source and sink organs of a vine are spatially separated and the vine has therefore needed to develop certain mechanisms whereby carbohydrate production and transport is controlled. The principle is analogous with the glycaemia regulation in mammals whereby sugar levels are sensed and are modulated according to the “feast and famine” response pattern (Agasse *et al.*, 2009). An excess of carbohydrates will favour the expression of genes which are involved in the biosynthesis and storage of reserves. High levels will additionally repress transcripts which encode for the up-regulation of enzymes related to photosynthesis and the mobilisation of reserves. Conversely, a depletion in carbohydrates will result in the up-regulation of genes involved in photosynthesis and carbohydrate transport, while concurrently decreasing the expression of genes encoding for storage and utilisation (Koch, 1996). Furthermore, a sink feedback mechanism was proposed by Quereix *et al* (2001) whereby a signal which originates

in the phloem tissue is able to influence the rate of photosynthesis in the vine. Signalling within the plant plays a vital role in the regulation of vine functioning. Further studies are needed to understand the complex communication between organs.

2.11 GIRDLING

Girdling refers to the complete removal of a ring of bark including phloem tissue from around the shoot, peduncle or pedicel. This practice hinders the flow of carbohydrates. (Figure 2.17) The wound however is capable of healing. When a shoot is girdled, the phloem is separated at the cambium level. Phloem tissue is able to regenerate after a certain amount of time. This is a common strategy for many plants in order to repair any damage caused to their tissues (Zhang *et al.*, 2011). The healing of the girdling wound includes the re-establishment of the vascular connection and depends on the cambial activity. Callus forms across the wound starting from the margins of where the tissue was removed. Cambium develops from the callus wherever the vascular tissue encroaches upon it. This vascular cambium forms new xylem and phloem tissue which is continuous with the same tissues in the uninjured part of the shoot adjacent to it. (Goren *et al.*, 2004). In grapevine, phloem regeneration will occur after two to three weeks.

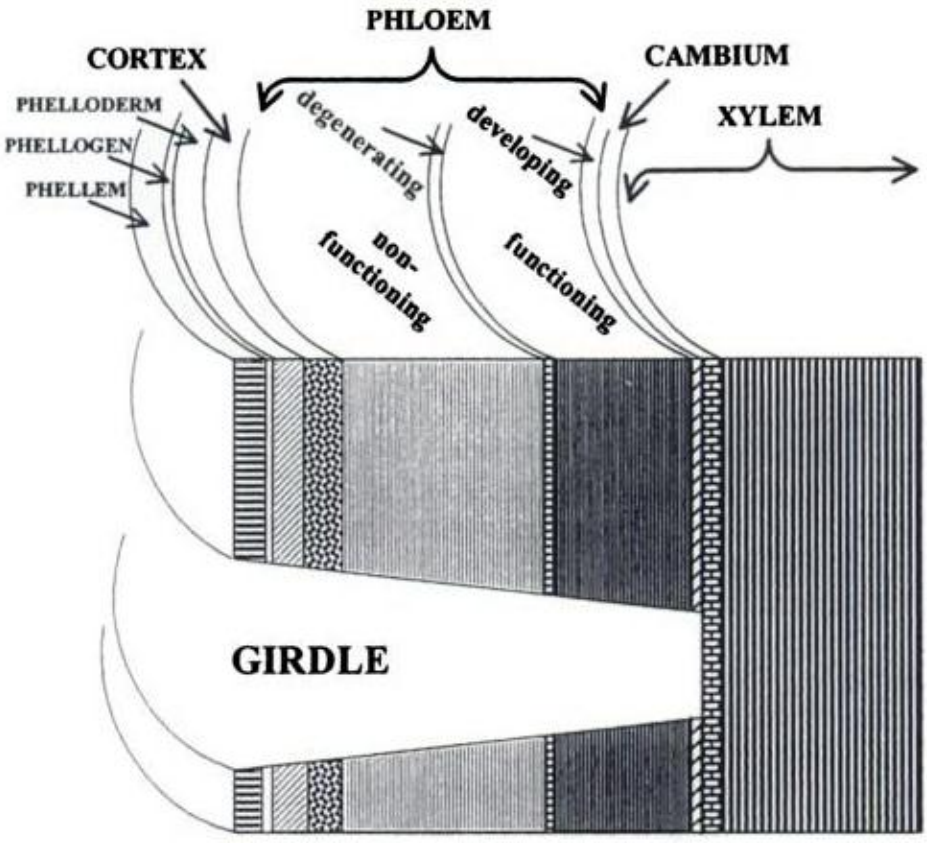

Figure 2.17 Schematic illiustration of the girdling technique. A ring of bark and phloem tissue are both removed by girdling (Schneider, 1954; Goren *et al.*, 2004)

Various trials have also been conducted using girdling to investigate source – sink relationships. The use of girdling has been essential in attempts to verify the hypothesis that there exists a sink feedback inhibition of photosynthesis (Neales & Incoll, 1968). The use of girdling effectively removes the root system as a potential source, making the leaves the only suppliers of carbohydrates. It has been noted that in this case, a build-up of assimilates occurs in the leaves leading to a reduction of photosynthesis (Kriedemann & Lenz, 1972; Harrell & Williams, 1987). The sink feedback inhibition of photosynthesis builds-up slowly as carbohydrates are accumulated. The increase in carbohydrate concentration may interfere with photosynthesis through various mechanisms including:

- The enlargement of starch granules may damage the chloroplasts
- Stomata may simply close (Goldschmidt & Huber 1992)
- The accumulation of phosphorylated intermediates leading to a decrease in the levels of inorganic phosphate
- The inhibition of the expression of genes which encode for necessary proteins involved in photosynthesis (Krapp & Stitt, 1995)

Girdling has also been used in past experiments to investigate the functioning of the vascular tissues by blocking phloem transport in plants. In 1993, Creasy and Lombard used peduncle girdling to determine its effect on pre and post véraison berry development and deformability. In another study, the effects of girdling on fruit set and vegetative growth was investigated (Caspari *et al.*, 1998). Girdling is also commonly used in the table grape industry to produce bigger, more appealing fruit.

Advances in plant sciences have shown that molecular regulation of gene activity is probably involved in the plants responses to certain manipulations. It can therefore be assumed that girdling also elicits a molecular response in the vine with regard to the regulation of genes. Further research to confirm this will lead to more conclusive insights into the far-reaching effects of girdling (Goren *et al.*, 2004).

Chapter 3

Materials and Methods

MATERIALS AND METHODS

3.1 EXPERIMENTAL SITE

The experiment was executed using *Vitis vinifera* L. cv. Cabernet Sauvignon clone CS 388C, grafted onto 101-14 Mgt (*Vitis riparia* X *Vitis rupestris*). The vineyard is situated (33 56' 42 S; 18 51' 44 E) close to the Eerste River with light to medium textured alluvial soils. The row orientation is North-South. The vines are trained on a seven-wire vertical trellis system with six movable canopy wires. The block was drip irrigated during main phenological stages, including berry set and véraison. Further irrigation was applied when need as dictated by stem water potential measurements.

3.2 GIRDLING EXPERIMENT

3.2.1 Design

A block comprising of three rows with seven panels each was selected in which to do the trial. Each panel included six vines bringing the total number of vines in the block to 126. Only half of the panels in the block were used due to the nature of the design. Vines in every second panel were treated, while vines in the other panels were left untreated. Two buffer rows were left on either side of the block as well as two buffer panels on either side of the rows.

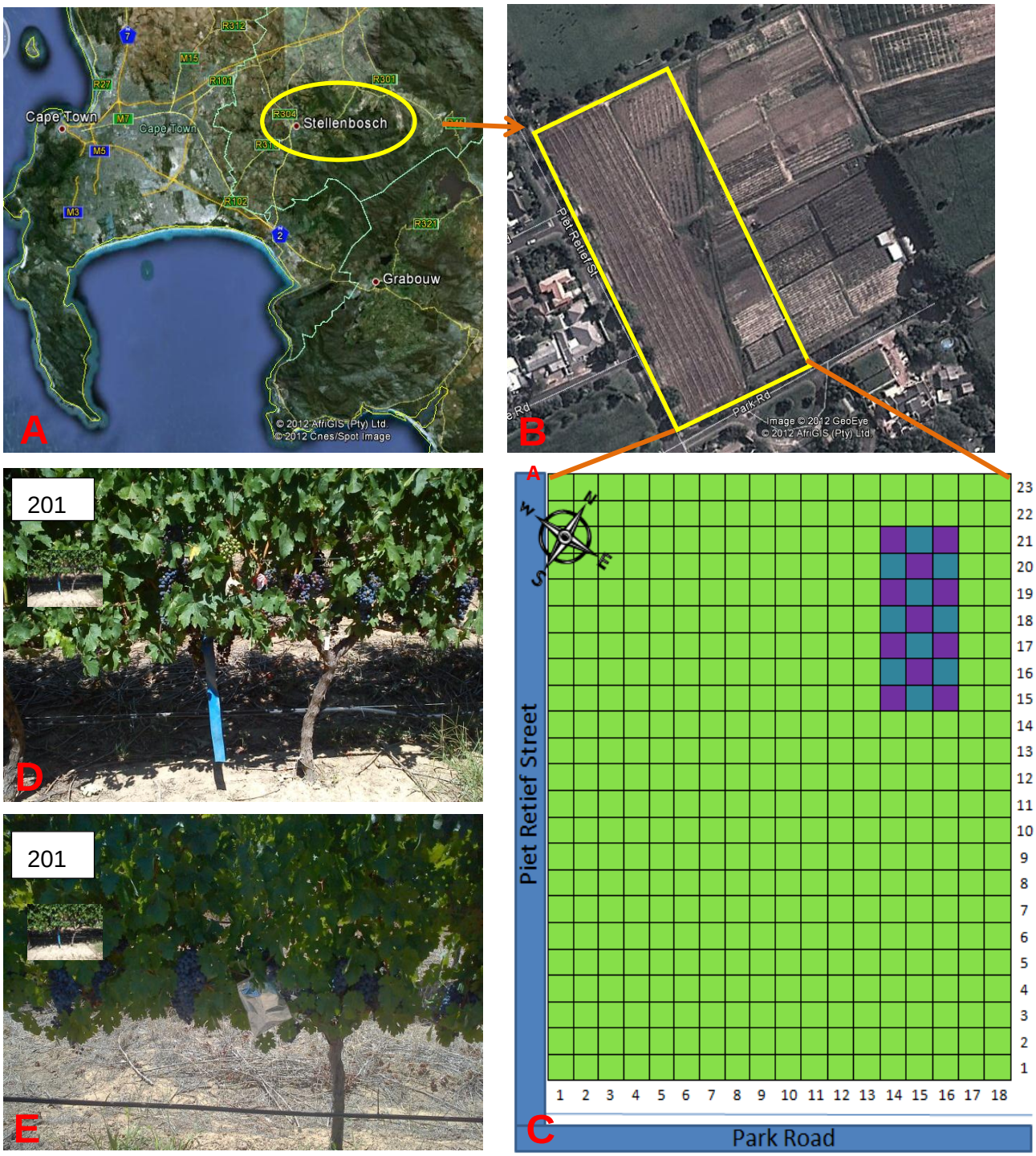

Figure 3.1 Experimental block layout. Figure A shows the viticultural region of the block (Stellenbosch). Figure B shows the vineyard in which the trial was conducted. Figure C depicts the experimental layout; the purple blocks indicate panels where vines were girdled and the blue blocks represent panels which were untreated. Figure D shows the canopy of the block in the first season (2011) and figure E shows the canopy in the second season (2012).

A single primary shoot on each cordon of every vine in the block was treated. A control shoot was also selected on each cordon of every vine (i.e. two girdled and control shoots per vine).

Figure 3.2 The shoots which were selected for treatment on a vine came from the middle of each cordon. The red ovals each encircle a treated and untreated shoot.

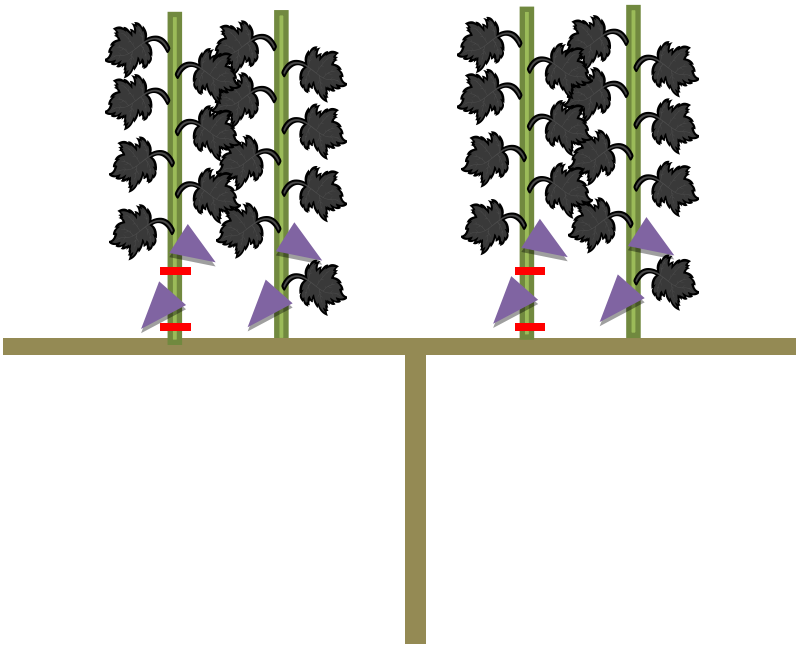

Figure 3.3 Two shoots on both cordons were selected for the trial; one treated and the other a control. The red lines indicate where girdling was carried out. The leaf opposite the girdled bunch was removed. The purple triangles represent bunches.

The trial was designed to include three replicates; each consisting of two bunches per primary shoot/treatment. A primary shoot represents a biological replicate. The trial was conducted over two years, 2010-2011 and 2011-2012, using different vines in each season.

3.2.2 Field procedures

3.2.2.1 Girdling

Girdling was carried out, using a girdling knife, on the lower bunch of a shoot bearing two bunches on every cordon of every vine in every second panel. This was done when 10 % of bunches in the vineyard had changed colour, therefore, at a point during véraison. The method involved removing a ring of bark and phloem tissue (5mm) from around the shoot, above and below the bunch, thereby isolating the bunch from any carbohydrate import from the leaves or the wood/root reserves, as well as from any additional compounds transported via the phloem tissue (Figure 3.4). The phloem was separated from the xylem at the cambium level. The leaf opposite to the girdled bunch was removed in order to prevent any potential carbohydrate input into the bunch from the opposite leaf. The girdled bunch was therefore completely isolated from sugar import.

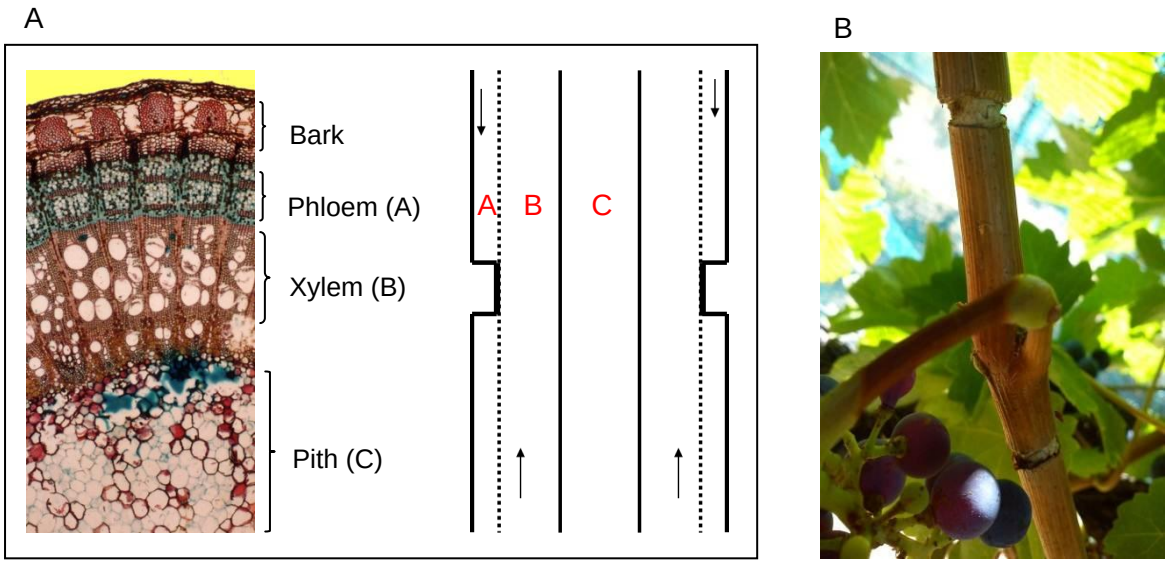

Figure 3.4 A ring of bark and the phloem tissue was removed above and below the bunch, thereby isolating it from carbohydrate import. The phloem was separated from the xylem at the cambium level. Figure A shows the tissue which was removed during girdling. Figure B shows the girdling wounds above and below a bunch.

Results obtained during the first year of the trial indicated a re-establishment of the phloem tissue after three to four weeks through a callus formation issued from the remaining cambium, therefore, the girdling process was repeated every two weeks throughout the ripening season in the following year to ensure that the wound was kept clear of any callus tissue development and new phloem cell differentiation.

Figure 3.5 Phloem tissue was able to regenerate due to callus formation which occurred after a few weeks. Figure A shows the development of callus at the wound margins. Figure B shows a completely callused girdling wound

A control shoot with two bunches was also selected on the same cordon as every girdled shoot. A girdled shoot as well as a control shoot were therefore situated on each cordon of every vine of each panel, in the experimental block. This allowed for the use of the primary shoot as a study unit as well as a biological unit.

Girdling was carried out in order to completely isolate a bunch from any carbohydrate import, thereby allowing one to test if berries could grow independently of sugar accumulation. Although effective, there were numerous problems experienced with this method. The fact that the girdling wound was able to heal so proficiently provided the challenge of ensuring that it stayed open during the trial. The utilisation of a tracer transported in the vascular tissue would have better demonstrated whether the girdling wound was wholly effective. Also, histological studies of the girdling wound shoot sections may have been useful to determine if the phloem tissue was indeed disconnected and if any damage had occurred to the xylem tissue.

3.2.2.2 Climate monitoring

For the duration of the trial, the mesoclimate of the experimental block was measured using Tinytag® data loggers (Gemini Data Loggers, West Sussex, United Kingdom) placed above the canopy. The loggers were set to measure maximum and minimum temperature, as well as maximum and minimum humidity every hour.

3.2.2.3 Vine water status

In addition, stem water potential (Choné *et al.*, 2001) was determined at mid-day on a regular basis to ensure that the vines were never subjected to any water constraints. The treated vines were not used, since removing the leaves would alter the source-sink relationship within these vines. Irrigation was applied if the pressure bomb (Scholander *et al.*, 1964) readings reached -900Kpa or below. Typically, between 8 mm and 10 mm of water would be applied for twelve hours in the case that irrigation was required.

3.2.3 Sampling and measurements

3.2.3.1 Leaf area measurements

In order to determine the total leaf area per shoot, all the shoots from which bunches were sampled were harvested and stored in the 4°C walk in fridge to preserve the leaves. Shoots were removed from the vineyard at the end of the season, prior to leaf senescence. The leaves were then stripped from the harvested shoots, with those from the primary shoots and lateral shoots kept separately. The number of leaves and total leaf area was determined using a planometer (Delta-T Devices, Cambridge, UK). This was done separately for each replicate of every treatment.

3.2.3.2 Sampling methods

Sampling of the whole bunches was carried out once a week with the first sample being taken on the same day that the bunches were girdled and the last sample being collected just before harvest. Bunches were selected randomly each time sampling took place. Every sample consisted of three replicates, each of which included two bunches respectively. The collected samples included both the girdled and ungirdled bunch on a shoot as well as two control bunches from an untreated shoot. The two control bunches were kept separate as “Control Top” and “Control Bottom” , this allowed for comparison between bunches at the same level as well as between the two bunches of the girdled shoot. All the shoots from which bunches were removed were marked with the date, the replicate number and shoot number using masking tape and a black Koki pen. After harvest, the marked shoots were removed from the vineyard and the total leaf area was measured.

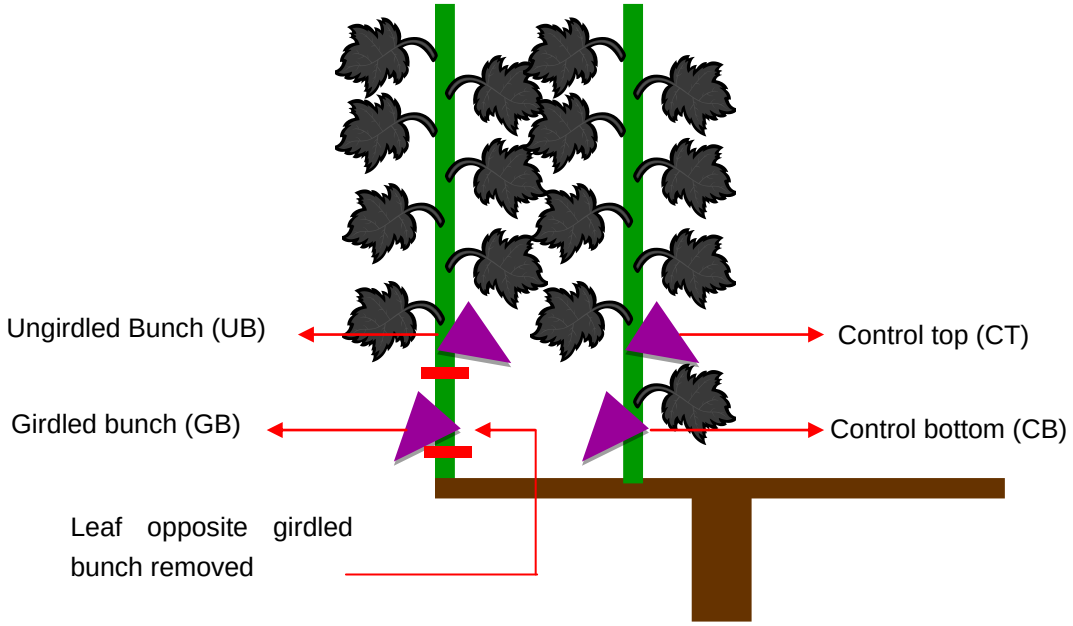

Figure 3.6 The bunches sampled during the trial

3.2.3.3 Berry classification according to diameter

Berry classification according to diameter was done separately on all the sampled bunches. This was achieved by removing all the berries from the bunches and separating them into different diameter classes using plastic grids in which holes of a specific size have been cut, ranging from 6.5 mm to 17.5 mm. The sphere shaped berries were removed by cutting through the pedicel just above where it was attached to the berry. The berries were sifted progressively through the plastic grids, starting with the lower diameter classes and proceeding to the larger diameter classes. All the berries which did not fit through the holes of a certain grid were kept aside and then again sifted through a grid with larger holes. In this way, the berries were sorted into different diameter classes. The diameter classes 11.5 mm and 12.5 mm were the major classes of berry diameter and were represented throughout the season, therefore these were chosen to work with. Berries of the same volume were chosen to eliminate size as a variable. This was important because we wanted to test the hypothesis that berry volume is independent of berry sugar accumulation. The number of berries per diameter class was counted, where after a portion of the berries were set aside to be frozen at -80°C for later analysis and the remainder was used to determine berry fresh mass and dry mass. These berries were counted and then weighed using a four decimal point scale, after which they were placed in a 70°C oven until dry. The dry mass of the berries was then noted allowing for the determination of the average water content per berry for each treatment and diameter class. This was ascertained by finding the average fresh mass and dry mass per berry and then calculating the difference between the two.

Figure 3.7 Plastic grids were used to classify the sampled berries according to their diameter (Ojeda *et al.*, 2001).

3.3 TRANSPIRATION EXPERIMENT

3.3.1 Field procedure

A second experiment was conducted in parallel with the girdling trial to follow the transpiration rate of the berries within the vineyard. This was a smaller trial with each treatment applied only to 24 bunches. A small block of two rows and two panels, situated two panels down the row from the bigger trial was utilized (Figure 3.8).

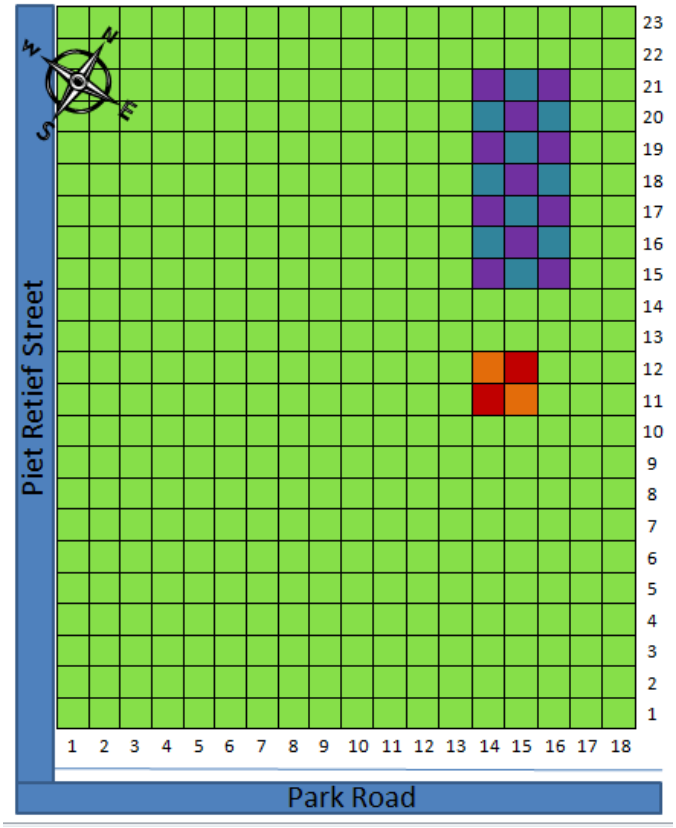

Figure 3.8 The same vineyard was used for the transpiration trial. The blue and purple indicate where the girdling experiment was carried out. The orange blocks indicate where bunches were cut, and the red blocks show where the bunches were bagged.

Two different treatments were applied to this block. Two bunches per vine were treated, either by removing bunches, or by enclosing the entire bunch in a bag. Both bunches on a vine received the same treatment. This was carried out on every vine in a panel. Bunches which were not treated in any way were sampled each time to serve as a control.

To determine the transpiration rate, bunches were cut from the vine and then suspended in the canopy using cable ties, thereby allowing them to remain in their typical microclimate for the duration of the trial. All wounds were sealed to prevent any water loss other than from the berries (Figure 3.9).

Figure 3.9 Bunches were cut from the vine and suspended in the canopy to determine the transpiration rate

Figure 3.10 Bunches were placed in foil bags to impede transpiration.

The second treatment involved using foil bags to cover bunches and thereby impede transpiration. The bags were made by covering re-sealable bags in foil and then wrapping them in contact to make them waterproof. The bags were carefully placed over the bunches and then sealed and secured using paper clips (Figure 3.10).

3.3.2 Sampling procedure

Sampling was carried out twice a week from véraison until harvest. Two bunches from each of the treatments were sampled as well as two control bunches. The berries for each treatment were removed from the bunch and then sorted into all diameter classes. The number of berries as well as the weight of the berries per diameter class was noted, where after they were placed in a 70°C to dry. Once dry, the berries were weighed again to determine the berry dry mass. The water content of the berries was determined by calculating the difference between berry fresh mass and dry mass.

3.3.3 Analytical procedures

The portion of the berries which were frozen were stored in labelled 50 ml falcon tubes and placed in a -80°C freezer. This preserved the sugars within the berry, allowing for analysis to be done at a later date. These berries were analysed for glucose, fructose and sucrose using the enzyme robot (Arena 20XT Photometric Analyzer, Thermo Electron, Oy, Finland). This machine has been used in previous experiments. (Sun *et al.*, 2009). To prepare the samples, ten berries per treatment and major diameter class, including 11.5 mm and 12.5 mm, were removed from the falcon tubes and placed in clearly labelled and pre-weighed 50 ml falcon tubes where they were allowed to defrost. Care was taken to prevent these berries from fermenting which would render them unusable for analysis; sample preparation was conducted while the berries were still cool. The tubes containing the berries were weighed again, allowing for the calculation of the berry weight by finding the difference between the weight of the empty tube and the tube containing the berries. A small amount of de-ionised water was added to the tubes in order to facilitate homogenisation. The berries were homogenised and the homogeniser cleaned with deionised water, ensuring that all berry matter left on the instrument was washed into the falcon tubes. The volume was then made up to 30ml using deionised water. The tubes were then placed in the ultrasonic bath for 20 minutes. This ensured that berry tissue was properly broken up to ensure maximum extraction of compounds. Thereafter, the berry material was centrifuged in the falcon tubes for 15 minutes at 10 000rpm at a temperature of 4°C. The clear supernatant was carefully transferred to clean labelled 15 ml storage bottles. The liquid from one falcon tube was divided between two 15 ml bottles; therefore, there were two storage bottles for each sample. These samples were stored at -20°C until analysis using the enzyme robot.

For analysis by the enzyme robot, one storage bottle per sample was allowed to defrost. A small amount of the liquid was transferred to labelled eppendorf tubes which were then handed to the analyst for assessment.

3.4 ANALYSIS BY THE ENZYME ROBOT

The prepared samples were analysed for glucose, fructose and sucrose. This was accomplished using the enzyme robot, a machine which is capable of rapid, accurate analysis with a capacity of up to 250 tests/hour (Arena 20XT Photometric Analyzer, Thermo Electron, Oy, Finland).

3.4.1 D-Glucose

The kit used for glucose analysis was the Enytec^{TM} *Fluid* D-Glucose Id-N^{o}: 5140 (Thermo Fisher Scientific Oy, Finland. Distributed by: R-Biopharm AG, Germany).The principle of the method for determining the concentration of glucose in the allotted amount of the sample is as follows:

D-Glucose + ATP <-------HK-----> Glucose-6-phosphate + ADP

G6P-DH

Glucose-6-phosphate + NAD^+ < ----------> Gluconate-6-Phosphate + NADH + H^+

ATP = Adenosine-5'-triphosphate
HK = Hexokinase
ADP = Adenosine-5'-diphosphate
NAD^+ = Nicotinamid-adenien-dinucleotide

Automation was carried out according to manufacturer's instructions as found in the document by R-Biopharm (Automated applications on biochemistry analyzers for Enytec^{TM} *Fluid* D-Glucose Id-N^{o}: 5140, updated: 16/03/2010).The calibration range was 0 – 100 g/L with an r^2-value: 0.999763. A dilution was built into the method for detection range of 0 – 300 g/L. The lowest standard used for calibration was 5 g/L, but a water sample was included in the calibration to enable quantification between 0 and 5 g/L.

3.4.2 D-Fructose

The kit used for fructose analysis was the Enytec^{TM} *Fluid* D-Fructose Id-N^{o}: 5120 (Thermo Fisher Scientific Oy, Finland. Distributed by R-Biopharm AG, Germany.) The principle of the method for determining the concentration of fructose in the allotted amount of the sample is as follows:

D-Fructose + ATP ----HK-----> Fructose-6-phosphate + ADP
D-Glucose + ATP ----HK-----> Glucose-6-phosphate + ADP
Fructose-6-phosphate <---PGI---> Glucose-6-phosphate
Glucose-6-phosphate + NAD^+ ---G6P-DH---> Gluconate-6-Phosphate + NADH + H^+

ATP = Adenosine-5'-triphosphate
HK = Hexokinase
ADP = Adenosine-5'-diphosphate
PGI = Phosphoglucose Isomerase
NAD^+ = Nicotinamid-adenien-dinucleotide
G6P-DH = Glucose-6-phosphate dehydrogenase

Automation was carried out according to manufacturer's instructions as found in the document by R-Biopharm (Automated applications on biochemistry analyzers for Enytec^{TM} *Fluid* D-Fructose Id-N^{o}: 5120 , updated: 02/06/2010).The calibration range was 0 – 125 g/L with an r^2-value: 0.997178. A dilution was built into the method for detection range of 0 – 300 g/L. The lowest standard used for calibration was 5 g/L, but a water sample was included in the calibration to enable quantification between 0 and 5 g/L.

3.4.3 Sucrose

The kit used for sucrose analysis was the Enytec^{TM} *Fluid* Sucrose Id-N^{o}: 5180 (Thermo Fisher Scientific Oy, Finland. Distributed by R-Biopharm AG, Germany.) The principle of the method for determining the concentration of sucrose in the allotted amount of the sample is as follows:

Sucrose + H_2O ----ß-Fructosidase-----> D-Glucose + D-Fructose
D-Glucose + ATP ----HK-----> Glucose-6-phosphate + ADP
Glucose-6-phosphate + NAD^+ ---G6P-DH---> Gluconate-6-Phosphate + NADH + H^+

The difference between D-Glucose determination with and without enzymatic conversion by ß-Fructosidase indicates the value for Sucrose

ATP = Adenosine-5'-triphosphate

HK = Hexokinase

ADP = Adenosine-5'-diphosphate

NAD^+ = Nicotinamid-adenien-dinucleotide

G6P-DH = Glucose-6-phosphate dehydrogenase

Automation was carried out according to manufacturer's instructions as found in the document by R-Biopharm (Automated applications on biochemistry analyzers for Enytec™ *Fluid* Sucrose Id-No: 5180, updated: 02/12/2010).The calibration range was 0 – 250 g/L with an r^2-value: 0.999549. A dilution was built into the method for detection range of 0 – 300 g/L. The lowest standard used for calibration was 5 g/L, but a water sample was included in the calibration to enable quantification between 0 and 5 g/L.

Chapter 4

Research results and Discussion

RESULTS AND DISCUSSION

4.1 SOURCE-SINK RELATIONSHIPS

The leaves are the main source organs for carbohydrates of the grapevine (amongst other things such as certain minerals and amino acids) and their structure and functioning is quite complex. During the growth period of the leaves, various abiotic factors including light, water and CO_2 levels as well as the genotype (interaction between scion and rootstock) will have an effect on stomatal size and density (Nadeau & Sack, 2002; Rogiers *et al.*, 2011). Once a leaf reaches maturity and the number of stomata is fixed, these same factors will induce a regulatory effect on the stomata. Both structural development and stomata regulation will have an effect on stomatal conductance which will in turn influence photosynthesis. Photosynthesis is responsible for the production of carbohydrates which are distributed throughout the plant (Figure 4.1).

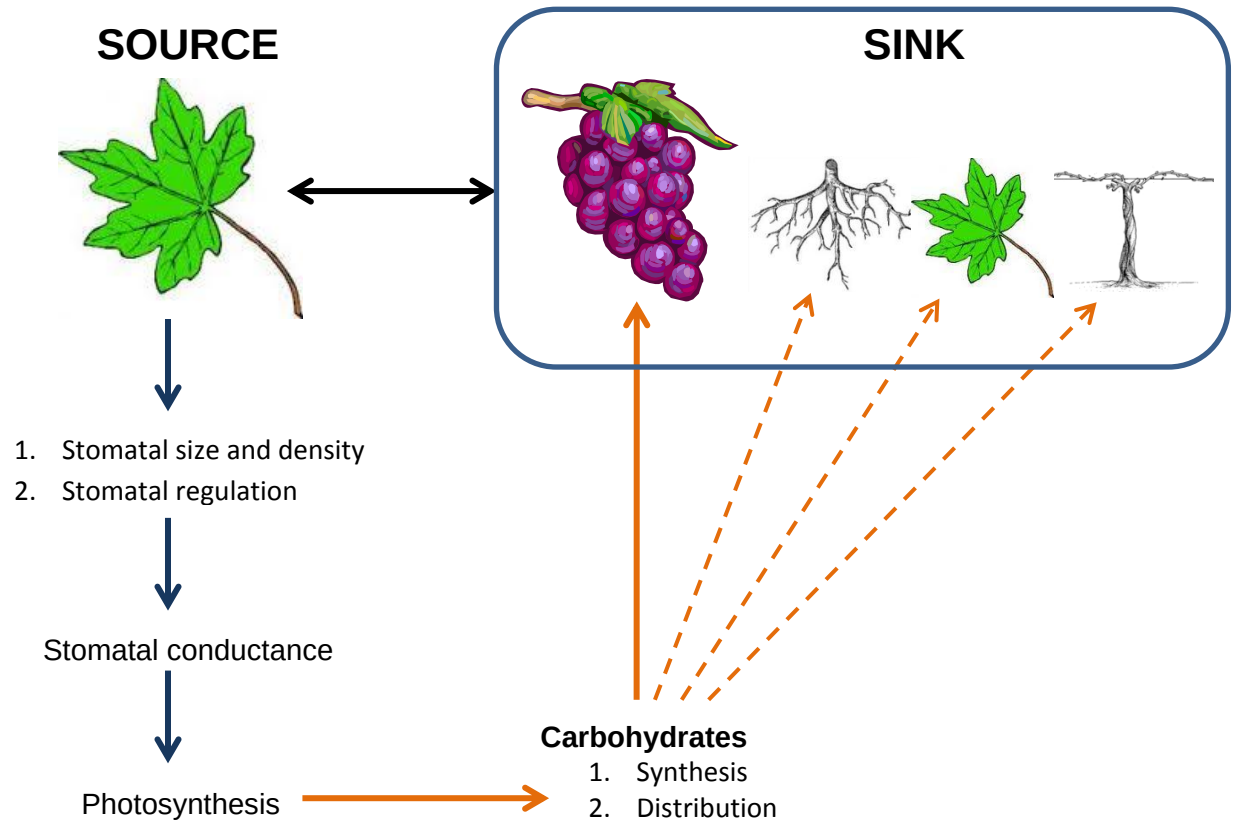

Figure 4.1 This scheme illustrates the relationship between the source and sink organs during the ripening period. During this time the main sink organ is the fruit, but other organs including the roots, stem and leaves also act as sinks

The partitioning of these carbohydrates is not a simple process as the needs of roots and leaves for respiration must be taken into account. Also, the energy requirements for both maintenance and growth of the vegetative part of the vine must not be neglected.

It is accurate to state that from véraison onwards the grape berry is a strong sink; however, it should not be assumed that it is the only sink. In order for the roots to survive, a certain percentage of carbohydrates must be allocated to them for various applications. A portion of the carbon is necessary for respiration for the maintenance of the existing biomass. A further fraction will be utilised for growth respiration, thereby allowing for the development of the root system and the replacement of damaged or dead parts. Respiration will also be required for ion uptake; however, this will be dependent on the nutrient requirements of the entire plant. Lastly, carbon may be lost due to leakages, stress conditions and root associations such as with mycorrhizal fungi (Buwalda, 1993). Leaves are to a large extent able to acquire the energy they need from photosynthesis, however, a certain percentage of energy is provided by respiration. (Cannell & Thornley, 2000). Stem respiration should also be taken into consideration. Escalona *et al* (2012) found that under irrigation, i.e., in a non-stressed environment, the estimated carbon losses due to respiration amounted to 47 g to 65 g per plant. This equated to 30% to 50% of the total estimated gains due to photosynthesis. Furthermore, respiration by the root system represented a percentage of 70 to 80 of the total carbon losses, illustrating the large requirements of this organ. The remaining percentage consisted of both leaf and stem respiration. (Escalona *et al.*, 2012).

In order to study source sink relationships, it is important to understand a few key concepts.

- The composition of xylem and phloem fluids is a crucial element in source-sink relationships. Xylem exudates from woody plants consist mainly of minerals, macro and microelements (cations and anions), as well as amino and organic acids, sugars and growth regulators (Glad *et al.*, 1992). Phloem exudates consist mainly of various organic compounds. Sucrose represents the main carbohydrate transported in the phloem, with lesser percentages of glucose and fructose. Organic acids, including predominantly tartaric and malic acid are present in phloem sap, however lower levels of citric acid can also be found. Additionally, amino acids are similarly transported in the phloem tissue, with glutamine making up the majority, followed by proline, with the rest making up a small percentage. Potassium has also been found in phloem exudate, with concentrations increasing until fruit set (Glad *et al.*, 1992).
- The role of invertase and sucrose synthase at the fruit level in terms of sugar partitioning in berry cell's vacuoles, i.e. the ratio between glucose and fructose as well as the sucrose content from véraison. The sugar which is imported is converted into hexoses and stored in the vacuole. This conversion could occur in the apoplast, cytoplasm or

vacuole itself (Coombe 1992). Hawker (1969a) found that the activity of invertase was higher than that of sucrose synthase, an enzyme also capable of hydrolysing sucrose. Invertase activity in the vacuole would suggest that sucrose transporters are present to move sucrose from the apoplast into the vacuole, where it would be converted. Sucrose could also be converted in the apoplast or the cytoplasm, where after hexoses would be transported into the vacuole by hexose carriers (Robinson & Davies, 2008)

- The abiotic and genetic control of leaf stomatal density and size during leaf growth. Various abiotic factors have been found to influence both stomatal size and density, including CO^2, water stress, light and temperature. Additionally, the interaction between the rootstock and the scion will determine size and density of stomata at a genetic level. Size and density are set once a leaf reaches the adult phase.(Nadeau & Sack, 2002)
- Abiotic and genetic stomatal regulation occurs once the leaves reach the adult phase Abiotic regulation is a process whereby plants are able to control the aperture of stomata to counteract certain effects caused by factors such as light or water stress. Genetic control again implies the interaction between the scion and the rootstock.
- Photosynthesis and photorespiration of adult leaves (75% of their full size) are also under abiotic (light, CO_2, temperature) and genetic control (cultivar, rootstock influence)
- Beside stomatal regulation and photosynthesis, it should be noticed that plant age and annual climatic variation, which will dictate to a certain extend annual primary and secondary shoot growth, leaf thickness and leaf area per vine, in relationship with the root system morphology and size (rootstock interaction), should play a role in vine water use efficiency and rate of photosynthesis.
- The vine is able, to a considerable degree, to compensate when leaf area and crop load are manipulated (Candolfi-Vasconcelos *et al.*, 1994; Petrie *et al.*, 2000).
- Carbohydrates distribution and storage during the growing season is complex and not totally understood.

The relationship between functioning leaves and sugar accumulation in fruit is complicated, but must be taken into consideration. It is also imperative that the production and distribution of carbohydrates be understood. New scientific approaches must be applied to find innovative ways of determining the relationship between source and sink tissues, as opposed to continuing to use the classical ratios such as the ratio between leaf area and yield or pruning mass and yield. One such method, which has been proposed, is the use of berry sugar accumulation as a physiological indicator to study the relationship between leaves and fruit. Sadras *et al* (2008) stated that the rate of accumulation is the key indicator related to timing of fruit maturity. This approach offers a more dynamic view of source-sink relationships over a longer period of time rather that a snapshot view at the end of ripening (Sadras *et al.*, 2008).

4.2 METHOD DEVELOPMENT

A study was conducted on Cabernet Sauvignon in order to address the following topics:

- The source-sink interactive relationship between leaves and grapes (post véraison) on a primary shoot.
- Dynamic of berry sugar and water accumulation during berry ripening (post véraison).
- Relation between the volume of a berry and its sugar content.
- The effect of the isolation of one sink from carbohydrate import using girdling on the remaining sink.

The study was executed using primary shoots as biological replicates. Girdling of the shoots was proposed as a method to try and find answers to some of these questions regarding the dynamic of berry sugar and water accumulation. Furthermore, berry classification according to diameter was carried out, thereby providing the unique opportunity to study berries of the same volume.

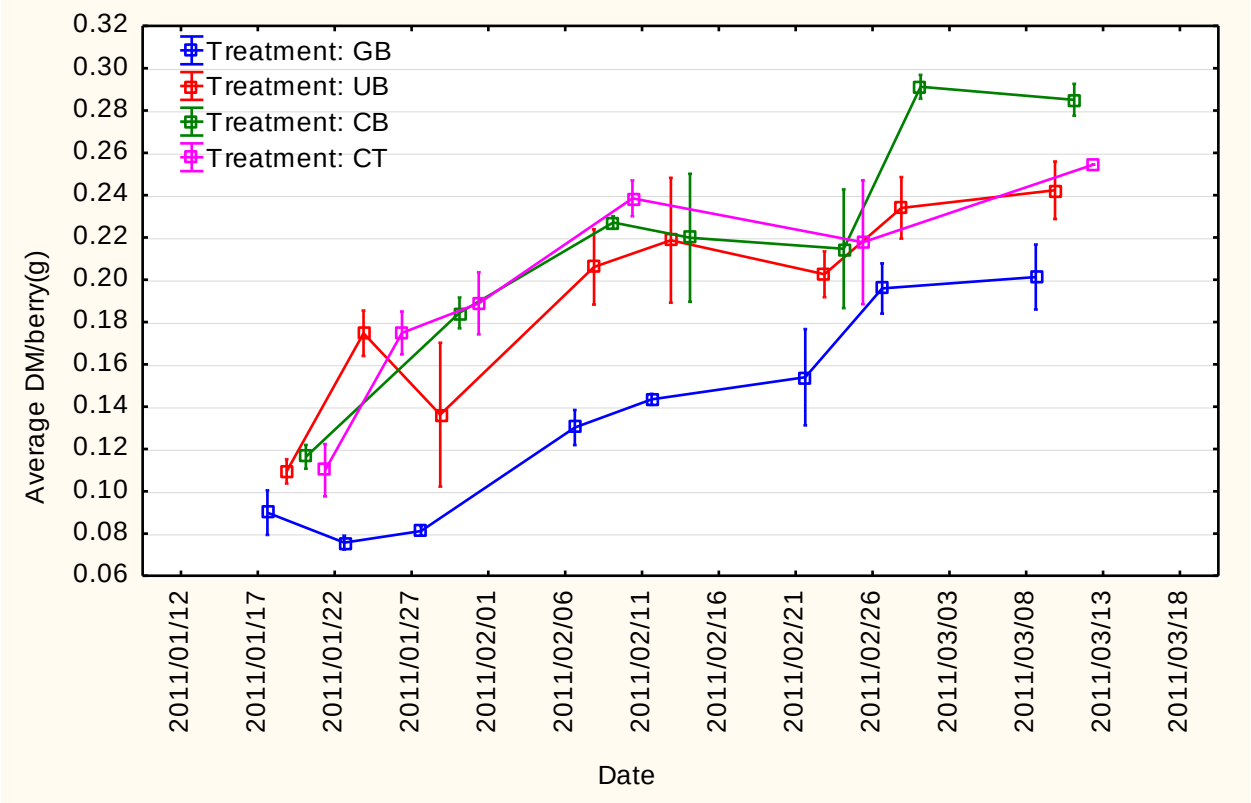

Figure 4.2 The mean plot of average berry dry mass measured in grams over the 2011 ripening period, between véraison and harvest, for the four different treatments. Only berries from the 11.5 mm and 12.5 mm diameter classes were used to create this figure. The bars indicate standard error.

Figure 4.2 indicates the changes in the average dry mass per berry over the 2011 ripening season. Only berries from the 11.5 mm and 12.5 mm diameter classes were used to create this figure. It is clear from figure 4.2 that girdling at the shoot level was able to interrupt carbohydrate transport into the berries, as a much lower average dry mass per berry was observed for the girdled bunch (GB) than the other three bunches. Nevertheless, it was observed that after two to three weeks, from between 01/02/2011 and 09/02/2011, an increase in dry mass occurred. When a shoot is girdled, the phloem is separated at the cambium level. Phloem tissue is able to regenerate after a certain amount of time due to cambial activity (Goren *et al.*, 2004; Zhang *et al.*, 2011). The formation of callus tissue at the margins of the girdling wound was seen in the vineyard after approximately two weeks (Figure 4.3). The observed increase in dry mass indicated a re-establishment of conductive tissue which resulted in an increase in sugar concentration over the ripening period.

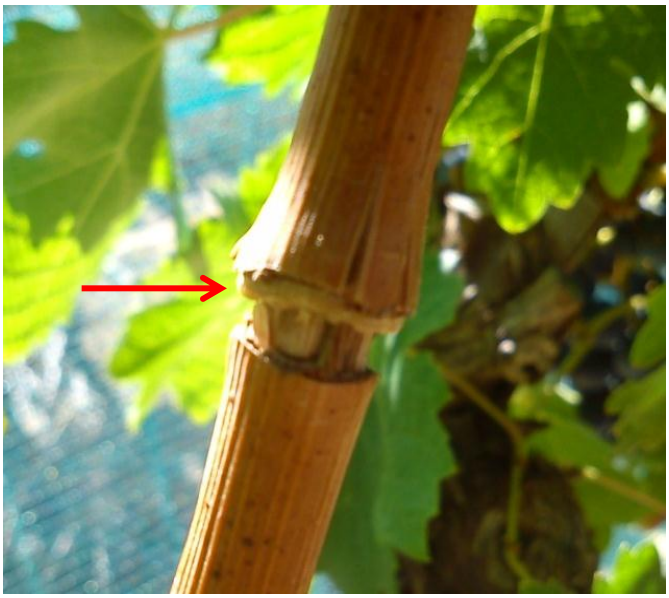

Figure 4.3 Callus formation occurred at the girdling wound margins after approximately two weeks

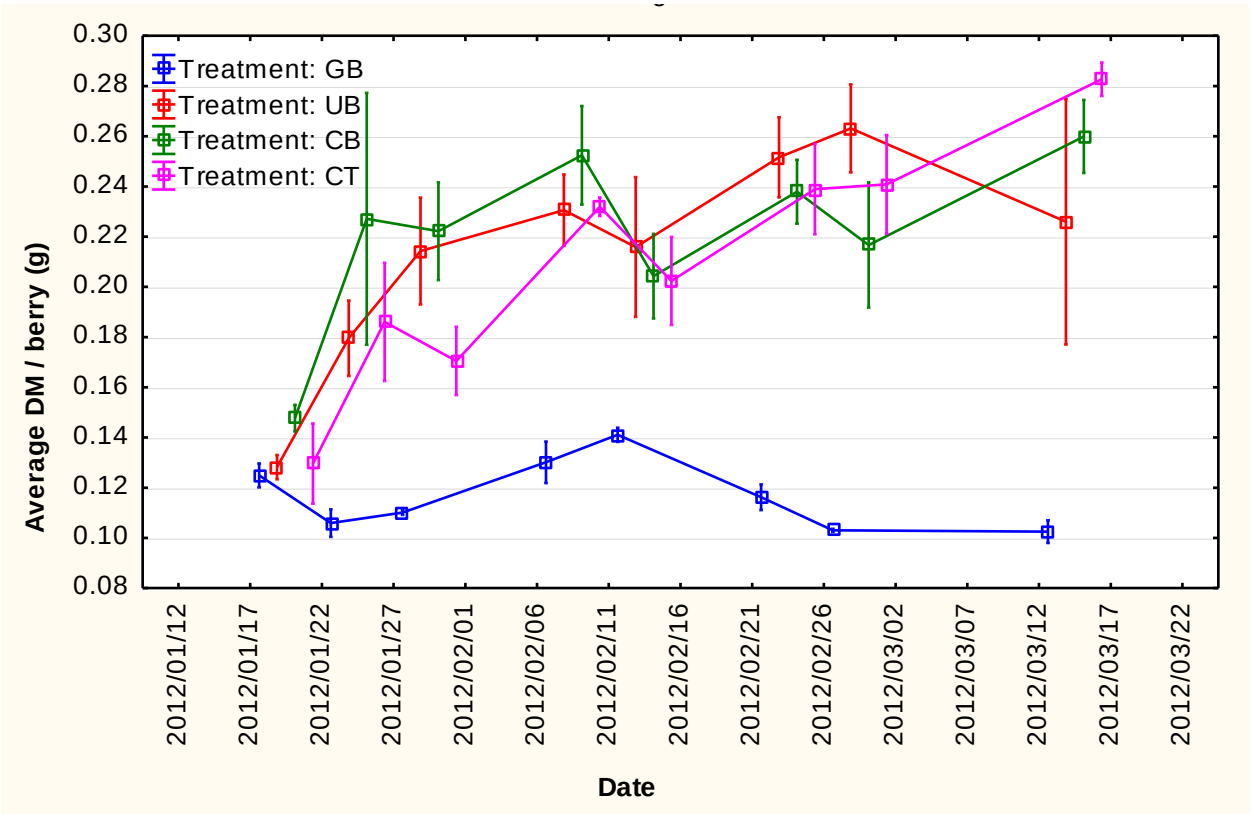

Figure 4.4 The mean plot of average berry dry mass measured in grams over the 2012 ripening period, between véraison and harvest, for the four different treatments. Only berries from the 11.5 mm and 12.5 mm diameter classes were used to create this figure. The bars indicate standard error

Figure 4.4 depicts the average dry mass per berry, measured over the 2012 ripening season, again using only berries form the 11.5 mm and 12.5 mm diameter class. A slight increase in average dry mass was noted in the girdled bunch after two weeks. At this point callus tissue formation at the girdling wound margins was observed in the vineyard (Figure 4.3). The girdling wound was re-opened at this point, every two weeks, which resulted in a more consistently low concentration of sucrose.

A varying average berry dry mass during 2012 could indicate that the girdling wound interruption was not wholly complete. This could be because the girdling method was not absolutely effective, or it could indicate that carbohydrate transport followed an alternative route other than via the phloem tissue. It has been stated that the xylem and phloem tissue are interconnected along their entire length and are therefore readily able to exchange water and solutes (Zwieniecki *et al.*, 2004). Solutes could therefore possibly have bypassed the wound via the xylem tissue. In order to conclusively determine whether the girdling method was effective or not, or if solutes were indeed able to circumvent the girdling wound by moving into the xylem tissue, it would be beneficial to use a tracer to follow the movement of sugar through the phloem tissue. In the outer pericarp of tomato fruit, 6(5) carboxyfluorescein and (^{14}C)glucose have been used as a symplastic tracer to study post phloem sugar transport (Ruan & Patrick, 1995).

Additionally, the application of $^{14}CO_2$ was used to study the distribution of carbohydrates within a vine (Candolfi-Vasconcelos et al., 1994). Similar methods should be employed to follow the movement of sugars through the vine and thereby determine the effectiveness of girdling.

4.3 BALANCE OF THE VINES IN THE EXPERIMENTAL BLOCK

Berry sugar loading in conjunction with the changes in sugar concentration may be considered a useful indicator of vine balance and also grape quality. This approach takes into account the accumulation of the sugar per berry (mg per berry) and therefore enables the kinetics of sugar concentration changes to be monitored. Kinetic monitoring of the quantity of sugar per berry may be considered as a method of measuring the plant's physiological functioning (Carbonneau *et al.,* 1998, Deloire *et al.,* 2004, Wang *et al.,* 2003), particularly photosynthesis, which is a reliable indirect indicator of the temperatures to which the vine is subjected under given conditions over a specific time period and with a certain grapevine water status.

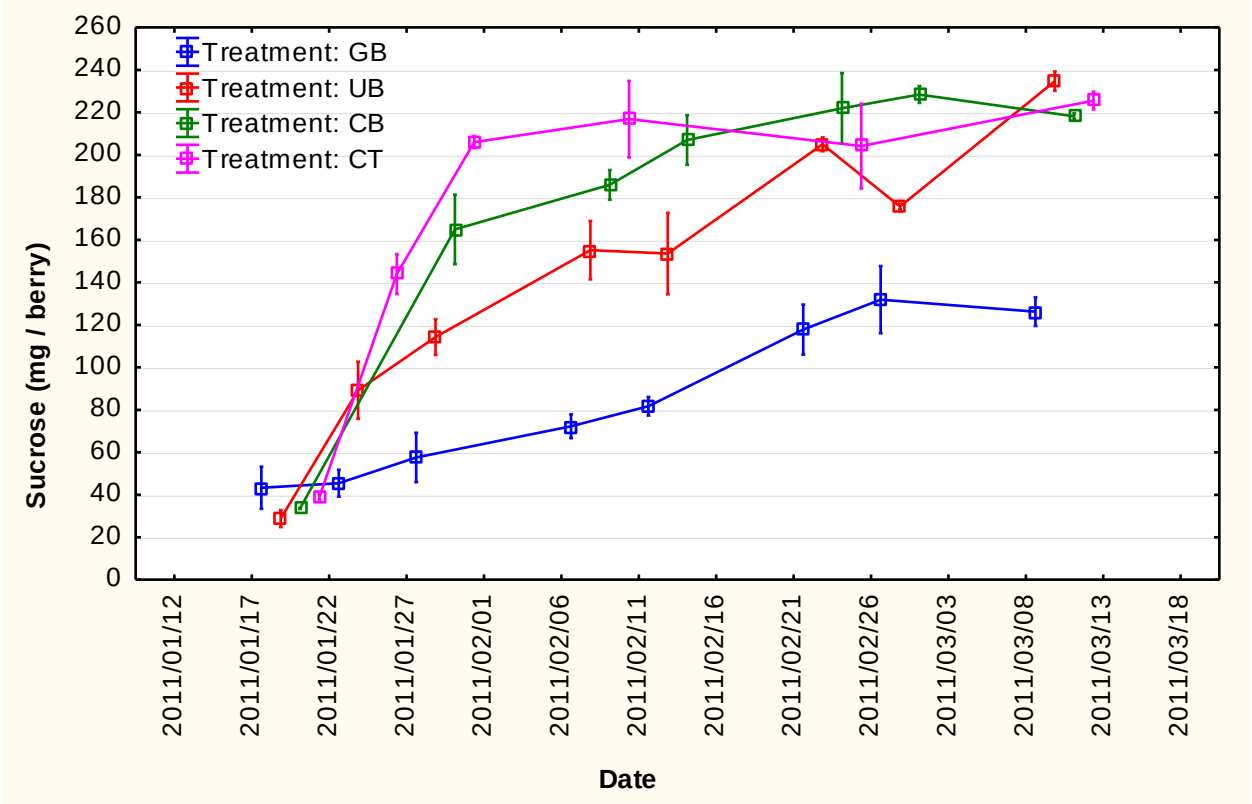

Figure 4.5 The mean plot of the average sucrose per berry measured over the 2011 ripening period for the four different treatments. All berries used to create this graph were from the 11.5 mm and 12.5 mm diameter class. The bars indicate standard error

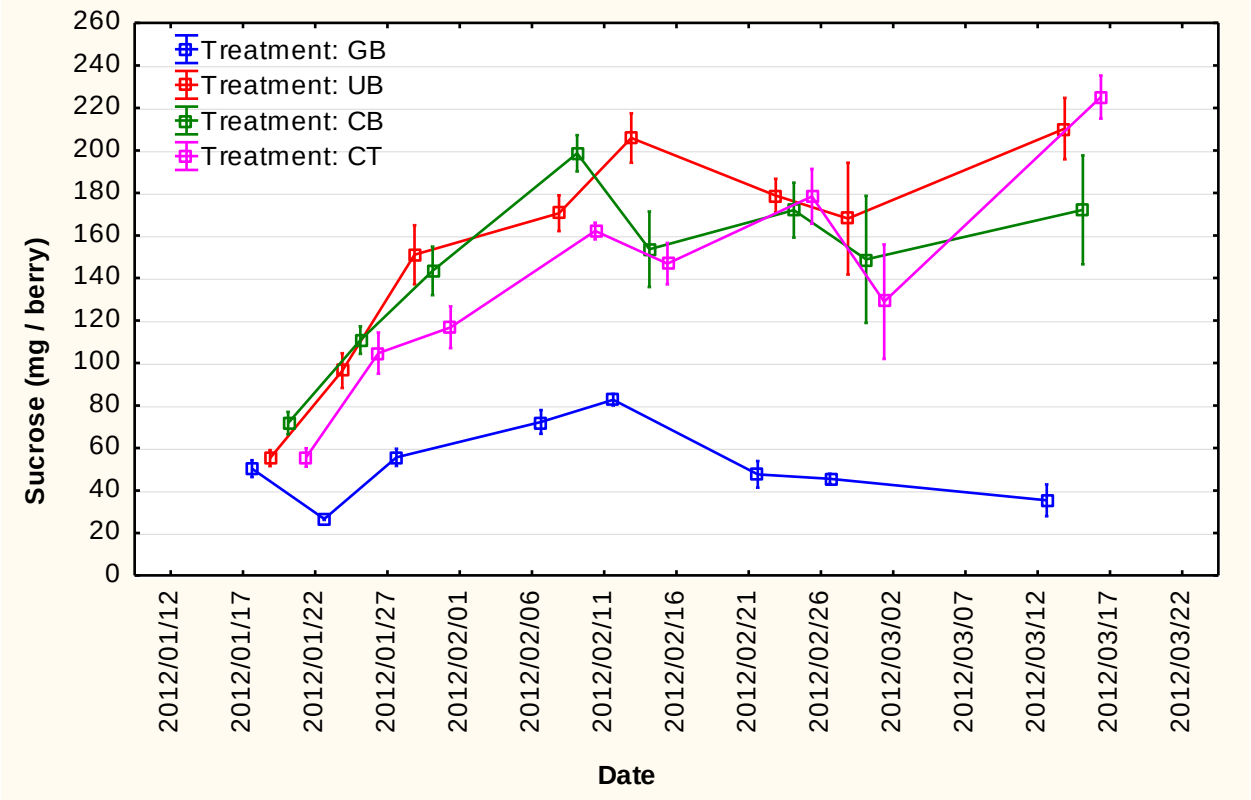

Figure 4.6 The mean plot of the average sucrose per berry measured over the 2012 ripening period for the four different treatments. All berries used to create this graph were from the 11.5 mm and 12.5 mm diameter class. The bars indicate standard error.

Figures 4.5 and 4.6 show the curves for sucrose content for both the 2011 and 2012 ripening seasons respectively. Berries from the 11.5 mm and 12.5 mm diameter classes were exclusively used at every date to create the curves. For Cabernet Sauvignon, it is considered normal if approximately 7 to 8 mg of sugar is accumulated per berry, per day until a plateau, or a slower rate of accumulation is reached (Deloire, 2011). In 2011, over a period of 22 days, from the 18/01/2012 to 09/02/2012 (where sugar accumulation slowed down), the average amount of sugar accumulation per day can be calculated from the CB curve as follows:

$$\frac{185 - 35\,mg/berry}{22\,days} = 6.82\,mg/berry\,/day$$

The same calculation can be applied to the 2012 figure using the CB curve. In this situation a daily rate of 6.36 mg sucrose/berry/day was observed.

$$\frac{200 - 60\,mg/berry}{22\,days} = 6.36\,mg/berry\,/\,day$$

Both correlate with the normal rate of sugar accumulation as stated in literature. Furthermore, sugar accumulation for both seasons plateaued between 160mg and 220 mg of sucrose per

berry, which also agrees with the literature (Van Leeuwen *et al.*, 2008). The fact that a plateau is reached indicates that a relationship possibly exists between the source and the sink. The plateau, or slower sugar accumulation occurs in relation to the capacity of a cell to accumulate sugar and also to the osmotic pressure of the phloem tissue. In addition, a signalling process between the leaves and the fruit may also be involved.

From figure 4.6, it can be seen that a late season spike in sucrose content was observed in the top control bunch (CT). This broaches the question of fruit functioning heterogeneity. The fluctuation is possibly due to the fact that sampling occurred across several vines which had inherently different levels of reserves, source-sink relations, etc. If repeat sampling had been possible on the same vine then this variability over time would have been overcome to some extent. Furthermore, working in a controlled environment may have helped in reducing or understanding this fluctuation.

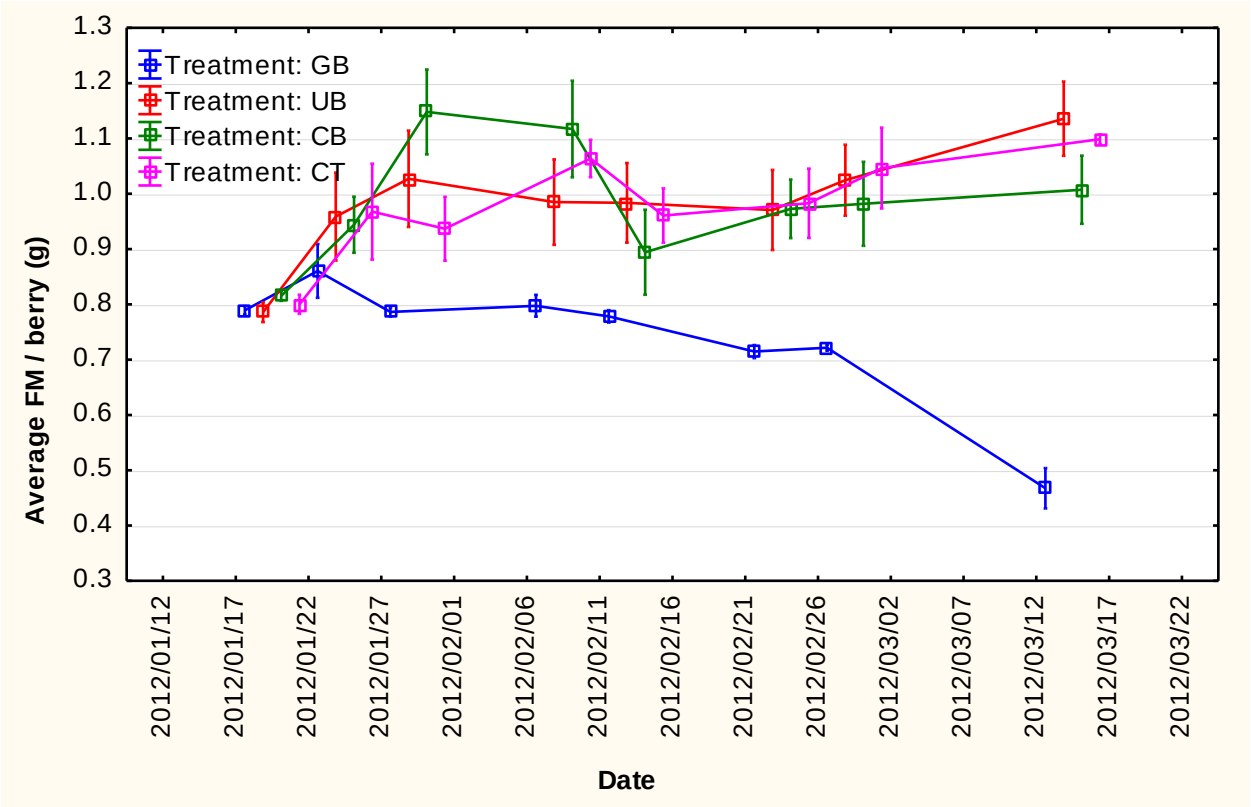

Figure 4.7 The mean plot of average fresh mass per berry measure in grams over the 2012 ripening period, between véraison and harvest, for the four different treatments. Only berries from the 11.5 mm and 12.5 mm diameter classes were used to create this figure. The bars indicate standard error

Figure 4.7 shows the evolution of the average FM per berry using only berries from the 11.5 mm and 12.5 mm diameter classes, for the 2012 season. The average berry fresh mass plateaued at approximately 1 g. This again coincides with what is stated in literature, indicating normal

development and proper water management. It must however be noted that berry fresh mass evolution is limited even in a well-watered situation due to fruit cell number and enlargement, both of which are limited. In a water-stressed environment however, smaller berries would be produced. Water was applied according to stem water potential readings, thereby ensuring that no water contraints were experience by the vine. The use of these curves to indicate vine functioning demonstates the value of this information and its potential use in developing a more dynamic physiological indicator.

4.4 GIRDLING

The rate of sugar accumulation can be divided into two phases. The first phase concerns a sharp increase in sugar loading, whereas the second phase demonstrates a more gradual sugar accumulation (Figure 4.8).

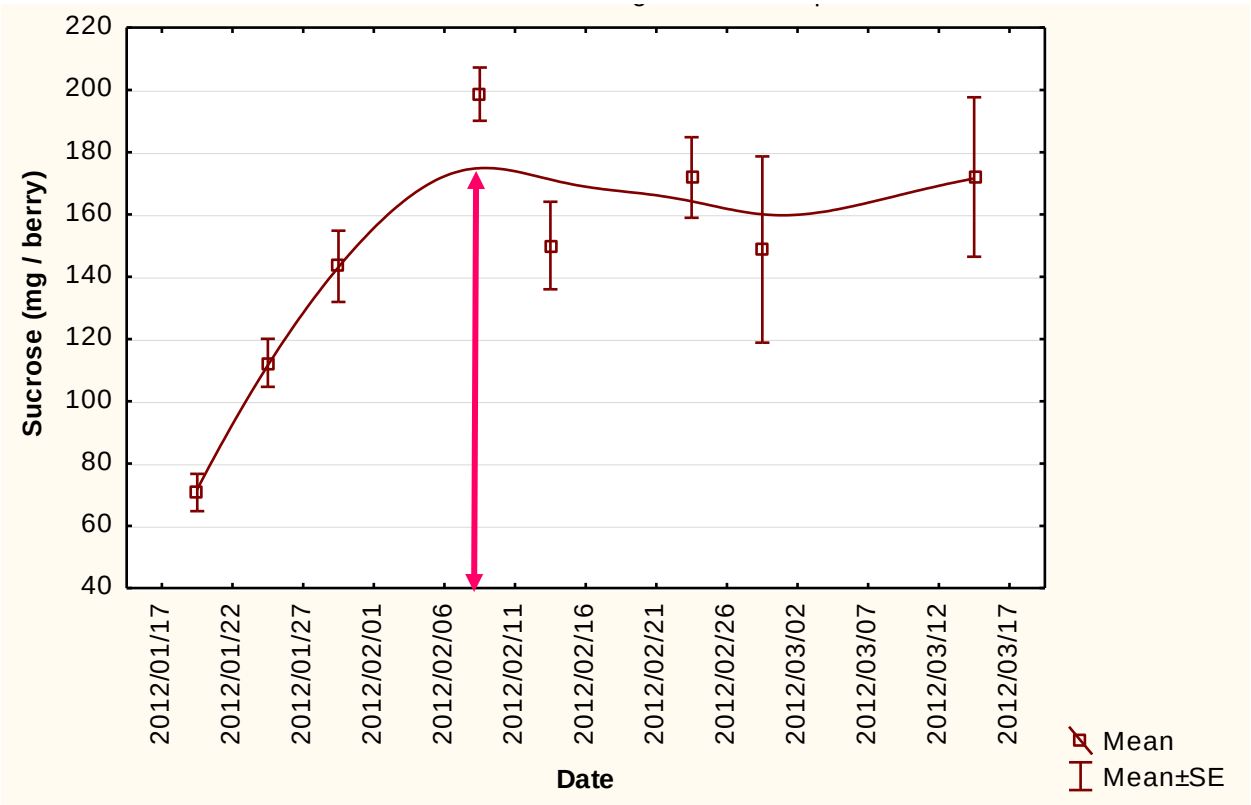

Figure 4.8 The mean plot of average sucrose per berry over the 2012 ripening period for the bottom control bunch. All berries used to create this graph were from the 11.5 mm and 12.5 mm diameter class. The bars indicate standard error. Mean = Distance weighted least squares. The pink arrow indicates the key point where sugar loading slows down.

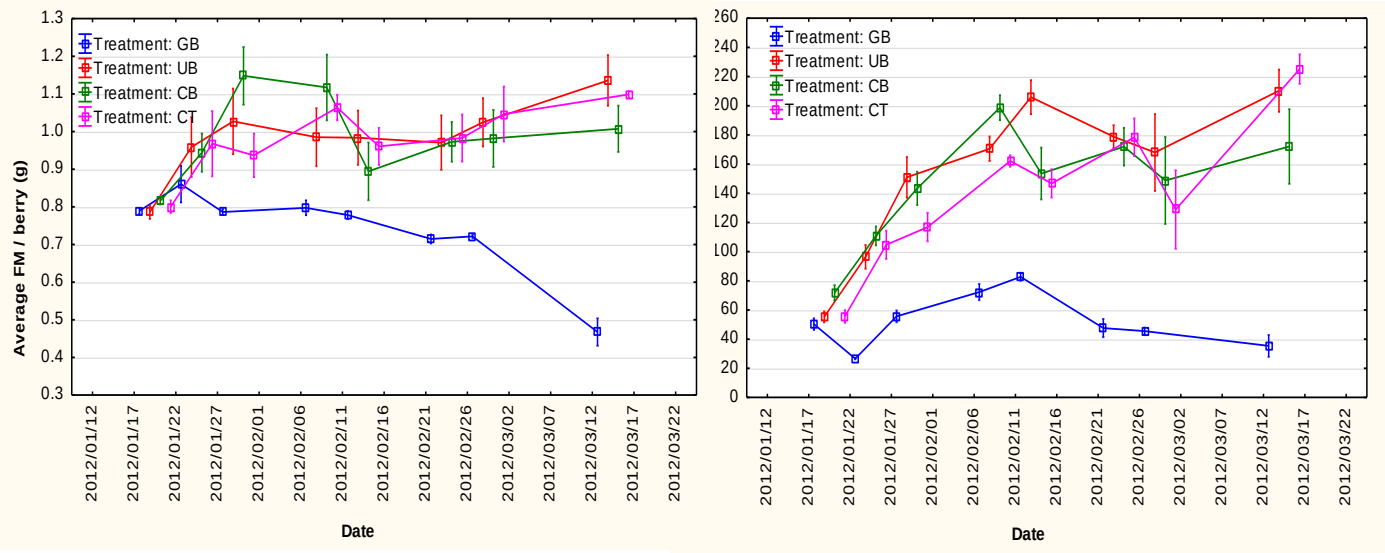

Figure 4.9 The mean plots of both average berry fresh mass and sucrose concentration for the 2012 ripening season. All four treatments are represented in both graphs using only berries from the 11.5 mm and 12.5 mm diameter classes. The comparison between the two graphs demonstrates that sugar accumulation had an effect on berry fresh mass. The bars indicate standard error.

It is clear from figure 4.9 (11.5 mm and 12.5 mm diameter class berries only) that the sugar accumulation affected the average berry fresh mass during the first phase. When the pathway of sugar loading was interrupted with girdling, there was a marked decrease in the average berry fresh mass. Furthermore, an increase in sugar will lead to an increase in fresh mass, as was demonstrated by the two control curves as well as the ungirdled bunch curve. This is due to the general growth of the berries according to the developmental curve. The two parameters can also be said to be linked due to osmotic pressure potentials. The increase in sugar will lead to the uptake of water which will result in an increase in fresh mass (in this regard, the role of berry evapotranspiration in conjunction with water and sugar accumulation is not clear).

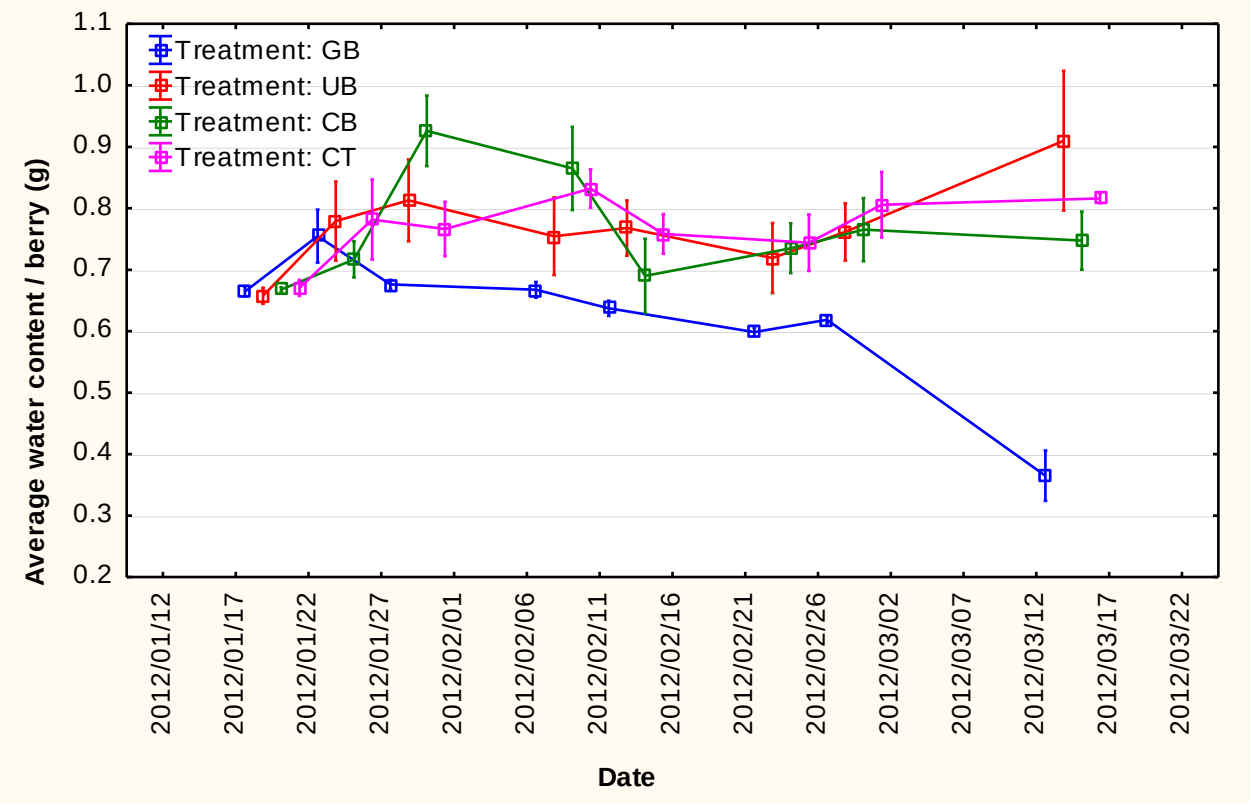

Figure 4.9 The mean plot of average water content per berry measured in grams over the 2012 ripening period, between véraison and harvest, for the four different treatments. Only berries from the 11.5 mm and 12.5 mm diameter classes were used to create this figure. The bars indicate standard error

Figure 4.9 shows the average water content per berry for the four treatments. To create the graph, the difference between berry fresh and dry mass was calculated using berries from the 11.5 mm and 12.5 mm diameter class. The average water content for the ungirdled bunch and the two control bunches remained relatively stable throughout the season. Water moves into a berry and is then lost due to transpiration, thereby keeping the amount of water within the berry generally stable. The process of transpiration is necessary to ensure a continued movement of water into the berry which has been hypothesised to be necessary for sugar loading. Certain literature states that from véraison onwards, the xylem tissue becomes dysfunctional (Findlay *et al.*, 1987); however, other studies have shown that it is still partially functional after véraison (Bondada *et al.*, 2005; Rogiers *et al.*, 2008). It is therefore possible that xylem flow contributed to the fresh mass of the berries. The fact that the water content in the girdled bunch was not zero could indicate that the xylem tissue was still functional to a degree. It could also indicate that the phloem disconnection was not wholly successful. To validate this, biological tracers could be used to follow the movement of water through both the xylem and the phloem. Fresh mass can be said to be a function of water input and water loss through transpiration and possible backflow. A correlation was found to exist between berry fresh mass and water content ($r = 0.94$, $r^2 = 0.88$).

Using girdling methods in combination with berry classification according to diameter, it can be demonstrated that there is no direct relationship between the volume of the berry and its sugar content, i.e. berries with the same volume can have different sugar concentrations. This is clearly demonstrated in figure 4.6 which shows the average sucrose concentration per berry during the 2012 ripening period. If the curve for the bottom control bunch (CB) is taken into consideration, it can evidently be seen that there exists a marked difference between the sugar concentration at the first and the last date. The final sugar concentration was approximately 170 mg per berry, whereas the initial sugar concentration was only 60 mg per berry. This indicates a 35 % difference in sugar concentration between the two dates. However, all the berries used to create this curve came from the 11.5 mm and 12.5 mm diameter class. This clearly shows dissociation between berry volume and sugar concentration. On the contrary, Dreier *et al*, 2000 claimed that there exists a close correlation between berry radius (i.e. berry size) and final sugar concentration. Hunter & Ruffner (2001) also claimed that osmotic gradient was the main driver of phloem translocation to berries during the ripening period. They also employed girdling as a method, however, this was only executed above the bunches at the primary shoot level, which did not isolate the bunch from the perennial part of the vine. The results showed an increase in berry sugar concentration with girdling, which could be attributed to berry concentration and/or to an import for the rest of the vine. By girdling above and below the bunch at the primary shoot level, bunches were completely isolated from any carbohydrate import in the current study. Furthermore, berry classification according to diameter provided the unique opportunity to study berries of the same global volume.

Recent studies on molecular biology of berry ripening have shown the involvement of sugar transporters in berry sugar loading. Protein transporters which facilitate the movement of sugars into the vacuole may regulate the accumulation of sugar. Both sucrose and hexose transporter cDNAs have been isolated from ripening grape berries. (Robinson & Davies, 2000). The processes of ripening are probably controlled at the level of transcription and translation and the numerous processes which are involved are coordinated through changes in the expression of relevant genes.

In parallel, the concept of berry water backflow (Tyerman *et al.* 2004) showed , in agreement with our results, that berry evapotranspiration is not the main driver of berry sugar accumulation. It is possible for the berry to carry on loading sugar, even in the instance of berry water backflow. Berry sugar accumulation will depend on the functioning of the source (current photosynthesis of the leaves and sometimes wood reserves) and will be mediated at the berry level by the expression and activity of sugar transporters. The shift of phloem unloading from a symplasmic to an apoplasmic pathway, which occurs after véraison, is accompanied by an increase of the expression and activity of cell wall invertases (Zhang *et al*., 2006). Movement

from the apoplast requires energy and the involvement of membrane located transporter proteins and therefore, a certain amount of energy. These transport proteins are necessary to transport sugar from the phloem and to facilitate the uptake and compartmentaion of sugars by moving them across the plasma membrane as well as the vacuole tonoplast. The up-regulation of genes encoding for these transporters occurs at véraison, when berry sugar accumulation increases (Davies *et al*., 1999; Manning *et al*., 2001). The expression of genes encoding both transporters and invertases indicate that the process of sugar accumulation is probably under regulation.

The use of molecular biology techniques has thus far been greatly beneficial towards better understanding plant functioning and further application will supplement current knowledge which would consequently lead to a better comprehension of berry ripening. (Robinson & Davies, 2000). This in turn will supplement the development of a more dynamic physiological indicator of vine balance and grape berry ripening.

4.5 DRIVERS OF BERRY VOLUME

Fruit volumetric growth is a direct result of water accumulation, as well as solute concentration and therefore, the maintenance of fruit growth relies on a combination of water and solute transport, both at the vascular tissue and individual cell level. Transpiration as well as the presence of an osmotic gradient are both necessary to ensure a favourable gradient for water uptake (Greenspan *et al.*, 1994).

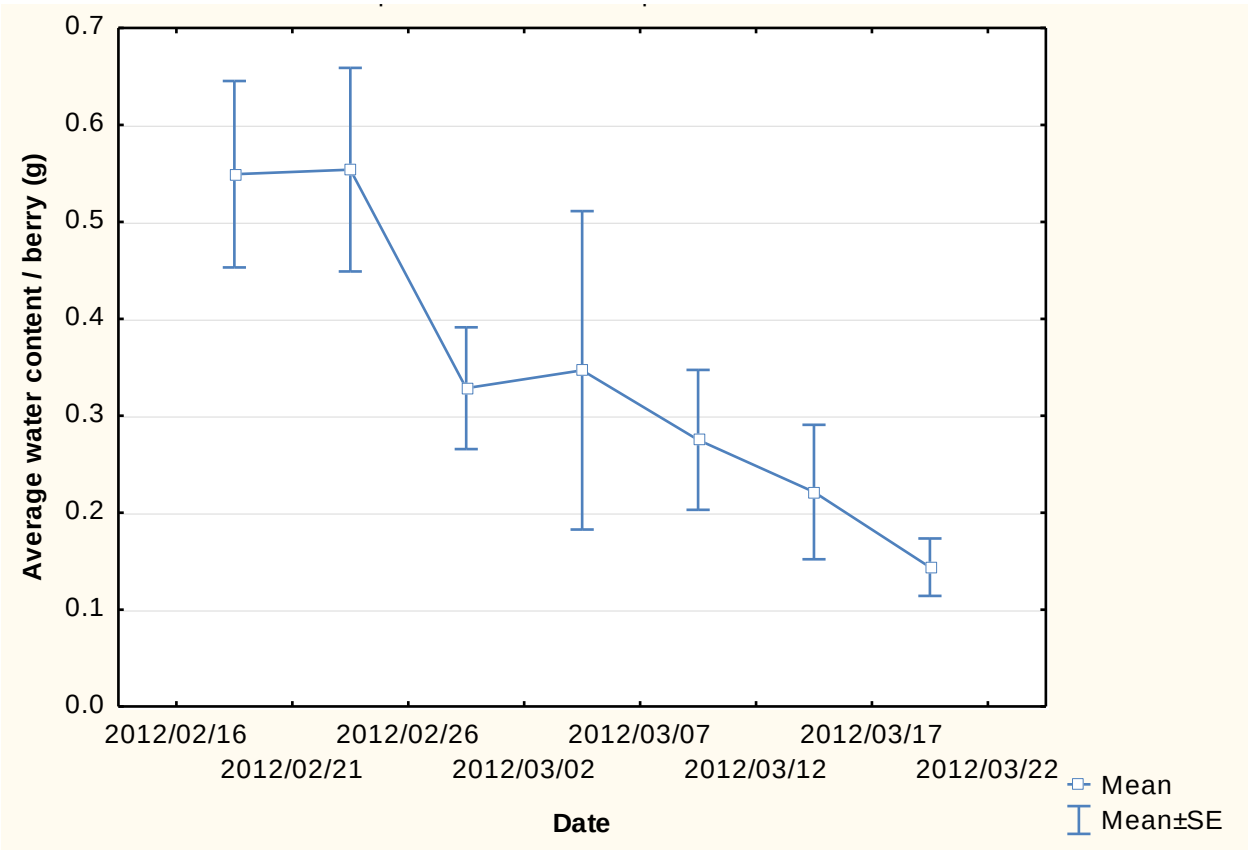

Figure 4.10 The mean plot indicating the average water content per berry over the 2012 ripening period from which the transpiration rate can be calculated. The data was attained using bunches which were cut from the vine and suspended in the canopy. A progressive loss of water from the berries resulted from the treatment. The bars indicate standard error.

Figure 4.10 shows the progressive loss of water from a berry population over the ripening period. The data was attained from bunches which were cut from the vine and then hung in the canopy. The transpiration rate for the duration of this period can be calculated as follows:

$$\frac{0.55 - 0.15\ g\ water}{29\ days} = 0.0138 g/berry/day$$

As an example, the average amount of water lost from a vineyard through the bunches which produces 10 ton per hectare can be calculated as follows, if the average berry weight of Cabernet Sauvignon is accepted to be 1.2 g:

$$10 T/ha = 10\,000\,000 g\ berries/ha$$

$$\frac{10\,000\,000}{1.2\ g} \times 0.0138 = 115\,000\ g\ of\ water$$

$$= 115\ kg\ water$$

$$= 115\ l\ water\ lost\ from\ the\ vineyard\ block\ per\ day$$

Although water uptake and berry water content are related to volume, berry volume is ultimately determined by cell multiplication and cell enlargement. Cell division occurs before véraison, mostly in the eight to ten days which follow flowering, and determines the potential for the final berry size. The size of a berry cell is not infinite, therefore cell enlargement is limited.

Figure 4.11 A few berries sampled from bunches which were encased in foil bags to impede transpiration displayed cracking, mostly around the pedicel. These berries were from the larger diameter classes, namely 13.5 mm and 14.5 mm. This was observed in both ripening seasons

Figure 4.11 shows berries removed from bunches which were covered with silver foil bags in the vineyard to prevent water loss via transpiration. These berries represented the larger diameter classes, namely 13.5 mm and 14.5 mm. The cracking which is clearly seen indicates that the berries cannot take up water indefinitely and that the volume of a berry and therefore sizes of the cells are predetermined. However, the amount of water which can be taken up by the berries also depends on the speed at which it enters the berries. Slower water uptake results in a lower susceptibility to splitting. Nevertheless, it is possible that the number of cells which are present could be the main factor determining berry size, in a situation where water is not the limiting factor. It is clear that some form of regulation between water evapotranspiration and water accumulation is needed to ensure that berries do not burst. This may be mediated at the molecular level by the involvement of aquaporins. Aquaporins are water permeable protein channels which are embedded in the cellular membranes. They are responsible for the regulation of water movement across these membranes and consequently control the rate of water flow through the grapevine (Tyerman *et al.*, 2009). Aquaporins present in the vacuole tonoplast are probably responsible for the osmoregulation of the cytoplasm, which is capable of rapidly losing or taking up water (Daniels & Chrispeels, 2007).

With regard to cell division, it has been found over many years that cultivars have a specific average berry weight and therefore possibly a regulated rate of cell division Shiraz berries typically weigh 2 g, Merlot usually weighs approximately 1.6 g and Cabernet Sauvignon has an average berry weight of 1.1 g, of course depending on the vine water status. This indicates the

maximum berry size per cultivar could be to an extent genetically dependant. Cell division, which is partly responsible for berry size, could therefore also be genetically dependant. Cell division could be pre-programmed to a point, i.e. a certain cultivar will have a limit to the number of cells which are present after cell division has ceased. However, studies done have shown that abiotic factors will also have an effect on cell multiplication (Tardieu *et al.*, 2000). Another factor which could affect cell division and cell multiplication are hormones. In terms of cell enlargement, it is well known that gibberellins are applied to seedless table grapes to increase their size via cell enlargement. Also, cytokinins have been used in the table grape industry to increase cell division. Hormones are released from grape berry seeds although not exclusively. In order for a berry to develop properly, at least one functional seed is required. There is no direct relationship between seed number and berry volume as berry volume will be dependent on water and nitrogen. The effect of hormones, including polyamines, needs further attention with regard to their possible influences on cell division and enlargement

A further factor contributing to berry volume is the number and size of the seeds within the berry. The heterogeneity which persists with regard to seed number may be attributable to a number of aspects, including fertilisation and those factors which may affect it. A berry of the same volume may have a variable number of seeds. This was observed when a few berries from a particular diameter class were cut open. The number and size of seeds will also affect berry fresh mass.

In order to more accurately determine fresh mass, the seeds should first be removed. This however poses various problems, including the inevitable loss of berry tissue and juice when seeds are removed. Their effect should nevertheless be noted.

4.6 FRESH MASS AND PHLOEM DISCONNECTION

Véraison is signified by the onset of berry softening. Girdling was done four days after véraison, at a point where 100% berry softening had occurred and 10% of berries had started to colour; therefore the sugar accumulated during the time before girdling could have influenced the results. In order to prevent any sugar accumulation, girdling should ideally be done before véraison.

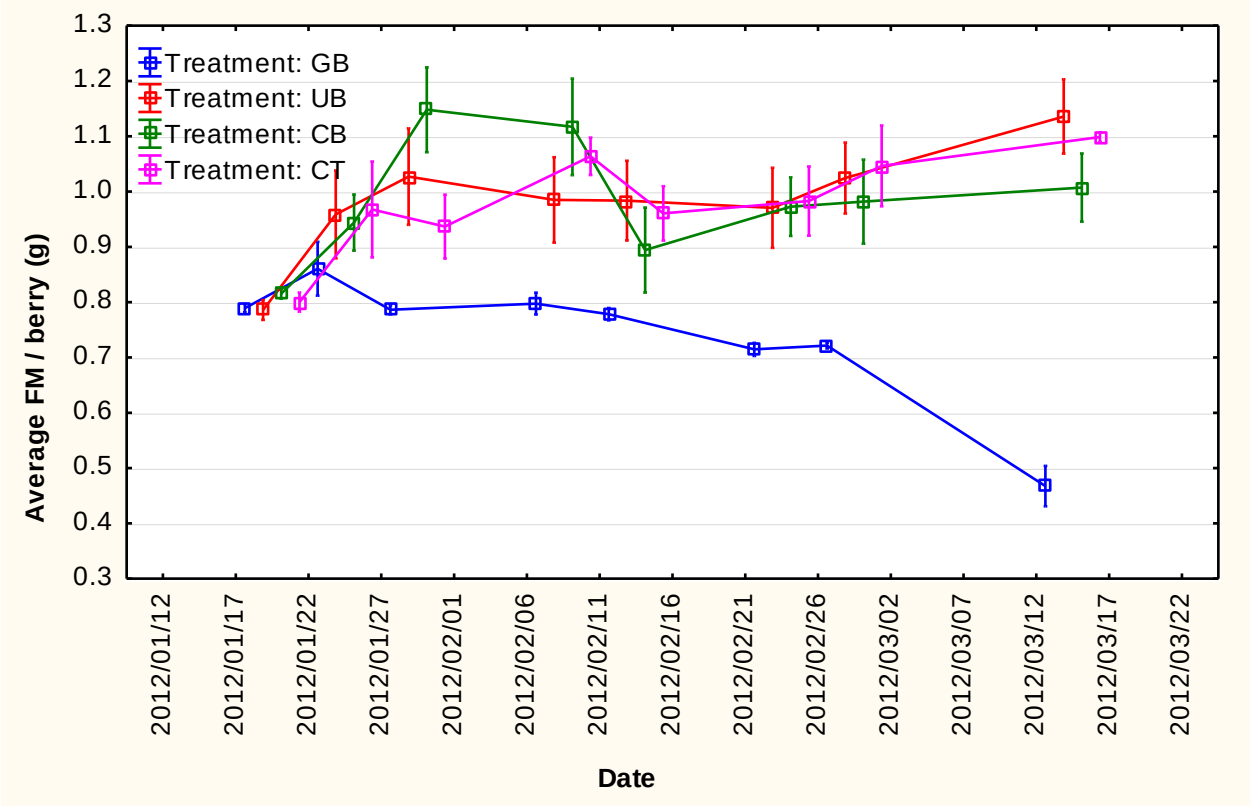

Figure 4.12 The mean plot of average fresh mass per berry measure in grams over the 2012 ripening period, between véraison and harvest, for the four different treatments. Only berries from the 11.5 mm and 12.5 mm diameter classes were used to create this figure. The bars indicate standard error

Figure 4.12 for 2012 shows the average fresh mass per berry measured in grams, again using only the 11.5 mm and 12.5 mm diameter class. Berry fresh mass remained stable since berries of the same volume were used. The lower initial berry fresh mass could possibly be explained by a lower sugar content, since berry fresh stabilised from about where the sugar loading plateau was reached. If the curve for the bottom control bunch (CB) is considered, it can be seen that a maximum average fresh mass per berry was reached at 1.1 g, where after it decreased slightly. Véraison started at approximately 0.8 g, therefore 73% of the maximum average fresh mass per berry was achieved before véraison. This means that only 27% of the maximum fresh mass was accumulated after véraison. This situation therefore corresponds to the norms of Cabernet Sauvignon development. Berry volume can therefore possibly be said to be pre-programmed in the sense that cell multiplication, which occurs before véraison, is mediated at a genetic level.

Furthermore, when looking at the control curves, it can be seen that an average fresh mass per berry of between 1.05 g and 1.15 g was attained. This conforms to the norms which have been observed for Cabernet Sauvignon, i.e. that the average maximum weight of Cabernet Sauvignon berries is 1.2 g, further suggesting that berry volume is pre-programmed (or not infinite) to an extent, in relation to cell multiplication.

In addition, it is interesting to note that the average fresh mass decreased slightly before harvest. Between 09/02/2012, which is where average fresh mass plateued, and 16/03/2012, just before harvest, a decrease of approximately 0.1 g occurred. This amounts to almost a 9% loss in average fresh mass per berry. The average fresh mass per berry began to decrease once sugar accumulation had plateaued, or slowed down. It can be accepted that berry fresh mass, to a certain extent, is related to berry sugar accumulation, as well as to water accumulation and the accrual of various other compounds. Furthermore, sugar which moves into the berry in contained within the berry vacuoles. Conversley, evapo-transpiration continues throughout the ripening season, therefore, the water which is translocated into the berry can be lost from it due to transpiration. At the point of average berry fresh mass decline, sugar transportation into the berry slowed down, however water movement into and from the berry continued as can be seen by the continued transpiration rate (Figure 4.10). It can therefore be hypothesised that the decrease in average berry fresh mass was due to the following possible reasons:

- a dysfunction of the phloem tissue at the berry level (peripheral vascular bundles)
- an imbalance between berry evapotranspiration and berry water accumulation
- possible water backflow .
- the possiblity of a decrease in cell vitality towards the end of the season (Tillbrook &Tyerman, 2008)

This phenomenon of phloem dysfunction has been proposed by a few other authors. (McCarthy & Coombe, 1999; Rogiers *et al.*, 2006). A disruption of the phloem tissue would have tremendous practical implications. Possibly the biggest problem which would be encountered would be the occurrence of berry shrivel. Not only would this lead to a more concentrated sugar level in the berry which would in turn result in higher alcohol wines, any decrease in berry volume would result in a decreased yield, resulting in significant financial losses. Furthermore, the resulting style of the wine would be changed due to the desiccation of the berries as these berries tend to give a more jammy character to a wine. In order to avoid this problem, harvest is normally carried out earlier in the ripening season; however, this practice often leads to the production of neutral wines as the grapes are harvested before they reach the mature fruit stage according to the berry aromatic sequence concept (Deloire, 2011; 2012). Deloire (2008, 2009, 2011) has shown that to achieve the berry ripening in terms of the berry aromatic sequence, it is necessary for the sugar per berry accumulation to plateau or to slow down, irrespective of the berry volume. Thereafter, the berries need to be left on the vine for an extended period of time in order to reach the desired wine aromatic phase. In red grape varieties, the aromatic sequence develops from fresh fruit aromas to neutral (or pre ripe in a particular site) and then to ripe fruit aromas through to mature fruit. The reactions involved are temperature dependant and

the evolution of the aroma compounds are highly dependent on bunch exposure and temperature and therefore, to climatic conditions over the ripening period. The time that berries are left on the vine will therefore be dependent on the region. Depending on when harvest is carried out, the wines will display a different aromatic profile. When the sugar per berry reaches a plateau or slows down, the practical target, which could be managed with precision irrigation using the leaf water potential thresholds, (Deloire *et al*., 2004; Chone et al, 2001) would be to maintain a constant berry volume and thus stabilise the brix levels or, more realistically, to get a slow brix increase while allowing aroma compounds and precursors to develop. However, the occurrence of phloem dysfunction at any point during the ripening season would negatively influence the endeavours to attain a more stable brix level. A cessation of water influx into the berry would inevitably result in berry desiccation, regardless of any irrigation applications. To determine whether phloem dysfunction is a definite reality and to consequently avoid further financial losses due to ineffective irrigation practices, as well as to find possible solutions to the problem, further studies on phloem function must be carried out.

4.7 SUGAR LOADING CURVES

Total sugars

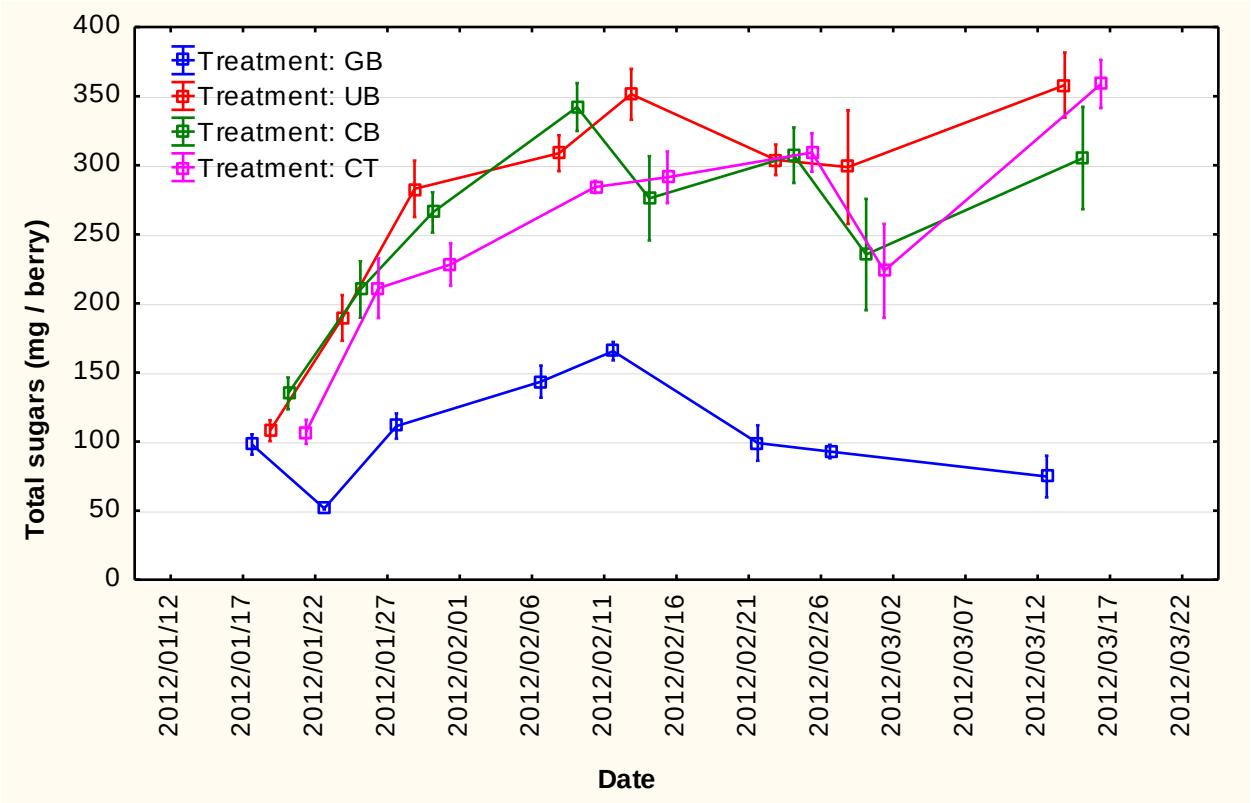

Figure 4.13 The mean plot of the average total sugars per berry measured over the 2012 ripening period for the four different treatments. The sugars include sucrose, glucose and fructose. All berries used to create this graph were from the 11.5 mm and 12.5 mm diameter class. The bars indicate standard error

Figure 4.13 shows the total sugar accumulation (sucrose, glucose and fructose combined) over the ripening period. Only berries from the 11.5 mm and 12.5 mm diameter classes were used to create the graph. From this figure it is clear that there was a rapid increase in sugar followed by a plateau or a slowed rate of sugar accumulation for the ungirdled bunch and two control bunches. The sugar content of the girdled bunch was always lower, but displayed some trends such as an initial increase in sugar accumulation and then a decrease towards the end of the season; however it remained lower than the other treatments. The increase could indicate that the girdling wound was not wholly effective, or possibly, a certain degree of sugar was able to bypass the girdling wound. This should be confirmed however using biological tracers.

4.7.1 Sucrose

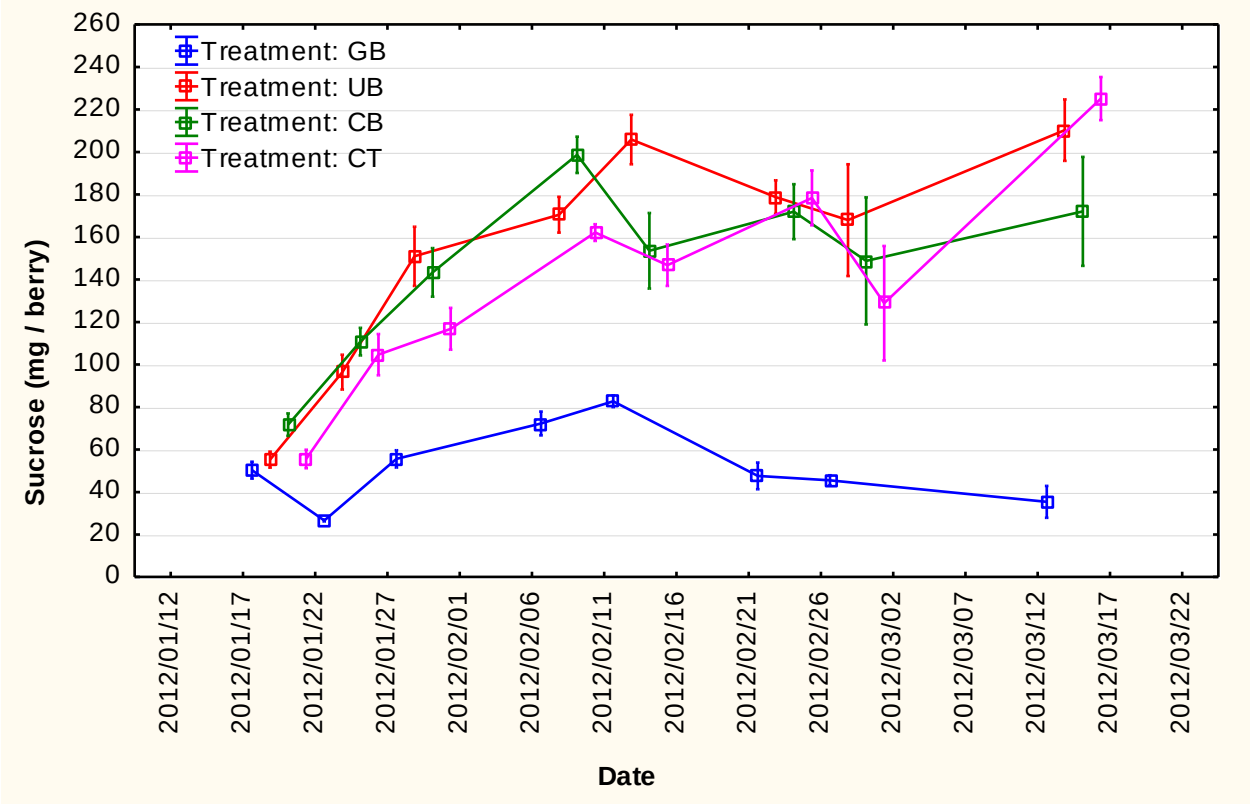

Figure 4.14 The mean plot of the average sucrose per berry measured over the 2012 ripening period for the four different treatments. All berries used to create this graph were from the 11.5 mm and 12.5 mm diameter class. The bars indicate standard error

Figure 4.14 shows the curves for sucrose evolution (mg/berry) over the 2012 ripening period for the various treatments. Over the season, berries were classified into diameter and only berries in the 11.5 mm and 12.5 mm class were used from every date to create the curve for sucrose evolution. As expected, due to girdling, the sucrose concentration of the girdled bunches remained low throughout the duration of the season. A slight variation in the sucrose concentration per berry indicated that the girdling wound might not have been wholly effective or

that sugars were transported via an alternative pathway. Regardless, the levels of sucrose per berry were still significantly lower than those of the other treatments. The other three curves including the bottom control (CB), the top control (CT) and the ungirdled bunch (UB), all displayed an expected sugar loading curve, i.e. a rapid initial sugar accumulation followed by a period of reduced sugar loading. The sugar loading plateau was reached around the 9 February 2012 for all three treatments at a concentration of between 160 mg and 200 mg per berry. Variation does exist between the top and bottom control bunches. The bottom bunch is usually a large bunch and the top bunches can sometimes be tendril bunches which are not as strong sinks for sugar. However, at the end of ripening, the top control bunch had a higher sucrose content. This could be attributed to the fact that sampling occurred across several vines which had inherently different levels of reserves, source-sink relations, etc. If repeat sampling had been possible on the same vine then this variability over time would have been overcome to some extent. Also, working in model vineyard with a controlled leaf to fruit ratio throughout the season might have aided in decreasing the variability.

What is interesting to note is that the ungirdled bunch followed the same trends as the two controls. By girdling the lower bunch on the shoot, the sinks of that shoot where effectively reduced to only one bunch. It can therefore be assumed that since the same leaf surface area was present for that shoot, that the ungirdled bunches could be speculated to have received the majority of the carbohydrates produced by the leaves. Since berries of the same volume can have different sugar concentrations, the berries of the ungirdled bunches would have higher sucrose concentrations than the two controls as it is the main sink on the primary shoot which previously had two sinks. What is observed however is a similar sucrose concentration (mg/berry) between the ungirdled bunch (from the girdled primary shoot) and the controls. At the end of ripening, the average sugar concentration for the bottom control was approximately 170 mg per berry, for the top control bunch it was approximately 220 mg per berry and the ungirdled bunch had a sugar concentration of 210 mg per berry. This implies some sort of regulation between the source and the sink, or a redistribution of carbohydrates to an alternative sink, namely the laterals as the growth point of the primary shoot had been tipped and there was no more growth. A further possibility would be that the sugars are stored within the leaves, or that they are used for the enlargement of the main shoot (i.e. primary shoot diameter), which is related to the functioning of the secondary cambium.

Firstly, with regard to the use of carbohydrates for the shoot diameter increase (functioning of secondary cambium); it is possible that enough sugar was available to facilitate the expansion of the shoot, however this cannot be confirmed. In an experiment on growth and dry matter partitioning, it was found that the removal of the crop led to an increase in shoot dry mass, suggesting that sugars could possibly be used for furthered shoot growth. This trial was

however conducted when the vegetative growth of the vine was still active (Petrie *et al.*, 2000). Shoot fresh mass and dry mass measurements as well as proper analysis should be conducted to ascertain whether a difference in shoot size was present. Source- sink relationships have both a spatial and temporal component and both factors must be considered. The spatial element refers to which organ is manipulated in the vine, whereas the temporal component refers to when this manipulation is carried out. Different effects will be noted should a certain applications be carried out at different times. This suggests that certain operations occurring in a vine may be regulated.

Secondly, it is possible for the carbohydrates to be stored in the leaves and stem. This can be determined by measuring the dry mass of the leaves and shoots for both treatment and control. A higher dry mass in the treatment organs could indicate higher sugar content. However, both leaves and shoots contain various sugars not exclusively limited to sucrose, glucose and fructose. Leave sugars also include inositol, sorbitol and rhamnose, and shoot sugars include raffinose, inositol, rhamnose and starch. All the roles of these sugars are not fully understood, however, they have been found to be utilised in defence mechanisms as well as in certain signalisations. In order to know the proportions of the various sugars found in leaves and shoots, accurate analyses should be conducted in a lab using proper extraction methods and analytical techniques.

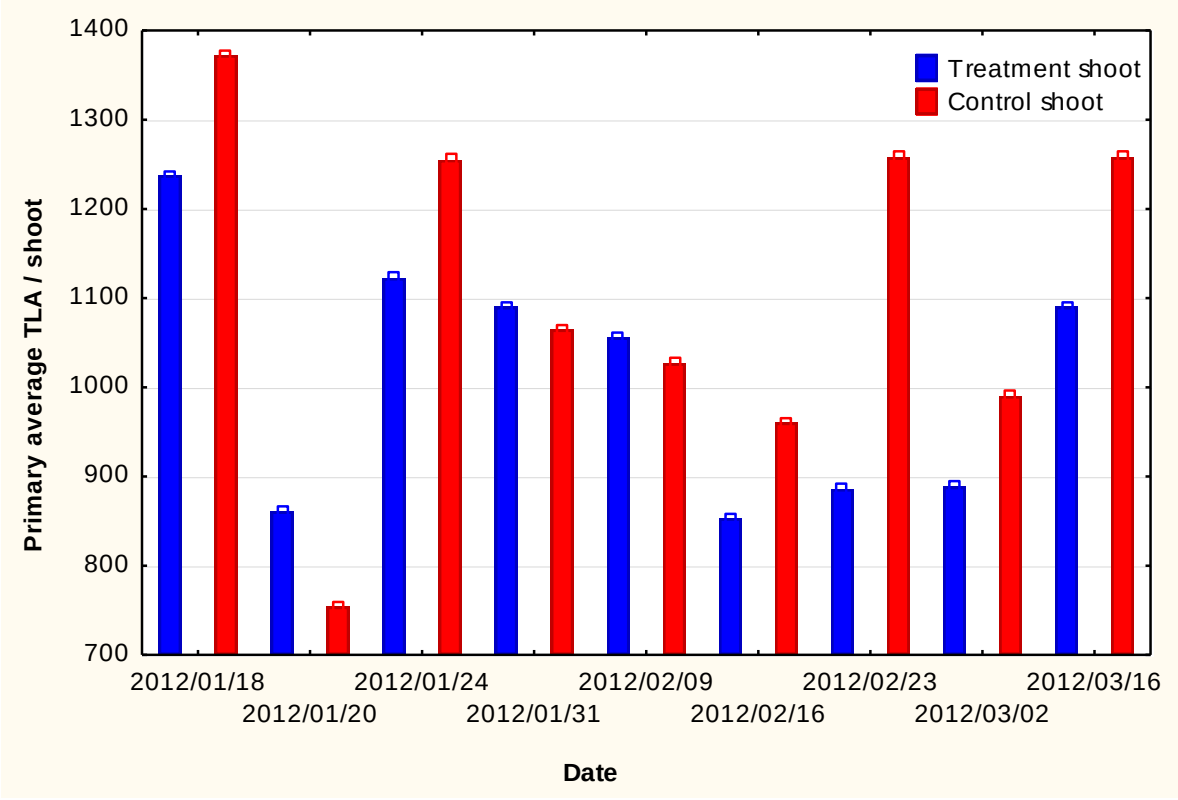

Figure 4.15 The mean plot of the average total leaf area for the primary shoots only over the ripening season. The blue bars indicate the shoots which were girdled and the red bars, the control shoots.

Figure 4.15 shows the average total leaf area per shoot for primary shoots only during the ripening season. The blue curve describes the treatment shoot (TMT) and the red curve, the control shoot (CNT). The treatment shoot is the girdled shoot from which the girdled (GB) and ungirdled bunches (UB) were sampled, whereas the control shoot included the two control bunches (CB and CT) which were sampled. In the beginning of the season, there is no visible difference in total area between the treatment and control shoots. Towards the end of the season, the control shoot had a higher total leaf area. This indicates that there was no primary shoot growth after véraison of the treatment shoots.

It could be possible for the carbohydrates to be utilised in alternative sinks, namely the laterals. Figure 4.16 shows the average total leaf area per shoot for lateral shoots only during the ripening season. The blue curve describes the treatment shoot (TMT) and the red curve, the control shoot (CNT). The treatment shoot is the girdled shoot from which the girdled (GB) and ungirdled bunches (UB) were sampled, whereas the control shoot included the two control bunches (CB and CT) which were sampled. It is clear from the graph that there is no definite difference in the average total leaf area for lateral shoots between the treatment and the control. A marked increase in lateral growth would have been evident in the case of the treated shoots, had any carbohydrates been reallocated to a different sink. This indicates that the idea that a vine is pre-programmed to make a certain amount of carbohydrates in fundamentally flawed.

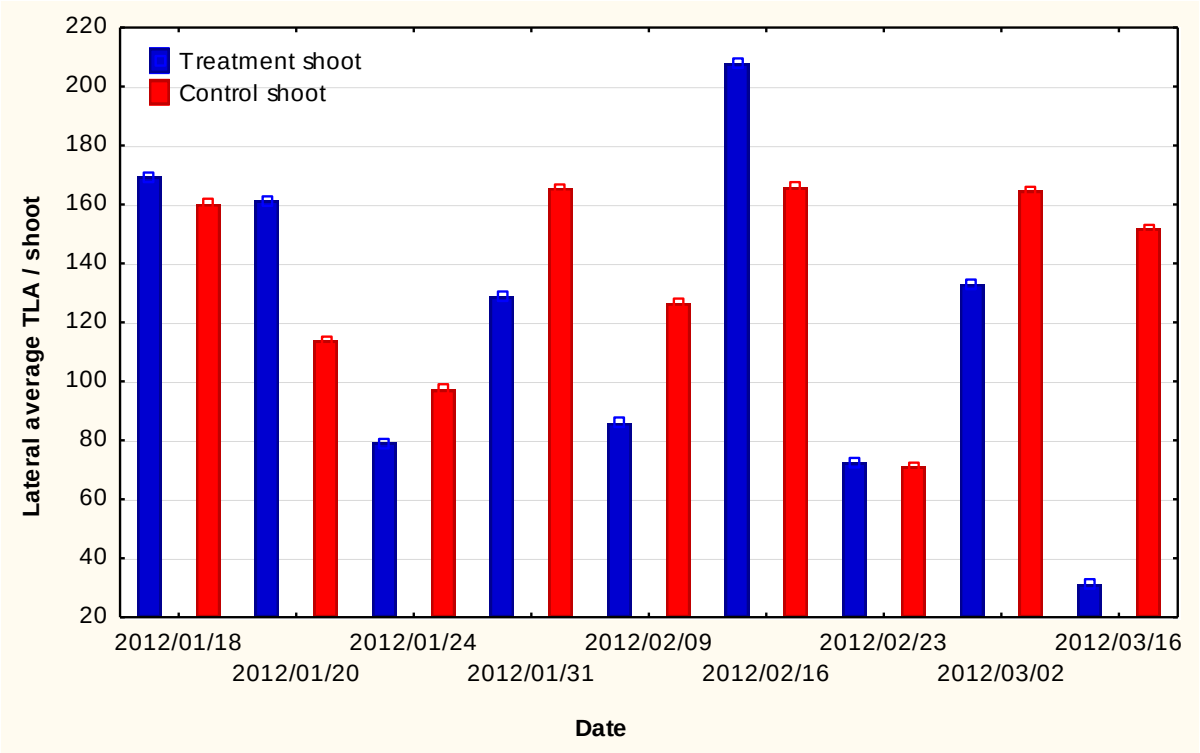

Figure 4.16 The mean plot of the average total leaf area for the lateral shoots only over the ripening season. The blue bars indicate the shoots which were girdled and the red bars, the control shoots

There is a degree of communication between the source and sink organs involving certain signals which relay information regarding the quantities of carbohydrates which are needed for a certain situation. Edson et al. (1993) stated that the capability of a vine to produce a certain dry mass results from an interaction between the vines inherent capacity for carbon fixation and the environment in which it grows. Management practices can influence where the produced carbohydrates will be allocated to, either vegetative growth or fruit production, but to increase the absolute amount of carbohydrates produced is difficult to achieve (Petrie *et al.*, 2000). Furthermore, in an experimental and modelling study conducted on source sink interaction, it was found that a phloem based sink to source feedback mechanism may exist in the grapevine. Results of the trial suggested that the stomatal regulation of photosynthesis was mediated by an internal factor. Model simulations supported the hypothesis of phloem-based feedback signal, although the detailed nature of the signal remained unknown. (Quereix *et al.*, 2001). This trial further provides substantial evidence for the existence of a regulatory mechanism between source and sink organs.

In addition, it is clear that a strong compensation effect is present within the vine and drastic changes must be applied before any significant consequences are noted within a specific environment. Furthermore, the removal of a sink will not result in the remaining sink organ loading more sugar. The volume of a berry is not infinite and the amount of sugar which can be accumulated may be regulated. The potential volume of a berry is dependent on cell division and also cell enlargement; however, a single cell cannot be filled beyond its capacity. The amount of sugar which can be loaded will therefore depend on how many cells are available to fill and maximum amount to which these cells can be filled.

Practically speaking, it therefore seems redundant to remove bunches in the vineyard to improve quality, if the exposed leaf area can support the crop. The removal of one bunch on a shoot will initiate a compensation effect due to the regulation within the vine, thus resulting in half the number of bunches with the same sugar concentration as would be present if the other bunches were not removed. Taking that into account, bunches can be removed to achieve a certain style of wine. For example, after the onset of véraison when the berries have attained a certain percentage of colour, those bunches with a majority of green berries can be removed to reduce the green character in a wine.

4.7.2 Glucose and Fructose

Fructose and glucose are transported in the phloem tissue, however to a lesser degree that sucrose (Glad *et al.*, 1992). The sucrose which is unloaded is converted by invertase enzymes into glucose and fructose. This is done to ensure that an osmotic gradient is maintained to facilitate the movement of sugars from the source organs to the sinks. Sugars can be stored in the cell vacuole or be used in various processes.

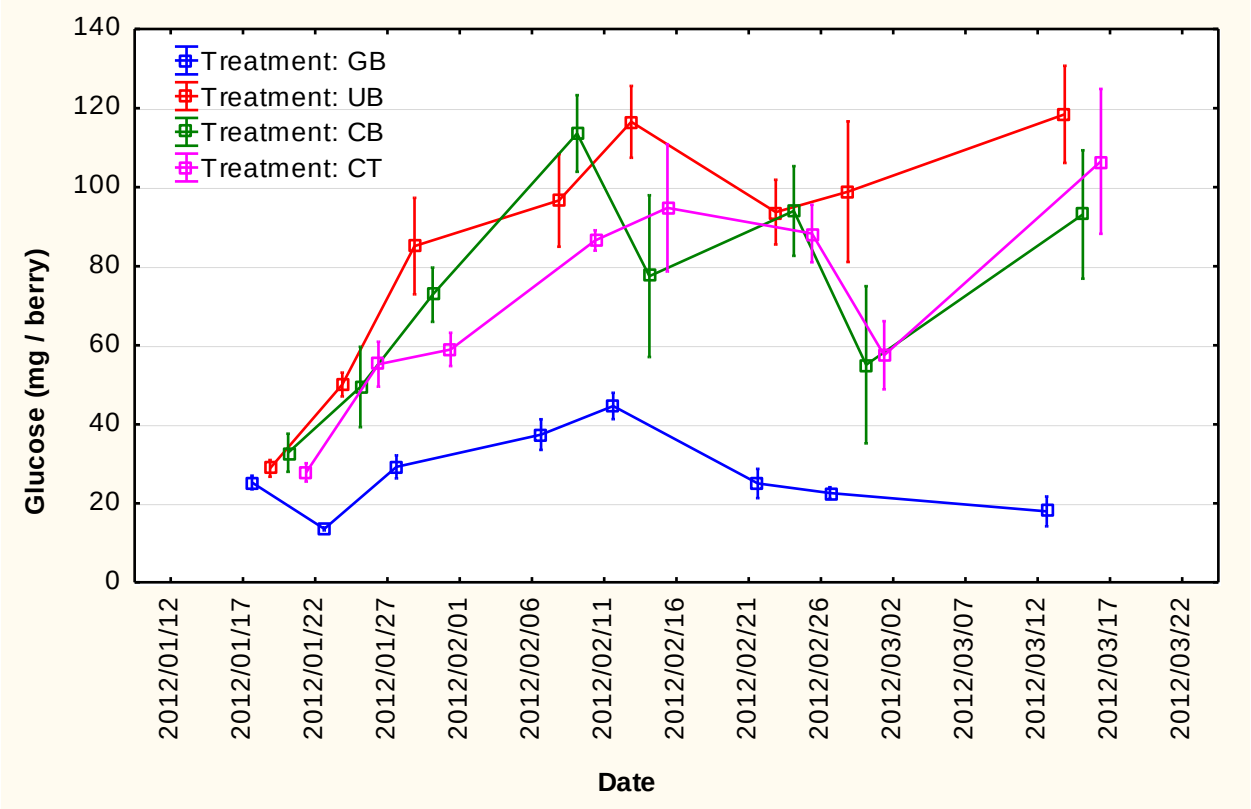

Figure 4.17 The mean plot of the average glucose per berry measured over the 2012 ripening period for the four different treatments. All berries used to create this graph were from the 11.5 mm and 12.5 mm diameter class. The bars indicate standard error

Figure 4.17 shows the evolution of glucose concentration per berry over the 2012 ripening season measured in milligrams. Again, only those berries with a diameter of 11.5 mm and 12.5 mm were used to create the curves. The trends follow those of sucrose concentration very closely. A correlation of between 0.81 and 0.99 was found between the glucose and sucrose contents per berry for all treatments (r = between 0.81 and 0.99, r^2 = between 0.66 and 0.98). . The girdled bunches showed a continuously low level throughout the season and the other curves seemed to reach a sugar loading plateau around the 9 February 2012. This concurs with the literature which states that the glucose to fructose ratio is 1:1 (Coombe, 1997; Robinson & Davies, 2008). Sucrose is cleaved into one molecule of glucose and one molecule of fructose

by invertases found in the berry. However, the curves for fructose indicate that the ratio between the two molecules is not 1:1; instead the ratio was observed to fluctuate between 2:1 and 4:1.

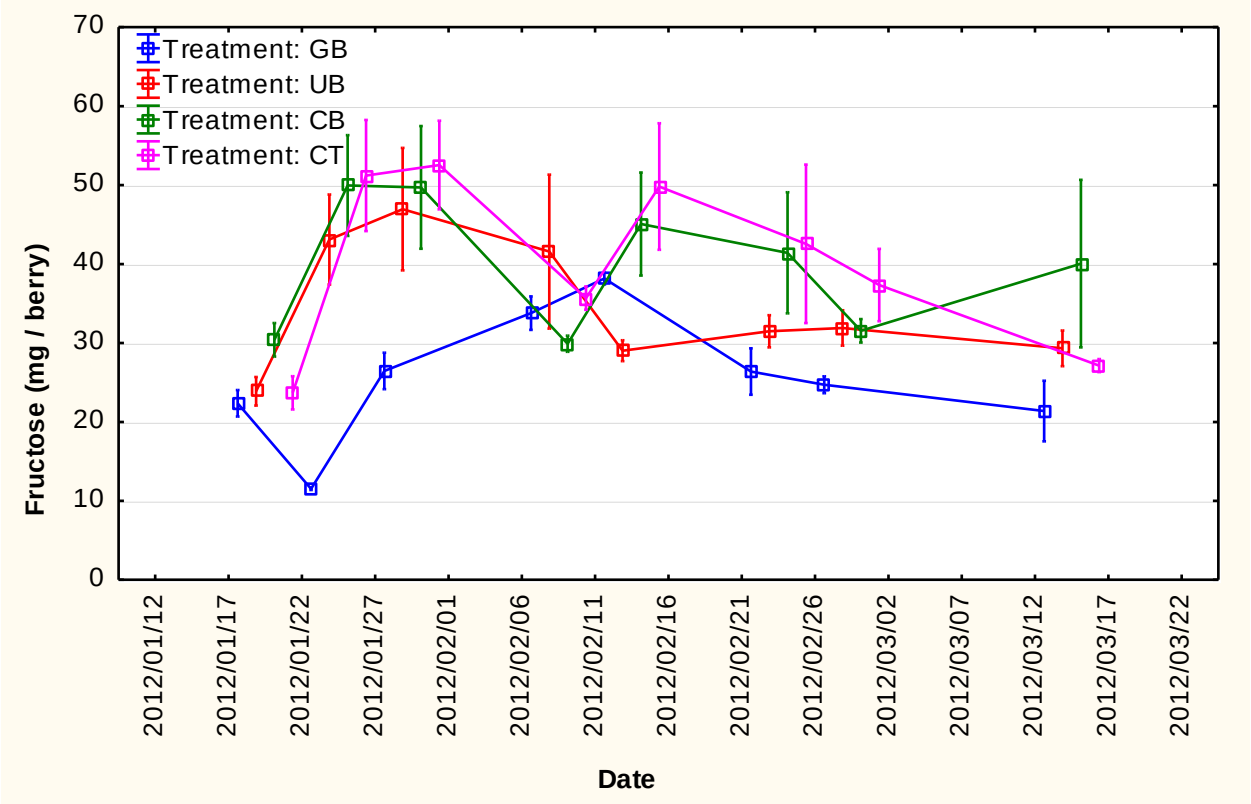

Figure 4.18 The mean plot of the average fructose per berry measured over the 2012 ripening period for the four different treatments. All berries used to create this graph were from the 11.5 mm and 12.5 mm diameter class. The bars indicate standard error

Figure 4.18 shows the curves for fructose content during the 2012 ripening season. Again, only berries from the 11.5 mm and 12.5 mm diameter class were used for every date to determine the concentration levels and therefore to create the figure. This does not comply with the notion of a 1:1 ratio between glucose and fructose as stated by a several authors (Coombe, 1997; Robinson & Davies, 2008). It seems that for the girdled bunches; the concentration levels for both glucose and fructose were similar, remaining between 10 mg and 40 mg throughout the season. A comparable pattern of where concentrations per berry either decreased or increased slightly can also been seen between the figure for glucose and fructose. However, the curves for the other three treatments do not follow the expected pattern of a rapid increase followed by a plateau or slow concentration increase. Furthermore, when considering the values for the bottom control (CB), it is observed that a maximum sucrose concentration level per berry was attained at approximately 200 mg/berry on the 9 February 2012. The maximum glucose concentration per berry was reached on the same date at a level of approximately 110 mg. This is about half of the maximum sucrose concentration per berry. It therefore stands to reason that a maximum fructose concentration should also be reached at approximately 100 mg per berry, if

one assumes the concept of the 1:1 ratio to be accurate. From the curve, it can be seen that a maximum fructose concentration of 50 mg per berry is attained, merely half of the maximum glucose concentration per berry. In addition, both the glucose and fructose concentrations reached 50 mg per berry by the 24 January 2012, before sugar loading plateaued. From this point, glucose continued to increase whereas the fructose did not.

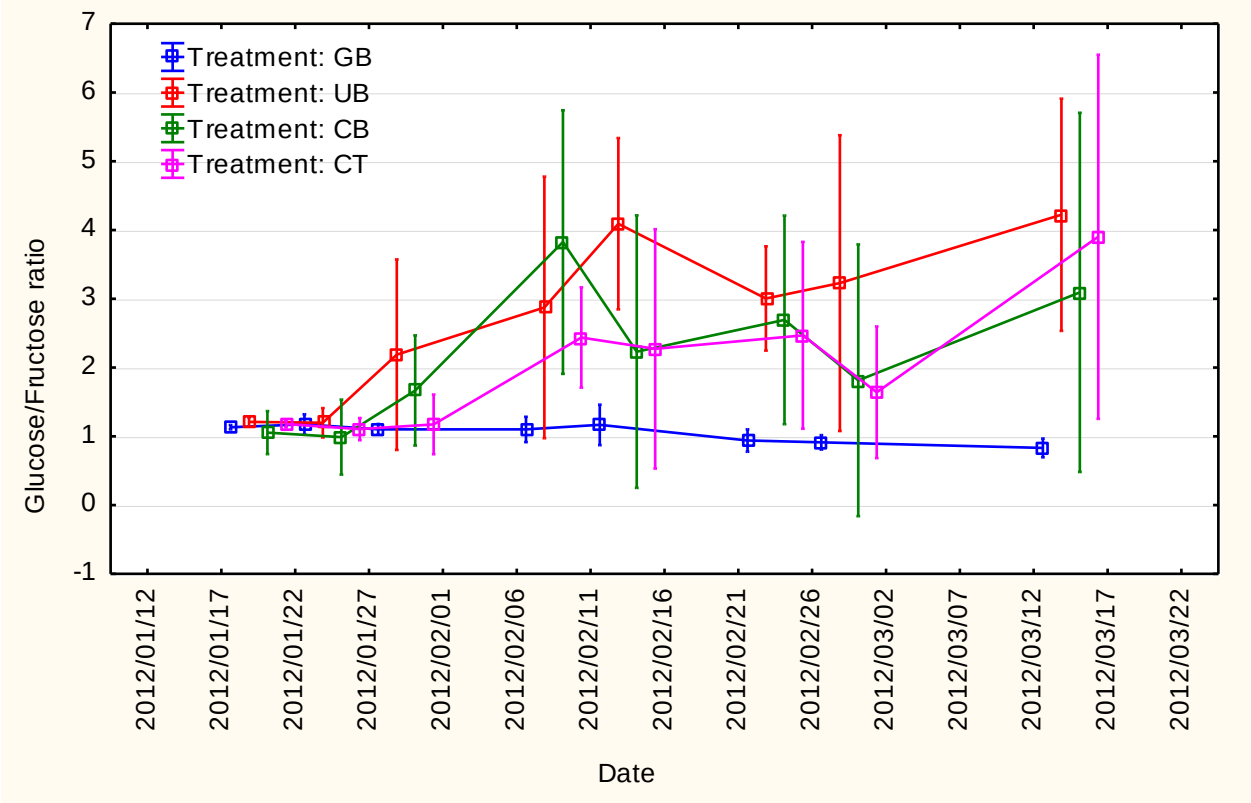

Figure 4.19 A mean plot showing the ratio between the average glucose and fructose per berry over the ripening season using only berries form the 11.5 mm and 12.5 mm diameter classes. The bars indicate standard error. The ratio is not 1:1 for the duration of the ripening period

Figure 4.19 shows the ratio between the glucose and fructose concentration levels for the 2012 ripening season using only 11.5 mm and 12.5 mm diameter berries for every date. It is clear that after véraison, but before the plateau of sugar accumulation, some incident occurs which influences the fructose concentration. The decreased concentration of fructose can be explained by a number of hypotheses, however nothing can be confirmed. The first thought would be that the invertase activity either stops or slows down; however, the presence of glucose, in what can be deemed normal concentrations, infers that there is no invertase dysfunction. The possibility of a dysfunction in the phloem transport is also not credible due to the incidence of glucose. A further hypothesis is that at a certain point, phloem is only able to unload glucose and not fructose, but this then raises the question of what happens to the fructose. The most plausible explanation would be that the fructose is used for some specific function, perhaps in the formation of certain secondary metabolites. Most literature states that

the ratio between the glucose and fructose is 1:1, therefore, it stands to reason that this trend where fructose levels decrease occurs under certain conditions or in certain situations. The answers are however not clear and further investigation is needed.

4.8 THE INTEREST OF USING THERMAL TIME INSTEAD OF CALENDAR DATES

The characterisation of balance within a vine/vineyard incorporates several crucial criteria which must be met. The relationship between the source and sink organs must be functioning with an appropriate ratio between the leaves and the fruit. Additionally, it is a prerequisite that an optimal microclimate exists within in a canopy with the further requirement that the vine not experience any form of stress. This will ensure an optimal rate of photosynthesis. (Wang *et al.*, 2003). Two phases can be delineated in the kinetic of sugar accumulation. The first phase typically extends for 25 to 30 days after véraison (i.e. start of berry softening) and is the most important in terms of sugar accretion. The second phase will commence once sugar loading reaches a plateau or slows down. It should be possible in a balanced situation to predict when the plateau or reduction in sugar accumulation occurs using thermal time. This would only be relevant for the first phase during which a certain amount of sugar must be loaded, which is limited to a certain extent. The second phase of sugar accumulation is physiologically different as this period is more related to the berry aromatic evolution in terms of berry ripening.

It is interesting to take into account the heat summation and use thermal time, instead of only considering a specific date over a ripening period. The incorporation of a link to a climatic parameter such as temperature allows for a more accurate comparison between seasons or vintages. Using growing degree days as opposed to normal calendar dates has the advantage of normalising temperature as a driving variable. Temperature is an abiotic factor which is known to be inextricably linked to stomatal conductance. This in turn has a direct influence on photosynthesis which is directly responsible for sugar production and therefore berry sugar accumulation.

The concept of degree days allows for a multi-seasonal comparison between growth rates, phenological, temperature driven events and also quality aspects (Christensen, 1969; Wermelinger & Koblet, 1990). This in turn may lead to the development of a model which could provide valuable information, thereby leading to better prediction of harvest dates as well as aiding in planning procedures carried out during and after the harvest season.

A comparable model has been developed in Australia using the relationship between total soluble solids and thermal time. The trajectory of total soluble solids was able to be modelled thereby providing a tool which allows the use of short term temperature forecasts to aid in

predicting the trajectory of total soluble solids for various management purposes. Furthermore, this model will allow for the profiling of maturity date using long term records of climatic data. It is a useful yet simple model which utilises known biological and viticultural drivers of berry ripening and the inputs which are required are readily available to industry(Duchêne *et al.*, 2012).

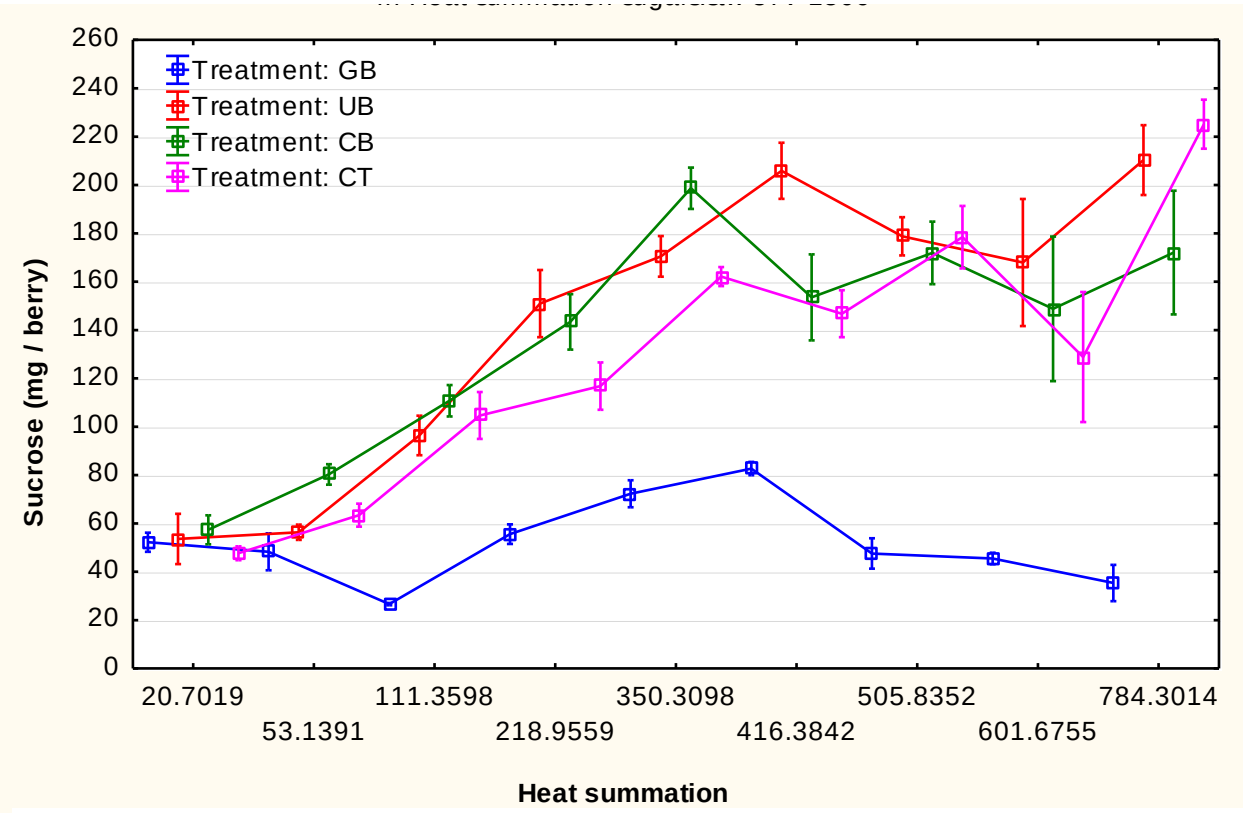

Figure 4.20 The mean plot of the average sucrose per berry measured over the 2012 ripening period for the four different treatments in relation to a heat summation or thermal time. All berries used to create this graph were from the 11.5 mm and 12.5 mm diameter class. The bars indicate standard error

As an example, figure 4.20 follows the accumulation of sucrose in mg per berry from véraison until just before harvest, with reference to the heat summation or thermal time. Véraison in this case indicates a 10% colour change. The thermal time was determined from climatic data collected from mesoclimatic loggers placed in the vineyard. When looking at the “Control bottom (CB)” curve, sugar accumulation reached a point where it slowed down at a thermal time of 350.31. The comparison of similar figures between vintages would give an idea of which thermal time is associated with the speed of sugar accumulation, the reduction in sugar loading or when the plateau is reached.

The growth of a plant is mainly related to the appropriation and distribution of resources, whereas development is linked to environmental factors such as temperature and light. Development can be defined as the continual change associated with both the form and

function of a plant (Loomis & Connor 1996). Temperature driven models which have been developed for the grapevine capture a significant portion of the phenotypic variation regarding the timing of budburst, flowering and véraison (Williams *et al.*, 1985; Duchêne *et al.*, 2010). Conversely, the biochemical and morphological changes which occur in the berry between véraison and maturity implicate processes which are both temperature and resource driven (Sadras *et al.*, 2008). A number of quantitative models which involve berry growth and development as well as various predictive models concerning grapevine maturity and harvest dates include a vast range of approaches and details. These models include empirical correlations between timing of berry maturity and climatic factors, as well as models which are more complex and involve certain physiological indicators. Furthermore, besides the biological interest in modelling berry growth and development, the prediction of the duration of berry sugar accumulation has become a point of decided interest as it is important for both logistical reasons during harvest as well planning of post-harvest operations. The application of frequent measurements of berry sugar concentration as well as the referral to various locally developed rules has been the main tactic in predicting berry maturity and harvest date. However, new and possibly more reliable options should be investigated, such as the use of sugar accumulation as a physiological indicator.

Chapter 5

Conclusions and Perspectives

CONCLUSIONS AND PERSPECTIVES

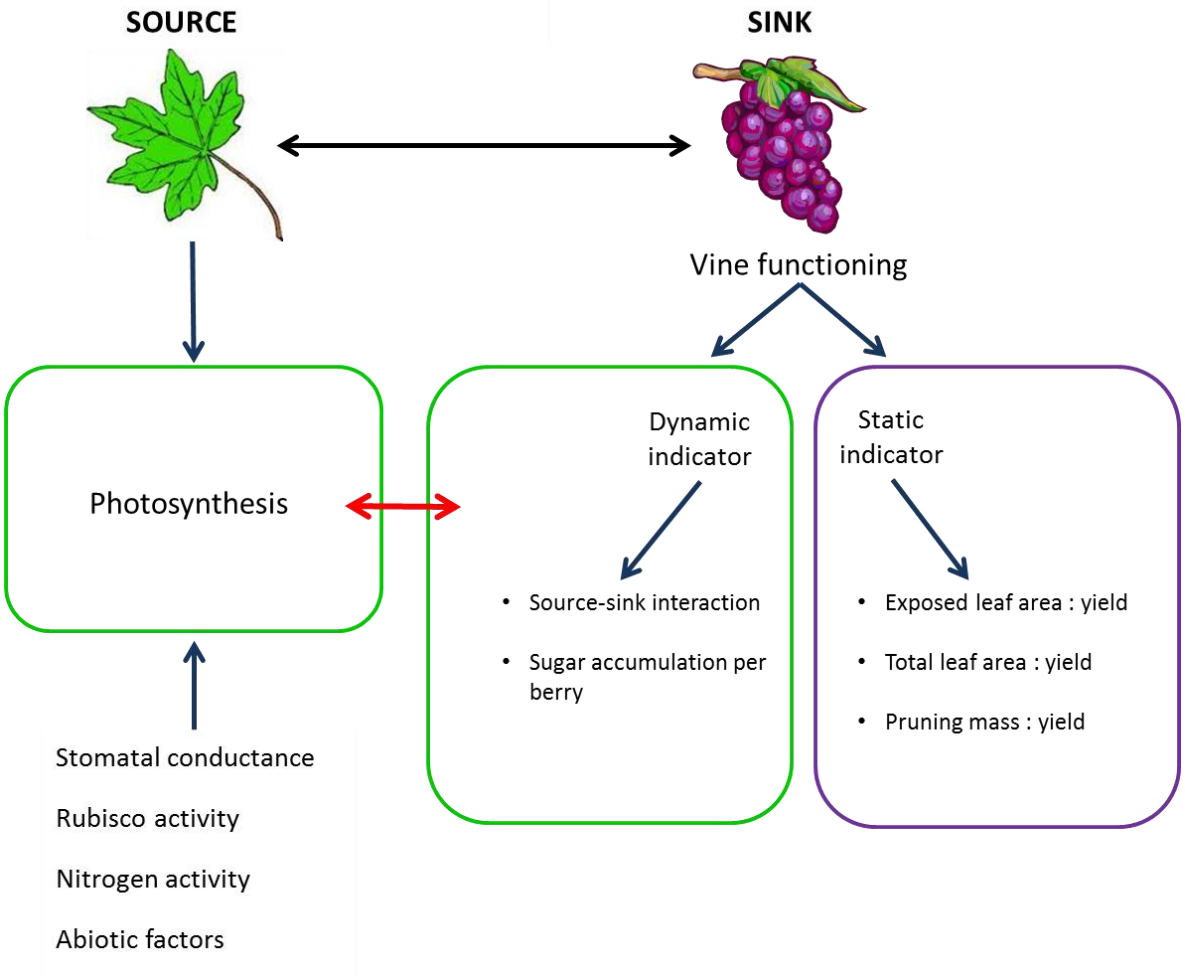

Figure 5.1 A diagram showing the relationship between the source and the sink organs with regard to indicators which could be used to determine vine functioning.

When taking the results of this study into consideration, it becomes clear that the classical ratios used to quantify the complex relationships between the fruit and the leaves may not be completely adequate to do so. The current way of looking at source-sink relationships and thereby determining whether a vine is balanced or not is over-simplified and there are numerous limitations involved in this approach. The vine is far more complex and various aspects must be taken into consideration before any claims can be made concerning source-sink relationships. The concept of source-sink relationships cannot be described using only simple ratios such as the ratio between exposed leaf area and yield or the ratio between pruning mass and yield. These ratios do not take into account the signalling processes which exist between the source and sink organs, nor do they include the strong compensation effect which results from this. These static, simple ratios exist more as guidelines for the farmer than to provide real informative indicators of vine balance. The value and thresholds of these ratios should be questioned. Practically, they should be re-addressed to provide farmers with more integrated

and dynamic physiological indicators of vine functioning and berry ripeness. Possible physiological indicators would be the evolution of sugar accumulation or the evolution of anthocyanins for red cultivars. This approach of considering berry sugar accumulation takes into consideration both the sources (leaves) and the sinks (berries). Sugar accumulation is directly linked to the leaves as they are responsible for the process of photosynthesis. Stomatal size, density, regulation and conductance can be considered of the main drivers of photosynthesis and therefore of sugar production, partitioning and accumulation in the fruit. Other important drivers include the genotype and rootstock influence. Furthermore, the sugar accumulation of a berry is an indication of sink functioning, related to the expression of hexose transporters. Using the kinetic of sugar loading and thereby taking into consideration the source operative, a more integrated and dynamic physiological indicator is provided for the duration of the fruit maturation phase. This gives a clearer idea of whole vine functioning as opposed to a “snapshot” view at one particular moment, which is what the classical ratios typically provide.

There are however a few potential drawbacks to this approach as well a few problems with the girdling method Firstly, the girdling method could be improved by using biological tracers to follow the movement of various compounds though the vascular tissue. This would enable one to determine if the girdling wound was complete or if any sugars were able to move past that point. Histological studies could be carried out on the girdled sections of the shoot to determine if the phloem was effectively removed and if any damage was done to the xylem. Girdling should also be carried out before véraison starts to prevent any sugar import. Furthermore, one of the biggest challenges of this trial was the variation in berry size over the season, in other words, there were more small berries in the beginning and more large berries towards the end of the trial. Perhaps some sort of statistical methods could rather be used to remove volume as a variable while using all the berries of a bunch. The drawbacks associated with using sugar accumulation as an indicator include the fact that the method requires repetitive sampling and cumbersome volume and sugar methods. It is also limited to the post véraison stage of development, while growers are likely to prefer to have earlier indicators so that they can more easily manipulate their management.

In order to develop this concept, more integrated studies using multi-disciplinary approaches are needed. To better understand vine functioning, a model vineyard should be used in further trials, thereby allowing for more precision in experimentation and leading to more accurate conclusions. Disciplines including anatomical, physiological, molecular and biological approaches should be incorporated into these studies to ensure that all the aspects of vine functioning are addressed.

In conclusion, it is clear that the classical ratios do not accurately provide the required information needed to determine vine balance. The vine functioning is far more complex since organs are able to communicate with each other, leading to strong compensation effects. More dynamic and integrated physiological indicators of vine balance and functioning should be considered such as the process of sugar and amino acids accumulation in a fruit. Further investigation using multi-disciplinary approaches is however needed to better understand these processes and thereby develop new and more useful methods or indicators which can ultimately be transferred to the wine industry.

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