Development and Change that Occurs in Table Grape Berry Composition During Growth.

by

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Summary

Grape quality is important for the producer, exporter and the consumer. Consumers judge table grapes according to their size, colour, taste and shelf life. The consumer's prerequisites will influence the producer. Therefore, it is essential to know how the table grape berry develops so that it can be manipulated, favouring the postharvest quality and shelf life.

This study was performed on Prime and Crimson Seedless, both grafted onto Ramsey, in the Paarl district of South Africa. The aim of this study was to describe and quantify table grape berry development and compositional changes taking place throughout growth and ripening. The effect of sugar:acid ratio on postharvest shelf life was also evaluated.

To evaluate whether berry size influenced the measured development parameters, three berry sizes were induced for both cultivars by using plant bioregulators such as gibberellic acid (GA_3) and forchlorfenuron – synthetic cytokinin (CPPU) or girdling. The following sizes were obtained for Prime: (i) small berries (<20 mm) with no treatment, which acted as the control; (ii) medium berries (20-24 mm) obtained by 15 ppm GA_3 application at 8 mm berry size; (iii) large berries (>24 mm) obtained by combination of 15 ppm GA_3 and 1 ppm CPPU application at 8 mm berry size. Crimson Seedless berry sizes were as follows: (i) small berries (<18 mm) with no treatment, which acted as the control; (ii) medium berries (18-22 mm) treated with 10 ppm GA_3 at 7 mm berry size; (iii) large berries (>22 mm) treated with 10 ppm GA_3 and vines were girdled at 7 mm berry size. To evaluate the effect of sugar:acid ratio on postharvest shelf life, grapes were stored for five weeks at -0.5 ˚C and another week at 7.5˚C. The bunches were evaluated for loose berries, browning, soft tissue breakdown, decay and berry split.

The following components were analysed for both cultivars to determine changes in berry composition throughout the season: berry fresh weight, total soluble solids (TSS), glucose, fructose, titratable acidity (TA), tartaric acid, malic acid, abscisic acid (ABA) and total phenols. Total and individual anthocyanins were analysed for Crimson Seedless.

Differences were obtained for the three berry sizes for both cultivars. Véraison, representing the start of ripening, started at the same time in successive seasons: 21 days after pea size berry (5 mm berry diameter) for Prime and 28 days after pea size berry (5 mm berry diameter) for Crimson Seedless. A lag stage was not observed, at seven day sampling intervals, for either of the cultivars.

Components such as TSS, glucose, fructose and TA content per berry were influenced by berry size in either one or in both seasons for both cultivars. Significant changes in component concentration were detected at the start of, or around véraison. Sugar concentrations (TSS) already started to increase for both cultivars before the start of véraison. At véraison, concentrations of glucose, fructose and ABA

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increased while concentrations of TA, tartaric acid, malic acid and total phenols decreased. Total anthocyanins in Crimson Seedless started to increase one week after véraison commenced. The main anthocyanin found in Crimson Seedless was peonidin-3-glucoside.

During ripening a 1:1 glucose:fructose ratio was detected in both cultivars. Prime tartaric:malic acid ratio was lower than Crimson Seedless tartaric:malic acid ratio in both seasons. Tartaric acid was the main organic acid found in Prime, while malic acid was the main organic acid found in Crimson Seedless.

No significant differences were found in the postharvest defects between the different berry sizes. However, tendencies for differences were observed which led to the assumption that medium size berries were more prone to loose berries in both cultivars. Large berries showed a higher percentage berry split for both cultivars. Crimson Seedless second harvest date took place 24 hours after rainfall which could have very likely led to the higher percentages berry defects compared to the first season. Greater berry decay was found with later harvest dates for both cultivars. No significant differences were found for the TSS:TA ratio between the three berry sizes for both cultivars. Postharvest defects were therefore found not only to be influenced by TSS:TA ratio but rather by harvest date and packing procedures. Environmental conditions prior to harvest also had an impact on postharvest shelf life.

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Opsomming

Druif kwaliteit is belangrik vir die produsent, uitvoerder en verbruiker. Tafeldruiwe word gekeur deur die verbruiker volgens grootte, kleur, smaak en raklewe. Die verbruiker se voorkeure sal dus die produsent beïnvloed. Daarom is dit belangrik om te weet hoe tafeldruiwe ontwikkel ten einde korrelsamestelling te manipuleer om na-oes kwaliteit en raklewe te kan bevoordeel.

Hierdie studie is uitgevoer op Prime en Crimson Seedless, beide geënt op Ramsey, in die Paarl distrik van Suid Afrika. Die doel van die studie is om vas te stel hoe korrelsamestelling gedurende groei en rypwording verander. Die effek van suiker:suurverhouding op na-oes raklewe is ook geëvalueer.

Om te kan meet of korrel grootte die gemete parameter beïnvloed is drie korrelgroottes verkry vir albei kultivars deur die gebruik van plant bioreguleerders, te wete gibbereliensuur (GA_3) en sintetiese sitokiniene (CPPU), of ringelering. Die volgende korrelgroottes is verkry vir Prime: (i) klein korrels (<20 mm) d.m.v. geen behandeling, geklassifiseerd as kontrole; (ii) medium korrels (20-24 mm) d.m.v. ‘n 15 dpm GA_3 behandeling by 8 mm korrelgrootte; (iii) groot korrels (>24 mm) d.m.v. ‘n kombinasie van 15 dpm GA_3 en 1 dpm CPPU by 8 mm korrelgrootte. Crimson Seedless korrelgroottes was soos volg: (i) klein korrels (<18 mm) d.m.v. geen behandeling, wat as kontrole gedien het; (ii) medium korrels (18-22 mm) d.m.v. ‘n 10 dpm GA_3 behandeling by 7 mm korrelgrootte; (iii) groot korrels (>22 mm) d.m.v. ‘n 10 dpm GA_3 behandeling en gelyktydige ringelering by 7 mm korrelgrootte. Om die effek van suiker:suur verhouding op na-oes houvermoë te kon evalueer was druiwe gestoor vir vyf weke by -0.5˚C en ‘n verdere week by 7˚C. Die trosse is geëvalueer vir loskorrels, verbruining, sagte weefsel afbreek, verval en korrelbars.

Die volgende komponente is geanaliseer vir albei kultivars om veranderinge in korrelsamestelling gedurende die seisoen te bepaal: vars korrelgewig, totale oplosbare vaste stowwe (suikerinhoud), glukose, fruktose, titreerbare sure, wynsteensuur, appelsuur, absisiensuur en totale fenole. Die totale en individuele antosianiene is ook vir Crimson Seedless gemeet.

Beduidende verskille tussen die drie korrelgroottes vir albei kultivars is verkry. Deurslaan, naamlik die begin van rypwording, het op dieselfde dag in opeenvolgende seisoene plaasgevind: 21 dae na ertjiekorrel grootte (5 mm korrel deursnee) vir Prime en 28 dae na ertjiekorrel grootte (5 mm korrel deursnee) vir Crimson Seedless. In teenstelling met die tipiese korrel ontwikkelingspatroon is ‘n rusfase nie waargeneem by beide kultivars nie.

Komponente soos suikerinhoud, glukose, fruktose en titreerbare suur inhoud per korrel is deur korrelgrootte beïnvloed in een of albei seisoene vir beide kultivars. Suiker konsentrasie van albei kultivars het reeds voor deurslaan begin toeneem. By deurslaan het die konsentrasies van glukose,

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fruktose en absisiensuur inhoud toegeneem, terwyl die konsentraies van titreerbare sure, wynsteensuur, appelsuur en totale fenole gedaal het. Totale antosianiene in Crimson Seedless het ‘n week na deurslaan begin toeneem. Die hoof antosianien in Crimson Seedless is peonidien-3-glukosied.

Gedurende rypwording was daar ‘n 1:1 glukose:fruktose verhouding gevind vir beide kultivars. In terme van sure is Prime se wynsteensuur:appelsuur verhouding laer as in Crimson Seedless vir albei seisoene. Wynsteensuur is die hoof organiese suur in Prime terwyl appelsuur die hoof organiese suur in Crimson Seedless is.

Geen betekenisvolle verskille vir na-oes houvermoë tussen korrelgroottes is waargeneem vir beide kultivars nie. Daar was egter tendense wat aanleiding gegee het in die aanname dat medium grootte korrels geneig is tot loskorrels in albei kultivars. Groot korrels het ‘n hoër korrelbars persentasie getoon vir beide kultivars. Crimson Seedless se tweede oes het plaasgevind 24 uur na reënval, wat aanleiding gegee het tot hoër persentasies korrelbederf. Hoër persentasie korrelbederf was ook gevind met later oesdatums. Geen beduidende verskille is gevind vir suiker:suur verhouding tussen die drie korrelgroottes vir beide kultivars nie. Dus word na-oes houvermoë nie net deur suiker:suur verhouding beïnvloed nie, maar ook deur oestyd en verpakkingsprodsedures. Omgewingsomstandighede voor oes kan ook na-oes houvermoë beïnvloed.

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Biographical sketch

Nastassja Sonnekus matriculated from Strand High School in 2006. In 2008 she enrolled at Stellenbosch University and obtained the BScAgric (Viticulture and Oenology) in 2011. In 2012 she enrolled for MScAgric (Viticulture) at Stellenbosch University.

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To the future table grape research students: Be curious for the sake of being curious.

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Preface

This thesis is presented as a compilation of 6 chapters. Each chapter is introduced separately and is referenced according to the style of the journal, South African Journal of Enology and Viticulture.

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Chapter 2	**Literature review** Development and change that occur in grape berry (*Vitis vinifera* L.) composition during growth – sugar, acid and phenol (anthocyanin) accumulation
Chapter 3	**Research results** Changes occurring in table grape berry composition during development: (1) Prime.
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Chapter 1

General introduction and project aims

Producers and exporters incur huge losses when (originally) good quality grapes are rejected on arrival in the market. Maintenance of quality of the grapes during transportation and cold storage is therefore extremely important. Quality of table grapes at harvest must firstly be ensured as basis for good berry shelf life. The quality of table grapes considered at harvest is determined by berry size, firmness, sugar concentration, titratable acidity (TA) and colour (Robredo *et al*., 2011). However, the extent of loss of berry quality after harvest is also believed to be affected by compositional changes that occur in the berry during growth and ripening.

Numerous studies have investigated wine grape berry development and compositional changes that occur (Coombe *et a*l., 2000; Kennedy, 2002). Since the end goal of table grapes is completely different from wine grapes, cultivation techniques are required that result in the adaptation of berry development. Few studies on the compositional changes of seedless table grapes however have been done.

Table grape producers use total soluble solids (TSS), titratable acidity (TA), minimum TSS:TA ratios and grape colour to determine optimal harvest dates (Jayasena & Cameron, 2008). The parameters for optimal harvest are set to ensure a high quality end-product. Consumer satisfaction is determined by taste, colour, maintenance of texture and the absence of defects like decay, soft tissue breakdown and browning. Berry quality in all respects might be determined by both primary and secondary metabolite concentration and their rate of accumulation or breakdown. Primary metabolites consist of sugars, organic acids and minerals while secondary metabolites consist of phenolic compounds like anthocyanins (Hayes *et al*., 2009). Although two populations of grapes might meet the criteria for optimal harvest according to primary metabolites (sugars and acids), different accumulation or breakdown dynamics might result in one population still lacking secondary metabolites (phenols like anthocyanins and tannins) and therefore is not completely ready for harvest. The need to understand changes in berry metabolite composition and how the final berry TSS:TA ratio influence postharvest quality was consequently expressed by producers. Research to clarify the changes that occur in berry composition during growth was therefore undertaken.

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This project endeavoured to provide basic information regarding table grape berry growth and composition and the possible link between internal quality and postharvest shelf life. This information will assist in decision making regarding harvest and export programs.

The aim of the project was firstly to investigate table grape berry development and compositional changes that occur during growth and whether it differs for berries of three different sizes (small, medium and large). Secondly, the relationship between berry size, change in TSS:TA ratio and postharvest defects was evaluated.

In order to accomplish the above mentioned objectives, the following methods were followed:

- Inducing different berry sizes through application of plant bioregulators (GA_3 and CPPU - forchlorfenuron – synthetic cytokinin) and girdling at appropriate times.
- Weekly sampling and berry size classification according to average berry diameter.
- Laboratory analyses for various compound concentrations.
- Evaluation of grapes for postharvest defects after cold storage.

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Chapter 2

Literature review: Development and change that occur in grape berry (*Vitis vinifera* L.) composition during growth – sugar, acid and phenol (anthocyanin) accumulation

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2.1. Introduction

Although the grape berry is a non-climacteric fruit, it is part of the fleshy fruit group, which includes fruits like banana, stone fruit, tomato, etc. (Coombe, 1976). The fruit flesh develops from the inflorescence (Coombe, 1976), which in itself is influenced by environmental and nutritional factors (Harris *et al*., 1968; Hrazdina *et al*., 1984), and therefore affects the entire developing process of the berry (Hrazdina *et al*., 1984).

The concept of berry development is discussed in order to understand aspects that will influence final berry quality and postharvest defects of table grapes specifically. These aspects include identifying the exact cultural practices needed to modify and achieve optimal quality grapes by predicting application times of plant bioregulators and estimating harvest dates. The final quality of each cultivar depends on the final goal of the product whether it is for wine production or consumer consumption (dried- or table grapes). Sugar, organic acid and anthocyanin concentration play an important role in the final quality of grapes. These elements change during berry development as follows: 1) Organic acids increase during the first growth stages (Hrazdina *et al*., 1984); 2) Sugars start to accumulate during onset of berry softening and deformation (Coombe, 1992; Kennedy, 2002); 3) Phenols, primarily anthocyanins, increase approximately one week after sugars start to increase (Pirie & Mullins, 1977; Coombe, 1992; Kennedy, 2002).

Numerous studies have investigated wine grape berry development and the compositional changes that occur during growth. Since the end goal of table grapes are completely different from wine grapes, adapted cultivation techniques are required which will presumably result in changed berry development. This is poorly studied and little is known about table grape berry development. General *Vitis vinifera* berry development was therefore discussed in this chapter, concentrating on the accumulation of sugars, acids and anthocyanins.

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2.2. Berry growth and development

Berry development and growth takes place in three stages which follow a double sigmoid curve as illustrated by the growth curve in Fig. 1. (Harris *et al*. 1968; Coombe, 1973; Pirie & Mullins, 1980; Coombe, 1992). The growth curve can also be divided into either two or four stages. The number of growth stages depends on environmental conditions, type of cultivar, cultivation practices, solar radiation, temperature and moisture received (Coombe, 1973; Hrazdina *et al*., 1984). According to Pratt (1971) Seedless cultivars (table grapes) usually do not depict a clear stage two (lag stage), which results in less definite stages in the growth curve. Cultivars with no or short lag stage, tend to ripen earlier than those with an clearly observable extended lag phase (Coombe, 1976).

All three stages of berry development represent a period in which specific changes occur as illustrated in Fig. 1. These various changes take place within a given time and therefore the berry needs roughly 90 to 120 days from anthesis to maturity and harvest (Liang *et al*., 2005). Each berry develops on its own and is not influenced by the adjacent berries that ripens earlier or later (Coombe, 1992).

2.2.1 First stage

The first stage of berry development occurs just after anthesis. It consists of rapid berry growth, seed formation and acid accumulation. The majority of pericarp (skin and pulp) cell division takes place within five to 10 days after anthesis (Pratt, 1971; Coombe 1973). Approximately seven to 11 days after anthesis, cell division firstly ends in the inner pericarp and placenta, then in the outer pericarp and lastly in the epidermis and hypodermis (32 to 40 days after anthesis) (Pratt 1971; Considine & Knox, 1981). When cell division subsides, the number of cells is permanent and the final size and shape of the berry is determined (Coombe & McCarthy, 2000). Further berry growth only occurs through cell expansion (Pratt, 1971; Coombe 1976; Kennedy, 2002; Liang *et al*., 2005).

Stage one of berry growth is important in that the final potential volume of the berry is established (Mullins, *et al*. 1992; Liang *et al*., 2005). This first stage usually takes place within 40 days from anthesis, as illustrated in Fig. 1 (Mullins *et al*., 1992; Kennedy, 2002). Large vacuoles in the grape berry cells are already developed two days after anthesis. The vacuoles start to enlarge the instant it starts to store tartaric, malic and citric acid – this increases the volume of the pericarp cells (Roubelakis-Angelakis, 2001). Tartaric acid accumulates during the early stages of berry development while malic acid starts to accumulate at the end of stage one until the start of véraison. Tartaric acid is usually higher on the outer part (skins) of the berry while malic acid is higher in the pulp (Coombe *et a*l., 2000; Kennedy, 2002). The

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organic acids that accumulate during stage one, are measured as titratable acidity (TA) (Coombe *et a*l., 2000; Kennedy, 2002). Hydroxycinnamic acids, precursors of volatile phenols and anthocyanins, also accumulate during the early stage of development and are distributed throughout the berry (Mullins *et al*., 1992; Kennedy, 2002).

During stage one, chlorophyll is the main pigment in the berry, which enables the berry to photosynthesise. The berry has high rates of respiration, rapidly accumulates acids and depicts a functional metabolism (Peynaud & Ribéreau-Gayon, 1971; Winkler *et al*., 1974). The vascular system, containing xylem and phloem components, supplies the berry with nutrients through the pedicel. The xylem transports water, nutrients, minerals and bioregulators upwards from the roots to the bunches and the rest of the vine, while the phloem transports photosynthates (sucrose) from the leaves to the vine and berries (Kennedy, 2002). Water in the xylem sap is the main component contributing to berry growth during stage one, since the accumulation of dry matter through the phloem is still low (Coombe & McCarthy, 2000).

2.2.2 Second stage

The next stage (lag stage) is a short period where no or slow growth of the pericarp occurs since the metabolism of the berry is slow. Photosynthesis and respiration rates, as well as chlorophyll concentrations, are reduced. The organic acids reach their maximum concentration, with malic acid at a higher concentration than tartaric acid (Coombe *et al*., 2000). According to Fig. 1, this stage starts approximately 40 days after anthesis and can last seven to 40 days depending on cultivar (Coombe & McCarthy, 2000).

The time of maturity, i.e. an early or late cultivar, is determined by the length of the lag stage (Pirie & Mullins, 1980; Mullins *et al*., 1992). Seedless cultivars, without a clearly defined or very short lag stage, tend to have a shorter ripening period compared to seeded cultivars (Pratt, 1971; Coombe, 1976; Farmahan & Pandey, 1976). However, the length of the lag stage of a cultivar could be influenced by the environment (Coombe, 1976).

2.2.3 Third stage

The start of the third stage in berry development is also known as véraison; the onset of ripening. Several physiological changes occur almost instantaneously, within 24 to 48 hours, during the transition from the second to the third stage. These physiological changes include re-initiation of berry growth, hexose sugar accumulation, berry softening, and change in berry colour (red cultivars), organic acid

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decrease, increase in pH cell sap and increase in proline and arginine concentrations (Mullins *et al*., 1992; Coombe & McCarthy, 2000; Roubelakis-Angelakis, 2001).

Most of the solutes that were accumulated during stage one (mainly tartaric and malic acid) remain until harvest, but since the volume of the berry increases its concentrations decrease considerably (Kennedy, 2002). The rate at which sugar per berry increase after véraison, is directly proportional to the volume of the berry. One week after sugar accumulation starts, total phenol and anthocyanin content increase significantly (Fig. 1), while chlorophyll content decrease entirely during the third stage. Potassium and anthocyanins accumulate in the skin, whereas hexose sugars accumulate in the skin and flesh (Pirie & Mullins, 1980; Coombe & McCarthy, 2000).

At the beginning of véraison, the berry starts to grow again and through the expansive swelling xylem flow is hindered (Coombe, 1992; Coombe & McCarthy, 2000). At approximately 6˚ to 7˚ Brix, the xylem flow is completely blocked – the tracheids in the brush zone are stretched and eventually the membranes start to disrupt (Coombe, 1992; Coombe & McCarthy, 2000). From here on berry growth and water uptake depends primarily on phloem sap movement as illustrated in Fig. 1. Consequently calcium accumulation decreases after véraison since it is transported through the xylem; this occurrence also proves that xylem becomes discontinued (Creasy *et al*., 1993). According to Creasy *et al*. (1993) xylem becomes discontinued at the onset of berry softening, suggesting that the xylem flow is terminated before berry regrowth occurs. Ollat *et al*. (2002) however found that xylem still contributes up to 20% of water import after véraison although it may be limited to the brush zone, since the peripheral network is detached. After véraison the xylem can even function as a water back flow mechanism. This occurrence depends on the water status of the berry (Ollat *et al*., 2002) and the cultivar, since table grape cultivars do not shrivel whereas Cabernet Sauvignon and Shiraz shrivel (Fuentes *et al*., 2010).

The length of the third stage range from 35 to 55 days as illustrated in Fig. 1 (Mullins *et al*., 1992). At the end of the third stage, the berry will start to deform (Pirie & Mullins, 1980) since the phloem sap movement is completely impeded two to three weeks after berries reached their maximum weight (Fig. 1). The berries start to shrink due to isolation from the xylem and phloem pathways while transpiration continues. The solutes per berry stay constant even though water is lost through transpiration (Coombe & McCarthy, 2000).

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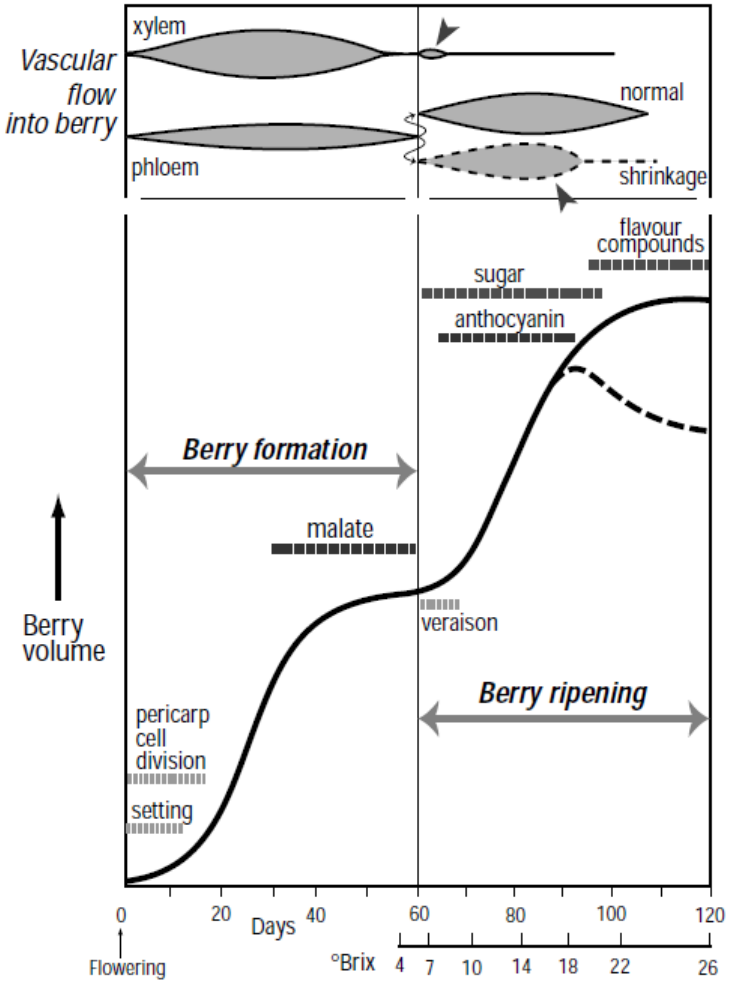

Figure 1. Illustration of berry development, solute accumulation and change that occur in the vascular flow of the berry (Coombe & McCarthy, 2000).

The quality of the final product depends on the third stage since final berry size, acidity, sugar, colour and flavour are determined during this stage (Roubelakis-Angelakis, 2001). Table grape cultivars depend particularly on sugar, pH and firmness for taste (Liang *et al*., 2005).

Indicators for harvest maturity that are used are titratable acids, total soluble solid content (˚Brix) and sugar:acid ratio (Guelfat-Reich & Safran, 1971; László & Saayman, 1991a; Jayasena & Cameron, 2008). Harvest dates for various table grape cultivars are determined by the acidity of the cultivar in the following way: a minimum °Brix level is used for low level acid cultivars, acidity content for high acidity cultivars and sugar:acid ratio for medium acidity cultivars (Guelfat-Reich & Safran, 1971). According to László & Saayman (1990) acidity fluctuates over seasons and can therefore not be used as a reliable harvest indicator. Therefore sugar concentrations should be the only indicator to ensure satisfactory palatability (László & Saayman, 1991b; Sonego *et al*., 2002).

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2.3. Factors influencing development

Several factors, namely cultivar and rootstock, production area, cultural practices, temperature, light, leaf area, crop size, application of plant bioregulators and seasonal changes influence berry development and anthocyanin formation (Jeong *et al*., 2004; Brar *et al*., 2008a). For instance, it was found that: (i) Anthocyanin accumulation increases with lower temperatures which can be initiated by sprinkler cooling and with a decrease in crop load: leaf area (Pirie and Mullins, 1977). (ii) Girdling at véraison improves ripening and anthocyanin accumulation (Winkler *et al*., 1974), but when done at berry set it results in lower soluble solid and anthocyanin accumulation (Dokoozlian *et al*., 1995). Contrary to this, Brar *et al*. (2008b) found that girdling at berry set causes an increase in anthocyanin accumulation. Brar *et al*. (2008b) also suggested that girdling promotes the activation of the enzyme F3', 5'-hydroxylase which drives anthocyanin formation in the skin. (iii) Vines infected by leafroll have a lower accumulation of anthocyanins since the anthocyanin pigment depends on photosynthates from the leaves. The virus infected vines have a lower photosynthate activity in the leaves. Anthocyanin development is also inhibited by the modification of enzymes involved in anthocyanin production in infected vines (Brar *et al*., 2008b). (iv) Plant bioregulators like ABA treatment applied at véraison can enhance anthocyanin synthesis, while NAA and shading decrease synthesis (Jeong *et al*., 2004).

2.3.1 Water

In the first four weeks of berry development after anthesis, the berry is prone to berry drop when brief periods of water stress occur (Alexander, 1965; Harris *et al*.,1968). When the berry reaches the third growth stage it is no longer susceptible to abscission (Alexander, 1965). Later berries start to shrink when the vine uses more than 80% of the plant's available soil water (Keller *et al*., 2006). Keller *et al*. (2006) found that before véraison, berries can regain their size when they receive water after a period of water stress. Contrary to this, Ojeda *et al*. (2001) found that even though pericarp cell devision is not susceptible to water stress, the pericarp cell volume will decrease and is irreversible during the first growth stage. The reduction in pericarp cell volume results in decreased berry size and weight (Ojeda *et al*., 2001). However Keller *et al*. (2006) found that only after véraison the berry diameter will not be restored after rewatering; rewatering will only prevent further shrinkage (Keller *et al*., 2006). Creasy *et al*. (1993) suggested that xylem discontinuity after véraison can be the reason why berries are not as susceptible to drought stress like before véraison, possibly because the water flow to and from the berry is restricted.

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2.3.2 Temperature

Ambient temperature affects berry development significantly. Berries grown at minimum daily mean of 20˚C developed faster than berries grown at min. 16˚C (Harris *et al*., 1968). Optimum temperature for berry development is 25˚C day temperature and 20˚C night temperature (Ollat *et al*., 2002). When the temperature exceeds 35˚C within the first two weeks of development, berries can be permanently underdeveloped since cell division is impaired (Harris *et al*., 1968; Ollat *et al*., 2002). Mori *et al*. (2005) found that high night temperatures (30°C) result in decreased anthocyanin content since anthocyanin biosynthetic gene expression are reduced during early stages of ripening.

2.3.3 Plant bioregulators (PBR's)

Plant bioregulators like gibberellic acid (GA_3) and forchlorfenuron, a synthetic cytokinin (CPPU), are generally used by table grape producers to enhance berry size and firmness (Retamales *et al*., 1995; Du Plessis, 2008; Zoffoli *et al*., 2009). Gibberellic acid promotes cell expansion in the berry and therefore decreased cell density, while CPPU causes cell division and increased cell density (Ben-Arie *et al*., 1997). Growth rate of berries treated with GA_3 increase drastically and do not depict the general double sigmoid growth curve of berry development (Du Plessis, 2008; Raath, 2012). Forchlorfenuron delays fruit maturity because of lower TSS, pH and slower colour accumulation (Retamales *et al*., 1995; Ben-Arie *et al*., 1997; Du Plessis, 2008). For example, a dosage of 3 ppm CPPU, applied to Crimson Seedless at 6 to 10 mm berry diameter, resulted in increased titratable acidity but lower anthocyanin concentrations (Strydom, 2013). However, 5 ppm CPPU applied in combination with GA_3 increased TSS of Redglobe (Strydom, 2013). Generally harvest date is delayed between seven and 21 days, depending on concentration of CPPU and GA_3 applied, but in the end will develop adequate colour and TSS (Retamales *et al*., 1995; Du Plessis, 2008). Berry drop during postharvest is also enhanced since pedicles are less flexible (Retamales *et al*., 1995; Strydom, 2013). Gibberellic acid treatment can decrease storage ability of certain cultivars e.g. Muscat Seedless (László & Saayman, 1991a). With an increase in CPPU dosages applied to Crimson Seedless postharvest defects increased (Strydom, 2013).

In many fruits ethylene concentration increase is considered to enhance ripening, since it causes a rise in respiration. This phenomenon does not appear in grape berries – rather the concentration of ethylene, before and during ripening, is low (Coombe & Hale, 1973; Gény *et al*., 2005). However, the exogenous application of Ethepon increases berry colour (Peacock *et al*., 1977; Gény *et al*., 2005). The timing of exogenous ethephon application is very important since the effect, accelerating or impeding ripening, in combination with other hormones depends on it (Coombe, 1989; Chervin *et al*., 2005).

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Abscisic acid (ABA) normally plays a role during stress, abscission and inhibition of seed germination (Coombe & Hale, 1973). Abscisic acid changes the hormonal profile and increases the ripening process (Gény *et al*., 2005). It is also involved in the change of cell wall permeability during véraison, consequently allowing water and carbohydrates to enter the cells more easily (Seymour, 1993). Gluconeogenesis, i.e. formation of glucose from non-carbohydrate compounds like malic acid, is also accelerated by ABA, enhancing ripening (Coombe, 1989; Coombe, 1992). Abscisic acid furthermore influences the transcription or translation of senescence-related genes (Coombe, 1989). When ABA is applied to grapes, the ripening stage is hastened by reducing chlorophyll quantities and enhancing colour change (Coombe & Hale, 1973; Gény *et al*., 2005). Once ABA has increased to a certain concentration, exogenous applied ethylene will promote ripening (Coombe, 1976). If ABA is lower than a specific concentration, the berry will not ripen and ethylene applications can inhibit the increase of ABA at this stage (Coombe & Hale, 1973; Gény *et al*., 2005).

Auxins, indole acetic acid (IAA), delays ripening by influencing the expression of genes necessary for ripening (Coombe, 1989; Gény *et al*., 2005). In addition, expression of cell wall-modifying proteins are also altered which influence cell wall loosening (Davies *et al*., 1997; Català *et al*., 2000; Gény *et al*., 2005). Indole acetic acid accumulates just before onset of véraison (when ABA starts to accumulate) but decreases during the ripening stage (Gény *et al*., 2005).

As berries ripen, calcium concentration decreases in the berries. Senescence is delayed if calcium concentration is maintained, since it plays a roll in the cell wall structure. Ripening therefore depends on the decrease of IAA and calcium concentration and increase of ABA in the berries (Gény *et al*., 2005).

2.4. Sugar

The major carbohydrate components in grapes are glucose, fructose and sucrose. Sucrose is produced through photosynthesis in the leaf mesophyll cells and transported to the berry where it is converted to glucose and fructose (Lavee & Nir, 1989; Horton *et al*., 2006). Glucose and fructose usually represents more than 99% of carbohydrates in juice. Fresh weight of mature berries can contain 12% to 27% glucose and fructose (Winkler *et al*., 1974).

The accumulation of glucose and fructose sugars show three distinct stages throughout the berry development (Hrazdina *et al*., 1984) as illustrated in Fig. 2. In the first few weeks of berry development the glucose and fructose remain constant at low levels. During the second stage, just after véraison, glucose and fructose concentrations increase substantially in both the flesh and skin (Coombe & Nii,

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1983). In the last stage the rate of sugar accumulation decreases (Coombe, 1980). However, final concentration of glucose and fructose depends on the length of time the berries are left on the vine as well as factors like disease status, dehydration, crop load and canopy size (Kennedy, 2002).

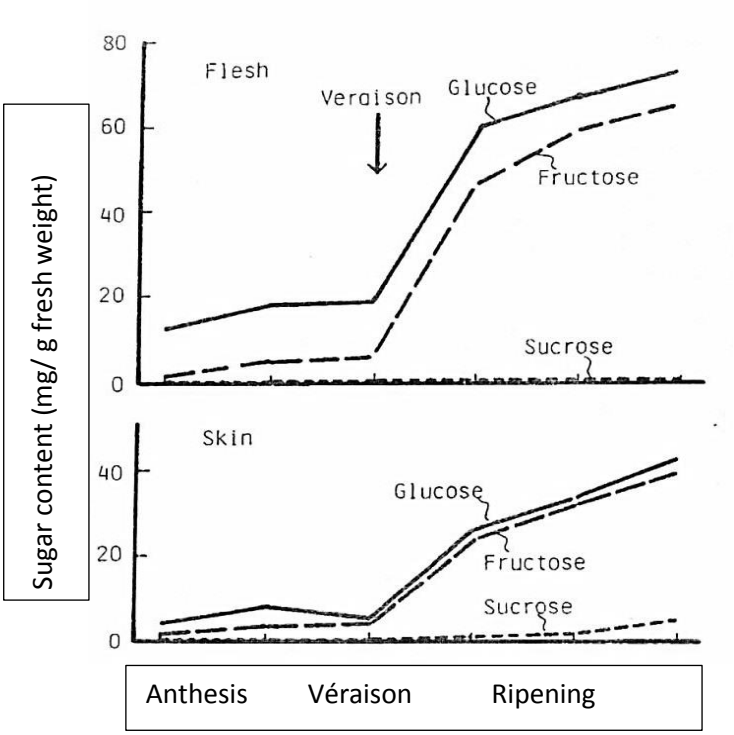

Figure 2. Change in sugar content (glucose —, fructose – – – and sucrose ----) in the various part of the berry (flesh, skin and pedicel) during berry development and ripening (Coombe & Nii, 1983).

2.4.1. Onset of sugar accumulation

Before véraison most of the sugars accumulated in the berry are metabolised, but at the onset of the ripening stage sugar storage in the berry vacuole commences. The amount of sugars stored depends on the cultivar since *Vitis vinifera* varieties store less sucrose than those of *Vitis labrusca* (Roubelakis-Angelakis, 2001).

Sugars used for metabolism and storage are obtained from photosynthesis in the leaves and from reserves in wood (Coombe, 1976). It is transported as a sucrose solution in water through the phloem and start to accumulate in the berry at onset of berry softening (Lavee & Nir, 1986; Coombe, 1989). In the berry sucrose is cleaved into fructose and glucose in either the cytoplasm or the vacuole (Lavee & Nir, 1986; Coombe, 1989). The cleaving process is catalysed by invertase (Robinson & Davies, 2000).

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The onset of sugar accumulation and cleaving activity creates a change in the source:sink relationship of the berry (Coombe, 1989). Last mentioned change occurs because berry cell growth causes the berry to become a strong sink, especially after véraison (Lavee & Nir, 1986; Coombe, 1989; Ollat *et al*., 2002). During the first six days after véraison; glucose and fructose accumulation follows a linear pattern (Fig. 2).

2.4.2. Sugar transport and accumulation

Sugar accumulates in both the skin and flesh. Once uptake has been activated the sugars will increase steadily and in an unstoppable way (Coombe, 1992). Sugar movement from the leaves to berry vacuoles can be divided in three stages, namely: 1) sugar loading, 2) sugar transport and 3) sugar unloading (Davies *et al*., 1999; Deloire, 2009). These stages are not separated from each other and therefore each stage flows into the next.

2.4.2.1 Sugar loading

Sucrose is produced in the mesophyll cells of leaves and pumped through the plasmamembrane into the phloem (Roubelakis-Angelakis, 2001; Lalonde *et al*., 2003). Lalonde *et al*. (2003) also found that symplastic- and apoplastic loading of sucrose into the minor veins of the vascular network can co-exist.

According to Deloire (2011) sugar loading in the berry follows one of three patterns: 1) quick and constant loading: from véraison onwards active carbohydrate uptake occurs from the leaves. This is associated with the second growth stage (ripening stage) of the berry and coupled with high rates of berry volume increase and vigorous growth. 2) Inhibited sugar loading (inhibited ripening): this loading is slow and sluggish which can eventually “block” ripening. It is normally caused by imbalanced vines, water deficit or high crop load. 3) Sugar loading with a plateau phase: a plateau is reached after an active sugar loading phase and is associated with maturity.

2.4.2.2 Sugar transport

Sucrose moves through the phloem by mass flow into the berry, where it is stored (Roubelakis-Angelakis, 2001; Lalonde *et al*., 2003). Because it is actively pumped into the phloem, it creates a high osmotic pressure leading to additional water inflow into the phloem. This increases the hydrostatic pressure, which results in a mass flow of phloem sap towards the sink i.e. the grape berry (Ollat *et al.*, 2002; Lalonde *et al*., 2003).

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2.4.2.3 Sugar unloading

According to Roubelakis-Angelakis (2001) sucrose unloading can occur via two methods; symplastically or apoplastically (Coombe, 1989; Roubelakis-Angelakis, 2001). It may be possible that the method of sugar unloading and transport at a given time depends on the development stage of berry and the type of tissue engaged (Davies *et al*., 1999).

During the symplastic unloading, sucrose moves through plasmodesmata (links between adjacent cells in the cell wall). Sucrose transport proteins then support the sucrose movement through the tonoplast into the vacuole. Invertase or sucrose synthase splitting enzymes in the vacuole split the sucrose in roughly equal amounts of glucose and fructose (Davies *et al*., 1999; Roubelakis-Angelakis, 2001)

During apoplastic unloading sucrose is released into the apoplastic space. Sugar transport protein pumps, located in membranes of cells, are involved in the transport and distribution of sugars into cells and tissue of the berry (Roubelakis-Angelakis, 2001). The phloem sugar unloading process through the apoplastic system requires energy, sugar transporters and enzyme involvement (Wang *et al*. 2003). This process is currently being considered as the main pathway for berry water and sugar transport after véraison, since the expression of the sucrose and hexose transporters increase significantly at véraison (Coombe, 1989; Coombe, 1992; Terrier *et al*., 2000; Wang *et al*. 2003; Zang *et al*., 2006). Either the sucrose can be transported into the vacuole where invertase occurs, or the sucrose can be divided by extracellular invertase in the apoplast. The glucose and fructose in the apoplast, are transported across the plasmamembrane with a monosaccharide symporter. It moves across the tonoplast to be stored in the vacuole (Roubelakis-Angelakis, 2001).

2.5. Acids

The acidity in the grapes plays a crucial role in the palatability of the grapes (László & Saayman, 1990), especially table grapes. Natural high acidity cultivars like Dawn Seedless and Sunred Seedless have a fresh taste at high acidity in combination with high sugar concentrations (>19% TSS). Low acidity cultivars like Redglobe already attained an acceptable taste at lower sugar concentrations (László & Loubser, 1995). While Mystery and Prime increase in taste with lower acidity (Sonego *et al*., 2002).

Malic, tartaric, citric and phosphoric acid are the main anion components in the grape berry (Hrazdina *et al*., 1984). More than 90% of acids in the grape berry are a combination of malic and tartaric acids while citric acid accumulates in low concentrations (Winkler *et al*., 1974). Tartaric and malic acid are mostly produced in the fruit from carbohydrate precursors (Ruffner, 1982). The accumulation of cations and

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the metabolism of the major acids create a change in pH and influence the osmotic pressure of the berries (Hrazdina *et al*., 1984; Ollat *et al*., 2002).

Several factors play a role in acid concentrations in the berry which include climatic conditions, type of rootstock, specific cultivar and mineral nutrients. In ripe berries malic acid is negatively correlated with temperature given that respiration rates are higher and acid breakdown are the main source for respiratory substrates. Grapes grown in cooler areas tend to have higher malic acid concentration than grapes grown in warmer areas (Kennedy, 2002) since malic acid concentration is more susceptible to environmental factors than tartaric acid concentration (Ollat *et al*., 2002). Vigorous vines usually have a higher malic acid concentration at the end of ripening (Kliewer *et al*., 1972; Kliewer, 1973; Ruffner, 1982).

There is a clear difference between tartaric and malic acid accumulation patterns during development, as illustrated in Fig. 3. Tartaric acid per berry increases within the first 20 days after anthesis and then remains constant until harvest (Fig. 3) (Ollat *et al*., 2002). Malic acid per berry normally starts to accumulate at the end of stage one of berry development for four to five weeks until véraison (Fig. 3) but one day after glucose and fructose accumulation commences, it starts to break down (Coombe & McCarthy, 2000; Roubelakis-Angelakis, 2001). During the ripening period malic acid can possibly either be diluted or metabolised and used as an energy source (Hrazdina *et al*., 1984; Sweetman *et al*., 2009; Zang *et al.*, 2011). Malic acid concentration therefore decreases and stabilise at low concentrations, approximately 2 to 3 g/L, which causes a change in pH in the vascular tissue (Hrazdina *et al*. 1984; Coombe & McCarthy, 2000; Roubelakis-Angelakis, 2001).

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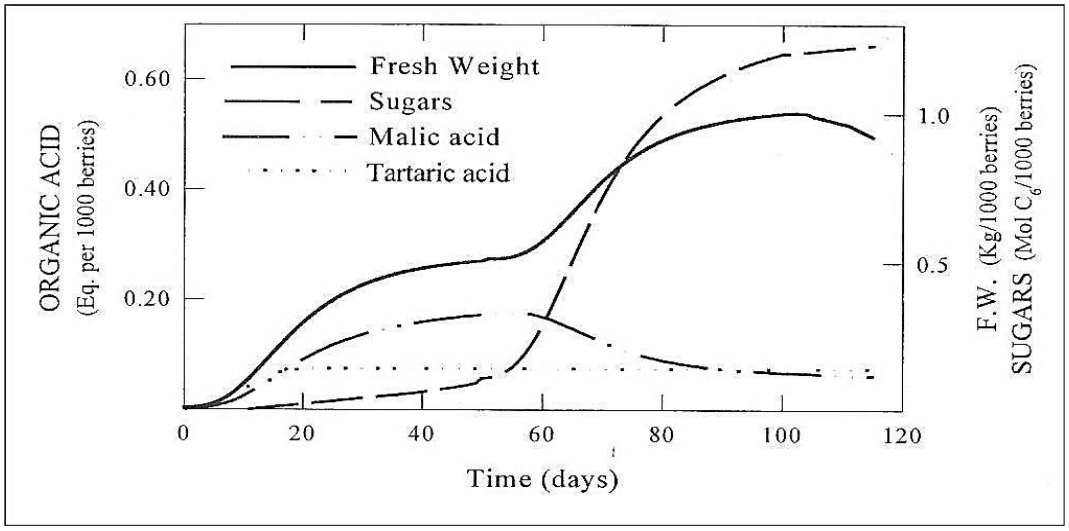

Figure 3. Change in tartaric acid, malic acid, sugars and fresh weight of the grape berry during development (Roubelakis-Angelakis, 2001).

According to Hrazdina *et al*. (1984) tartaric acid concentration stays constant in the early berry development (from 4mm berry diameter). After four weeks tartaric acid concentration will decline rapidly for three weeks where after it is synthesized again for another three week period. From here on tartaric acid will be metabolised until harvest (Hrazdina *et al*., 1984) therefore the concentration remains the same. This differs from the results obtained by Iland & Coombe (1988) who found that tartaric acid concentration decreases during ripening.

Tartaric acid per berry volume however remains constant in the berry's flesh and skin during ripening (Fig. 4). Illand & Coombe (1988) describe this decrease in concentration as a dilution of tartaric acid since the volume of the berry increases, and not due to metabolism of tartaric acid as Hzardina *et al*. (1984) explained previously.

Malic acid concentration per berry and volume per berry decreases in the flesh, but increase in small amounts in the skin during ripening as illustrated in Fig. 4 (Iland & Coombe, 1988). Contradictory to these results other studies have revealed that malic acid concentration is higher in the inner flesh than the outer flesh, near the skin, since malic acid respiration occurs in the outer flesh cells (Possner & Kliewer, 1985). Coombe (1987) suggested that vascular bundles might be involved in the malic acid respiration.

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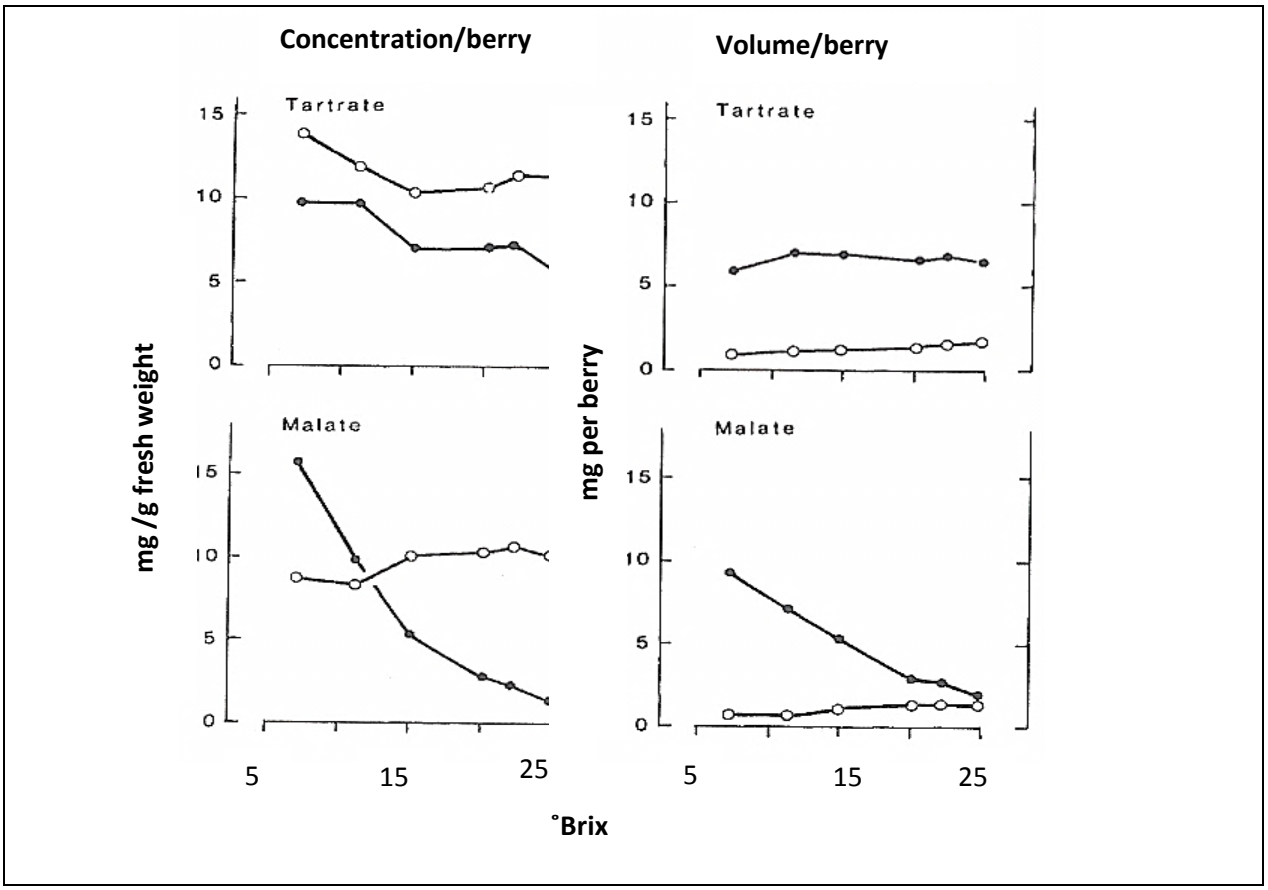

Figure 4. Change in tartaric and malic acid in the skin (○) and flesh (●) during the ripening of Shiraz grapes. Results are expressed as concentration per berry (left) and volume per berry (right) (Iland & Coombe, 1988).

Before véraison, carbohydrates are used as an energy source, while carboxylic acids are used as the energy source during ripening (Mullins *et al*., 1992). Organic acid concentration therefore decreases during berry ripening due to berry volume increase (dilution of organic acids), acid break down, salt formation, inhibition of acid production, as well as acid transformation into sugars and energy sources (Roubelakis-Angelakis, 2001).

Tartaric acid concentration decreases during ripening. This results from berry water and cell volume increase as sucrose accumulates. However, when tartaric acid is measured on a per berry basis the levels stay constant during ripening. It might even increase slightly during berry water loss (Mullins *et al.*, 1992; Roubelakis-Angelakis, 2001).

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2.6. Anthocyanins

The plant creates self defence mechanisms against the harsh UV-A and UV-B radiations which involves the production of hydroxycinnamic acids and flavonoids, (Zang *et al*., 2011; Azuma *et al*., 2012). The phenolics in grapes contain end products of hydroxycinnamic acid which includes the following: coumaric- and caffeic acid, anthocyanins, tannins and flavonols (Cantos *et al*., 2002). The flavonoid phenolics accumulate in the dermal cells while the non-flavonoid phenolics accumulate in the vacuoles of mesocarp cells in the berry (Mullins *et al*., 1992). The total phenol concentration in skins of coloured berries decline until véraison, but rise again during anthocyanin accumulation (Mullins *et al*., 1992). Anthocyanin pigments are present in red and black cultivars (Jeong *et al*., 2004) while carotene and xanthophyll are the colour pigments present in white and yellow cultivars (Mullins *et al*., 1992).

Tannins and colour pigments are located mainly in the skin of the berry (Cantos *et al*., 2002; Adams, 2006), while the seeds only contain tannins (Adams, 2006). The seeds accumulate polyphenols during the first growth stage of the berry and the reach a maximum at véraison. During ripening, the seed experience water loss and the polyphenols are oxidised, giving the seed a brown colour (Adams, 2006). The skin consists of two cell types: The first single layer on the outside of the berry is made up by clear epidermal cells and the six layers of hypodermal cells are found underneath the first layer. The amount of hypodermal cells present depends on the specific cultivar (Considine and Knox, 1979). Tannins and anthocyanins are located in the vacuoles of the first three to six hypodermal cell layers of the skin (Hrazdina *et al*. 1984; Mullins *et al*., 1992; Adams, 2006). The amount of anthocyanins accumulating in the hypodermal cells is indefinite – each cell contains various amounts of anthocyanins (Adams, 2006).

2.6.1 Onset of phenol and anthocyanin accumulation

Pirie & Mullins (1977) noted that anthocyanin accumulation coincides with, or just after, sugar accumulation. With these findings Pirie & Mullins (1977) concluded that the accumulation of sugar in the skin of the berries act as regulator in the production and accumulation of anthocyanins, but in later revised research they explained that anthocyanin generally start to increase one week after sugar accumulation (Pirie & Mullins, 1980). Therefore, there is only a positive correlation between anthocyanin- and sugar accumulation in the skin and the presence of sugar does not act as the trigger mechanism for anthocyanin accumulation.

Other research showed that anthocyanin biosynthesis is genetically regulated in the flavonoid pathway (Carreño *et al.*, 1997; Hiratsuka *et al*., 2001; Mori *et al*., 2005). Some production of anthocyanins occurs

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just after fruit set; this amount is usually below the threshold levels of most methods used in research. These anthocyanin pigments are located in small areas during early stages of berry development (Hrazdina *et al*. 1984). Fujita *et al*. (2005) found that anthocyanidins accumulate in the skins and seeds during stage one of berry development and decreases in concentration after véraison. Its accumulation is in preparation for the production of anthocyanins when the *ufgt* (UDP-glucose: flavonoid 3-O-glucosyltransferase) gene is expressed (Roubelakis-Angelakis, 2001; Azuma *et al*., 2012). Last mentioned is only expressed after véraison and is considered by Roubelakis-Angelakis (2001) to trigger anthocyanin accumulation. No clear evidence, however, of this has been found yet, although the *ufgt* gene is absent in white grape cultivars where anthocyanin is not produced (Roubelakis-Angelakis, 2001). The presence of phenylalanine-ammonia lyase (PAL) is also described as the control point for anthocyanin production (Hrazdina *et al*. 1984; Mullins *et al*., 1992). It is produced from sugars through the shikimate pathway (Pirie & Mullins 1980; Hrazdina *et al*. 1984). The genes required to transport the anthocyanins into vacuoles of the skins, are expressed after onset of véraison (Roubelakis-Angelakis, 2001).

Enzyme activity involved in phenylpropanoid and flavonoid pathways are induced by light (Hiratsuka *et al*., 2001; Azuma *et al*., 2012). Low night temperatures (15˚C) and light stimulate the expression of all the genes contributing to the various pathways and encourage higher total flavonol content than in berries grown at higher night temperatures (35˚C) (Mori *et al*., 2005; Azuma *et al*., 2012).

2.6.2 Types of anthocyanins

Anthocyanins derive from anthocyanidins which includes malvidin, cyanidin, petunidin, delphinidin, and peonidin (Cantos *et al*., 2002; He *et al*., 2010). Cyanidin is considered to be the main precursor pigment of these anthocyanidins (Carreño *et al*., 1997; Brar *et al*., 2008a). Anthocyanins are formed when a glucose molecule are attached to the aromatic ring of anthocyanidin. The anthocyanidins usually forms 3-monoglucoside, 3-*p*-coumaroylglucoside and 3-acetylglucoside when glycosylated (Roubelakis-Angelakis, 2001; He *et al*., 2010). There are more anthocyanins than anthocyanidins, i.e. there is only five anthocyanidins, which can be acylated and glycosylated with a range of sugars and acyl groups at various sites on the ring as described in Fig. 5 (Mullins *et al*., 1992; Roubelakis-Angelakis, 2001; He *et al*., 2010).

Carreño *et al*. (1997) describes the final steps in anthocyanin production by means of Fig. 5: Cyanidin is converted into either peonidin, by 3’-O-methyltranferase enzyme or delphinidin, by flavonoid-3’-hydroxylase enzyme. Delphinidin is furthermore methylated by 3’-5’-O-methyltransferase into petunidin and later on into malvidin. The activity of enzyme 3’-hydroxylase is very important in determining the

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ratios of di- and trihydroxysubstituted anthocyanins produced – these results are used in classifying grape cultivars. Di-hydroxy substituted anthocyanins, cyanidin and peonidin, are low in colour while tri-hydroxy substituted anthocyanins like malvidin, petunidin and delphinidin have intense colours (Carreño *et al*., 1997).

The most common anthocyanin found in red-wine grapes is malvidin based which enhances the blue pigments in the skin and is improved during maturity since malvidin-3-glucoside increases as the berry mature (Roubelakis-Angelakis, 2001). Various table grapes cultivars like Crimson Seedless, Redglobe and Flame Seedless contains di-hydroxy substitute anthocyanins (Carreño *et al*., 1997; Serrano *et al*., 2006), with peonidin-3-glucoside as the main anthocyanin (Cantos *et al*., 2002). Cyanidin-3-glucoside and malvidin-3-glucoside are considered as the following main anthocyanins (Carreño *et al*., 1997; Cantos *et al*., 2002; Serrano *et al*., 2006). Cantos *et al*. (2002) found that acylated anthocyanins like peonidin-3-*p*-coumaroylglucoside and cyanidin-3-*p*-coumaroylglucoside were absent in Flame Seedless but were present in both Redglobe and Crimson Seedless.

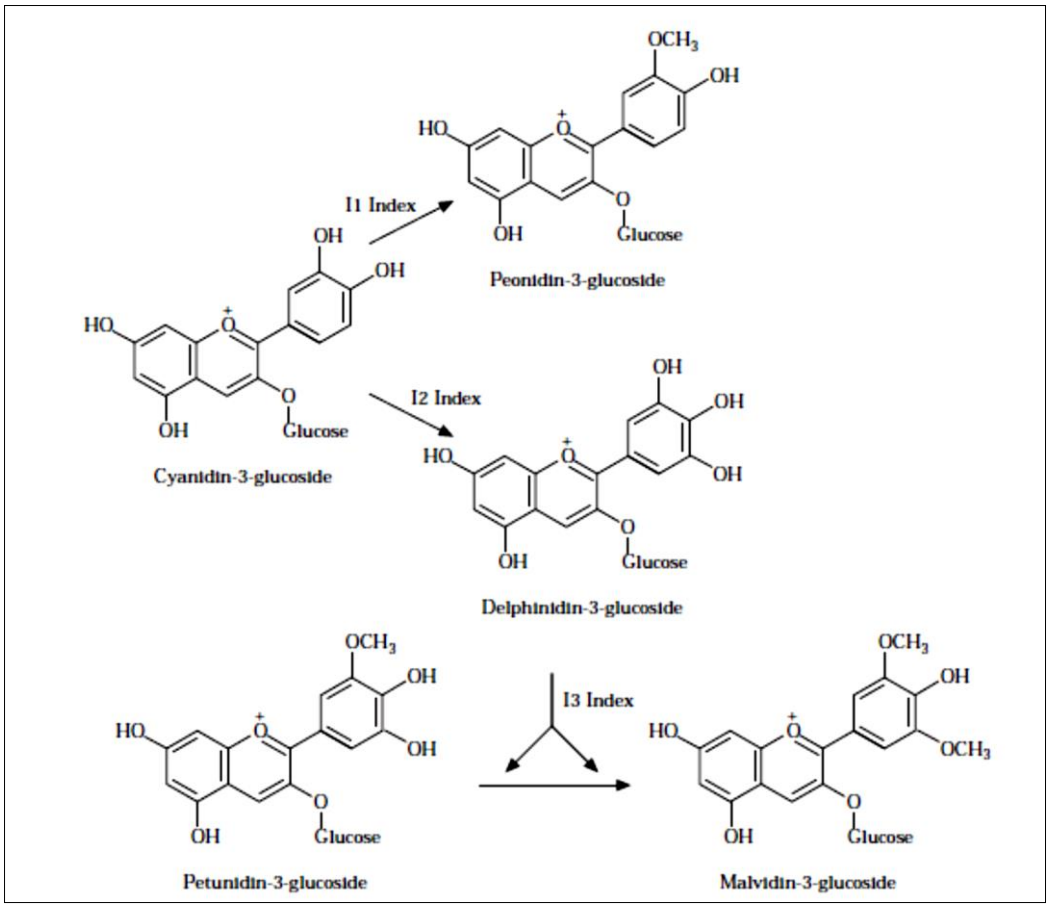

Figure 5. Final steps in anthocyanin production according to Carreño e*t al*. (1997)

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Each cultivar has its distinctive set of anthocyanins with its own colour spectra, which influences the quality and quantity of colour in red or black skinned cultivars (Roubelakis-Angelakis, 2001). Table grapes of *V. vinifera* species are categorised into groups according to their skin colour: green-yellow, pink, red, red-grey, red-dark violet, red-black and blue-black (Carreño *et al*., 1997). The type of anthocyanin present in the skin depends on the number of hydroxyl groups and their position on the ring, the amount of sugars attached, attachment of aromatic or aliphatic acids on the sugars or the methylation of hydroxyl groups (Roubelakis-Angelakis, 2001). In cultivars where the acylated pigments are absent, the grape can only produce the five basic anthocyanins. However, when all three acylated pigments are present, the grape can produce up to 20 anthocyanins (Adams, 2006). Anthocyanin changes colour as the pH of the cell changes. When the pH is above 4.5 the anthocyanins are purple (anhydrobase), at pH 4.0 it is colourless (carbinol base) and under acidic conditions the colour is red (flavilium salt). Alkaline conditions create a blue colour (Mullins *et al*., 1992; Adams, 2006).

2.7. Conclusion

A large amount of research has already been conducted on the development and accumulation of various compounds in the *V. vinifera* berry. Most of these studies focused on wine grapes, where the goal of production practices are to obtain small berries, while research on berry development in conditions that promote the development of large berries e.g. table grapes, is limited.

From these studies *V. vinifera* berry development can be summarised by the following general assumptions (i) organic acids increase during the first growth stage of the berry. Tartrate accumulates just after anthesis while malate accumulates at the end of the first growth stage. After véraison, malic acid is metabolised, but tartaric per berry stay constant. (ii) After véraison, sugars start to accumulate. The most commonly accepted sucrose transport method is the apoplastically phloem unloading pathway. Sucrose is transported throughout the vine and is converted to glucose and fructose in the vacuoles of the berry. (iii) Approximately one week after sugars started to increase, anthocyanins will start to accumulate. The type and amount of anthocyanins present in the berry are determined by the cultivar, which result in unique colour spectra for each cultivar.

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While general berry development can easily be summarised, the complete developing process is complex. Therefore, further research is required to fully understand what changes occur in berry composition during growth in both wine grapes and table grapes.

Possible differences between wine grape and table grape berry development should provide insight to assist producers to manipulate physiological processes to benefit final quality.

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Chapter 3

Changes occurring in table grape berry composition during development: (1) Prime

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3.1 INTRODUCTION

Table grapes are considered one of South Africa's main deciduous fruits. Prime is the fourth major cultivar grown in South Africa and accounts for 9% of total area planted with table grapes (in 2010). It is an early cultivar producing good sized berries with crisp taste (Anon, 2011). To obtain the required characteristics and optimum postharvest quality, it is necessary to understand Prime berry development so that it can be favourably manipulated.

Table grapes are judged by consumers according to colour, crispness, flavour and postharvest shelf life (László & Saayman, 1990; Muñoz-Robredo *et al*., 2011). These parameters are influenced by sugars, organic acid and phenols (primarily anthocyanin). During the 90 to 120 days of berry development (Liang *et al*., 2005) three key processes occur consecutively, namely: organic acids increase in the first growth stage (Coombe & McCarthy, 2000; Roubelakis-Angelakis, 2001; Kennedy, 2002), sugar accumulates at berry softening and ripening (Mullins *et al*., 1992; Coombe & McCarthy, 2000; Roubelakis-Angelakis, 2001) and anthocyanins start to accumulate one week after sugar accumulation commence (Pirie & Mullins, 1980; Coombe & McCarthy, 2000).

Berry development and maturation are influenced by various natural components including light, temperature and plant water status (Hrazdina *et al*., 1984; Liang *et al*., 2005) as well as the genotype of each cultivar (Liu *et al*., 2006). Berry total soluble solids (TSS), measured as ˚Brix, titratable acidity (TA) and TSS:TA ratio are considered as the maturity indices used to establish optimal harvest time for grapes (Guelfat-Reich & Safran, 1971; Liang *et al*., 2005). Berry TSS and pH shows the same pattern during development – slow increase at first, followed by a rapid increase from véraison until harvest. Major acids are metabolised after véraison, lowering the TA (Hrazdina *et al*., 1984).

Organic acids are key factors influencing the taste of table grapes (Muñoz-Robredo *et al*., 2011) and therefore, collectively have an effect on consumer acceptability. The main organic acids in the grape are tartaric acid and malic acid, representing more than 90% of total acids (Ollat *et al*., 2002). The ratio between tartaric and malic acid is determined by genotype of cultivars since wine grapes tend to have higher tartaric acid content than table grapes (Liu *et al*., 2006). Liu *et al*. (2006) found significant differences in tartaric acid, malic acid and total acid content within two consecutive seasons and therefore concluded that organic acids are sensitive to climate change over years. Tartaric acid is more stable during maturation while malic acid is more sensitive to climatic changes for particular cultivars (Ollat *et al*., 2002; Liu *et al*., 2006).

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Numerous studies focused on wine grape berry development (Coombe, 1976; 1980; 1992; Kennedy, 2002) and its compositional changes. Few studies, however, have been undertaken on table grape development (László & Saayman, 1990; Cantos *et al*., 2002; Brar *et al*., 2008), and even less on seedless cultivars. Coombe (1976) found that seedless cultivars do not illustrate definite stages in the growth curve, which could lead to early ripening. In line with this, Prime berry growth dynamic differs from the standard double sigmoid berry development growth pattern by not showing a clearly defined lag stage (Raath, 2012).

This study focused on the Prime development. The aim was to obtain a model of ideal development and accumulation of various compounds including total soluble solids (^{o}Brix), pH, titratable acidity (TA), organic acids (tartaric acid and malic acid), carbohydrates (glucose and fructose), total anthocyanins and phenols and abscisic acid.

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3.2 MATERIAL AND METHODS

3.2.1 Experimental vineyards

The experiment was performed on Prime (*Vitis vinifera* L.) grafted onto Ramsey (*Vitis Champinii* Planch) rootstock. The experimental block was a 15 year old vineyard situated in Slot van die Paarl farm (33˚67'S, 18˚94'E), Berg River Valley, South Africa. The vines were spaced 3 m x 3 m and trained on a flat roof trellis system. Soil water was measured with a tensiometer and drip irrigation was applied as needed during the growing season. Vines were pruned with long bearers, 10 – 12 buds per cane, with an average of ten canes per vine, evenly spaced. Seasonal canopy management included tie-back of shoots, suckering, leaf removal and topping. Leaf removal was done before plant bioregulators were applied as well as during véraison, while topping was done only after the application of plant bioregulators. No lateral shoots were removed. During crop control the yield was reduced to 35 bunches per vine. Standard cultivation practices were followed as recommended by Greyling (2007).

Fig. 1 displays the average monthly minimum and maximum temperatures, while Fig. 2 shows the average monthly rainfall and relative humidity (% RH) for the Paarl area during the period in which the trial was conducted. Both seasons show similar trends in minimum and maximum temperatures. However, maximum and minimum temperatures were lower in November and December in 2011/12 season than in 2012/13 season. Correspondingly to the temperatures, greater rainfall occurred during November and December in 2011/12 compared to 2012/13 season. During 2012/13 harvest month (January), average 2.5 mm rainfall occurred.

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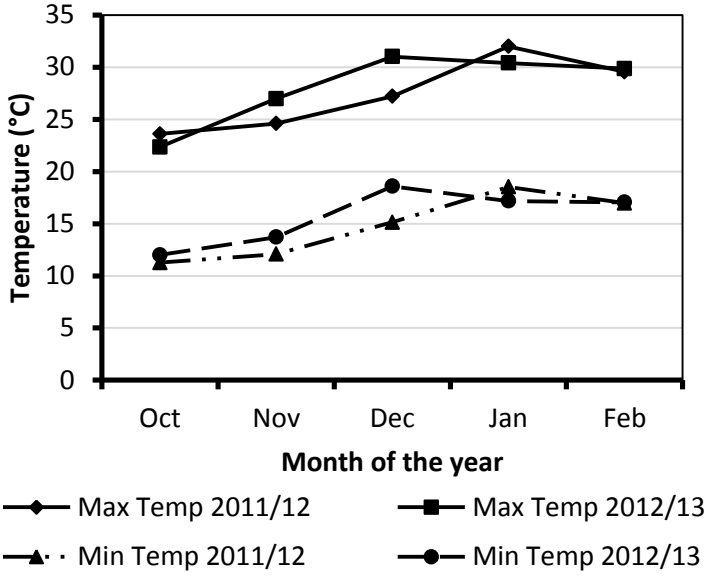

Figure 1. Average minimum and maximum monthly temperatures for Paarl, Berg River Valley, for 2011/12 and 2012/13 seasons (Source: ARC-ISCW).

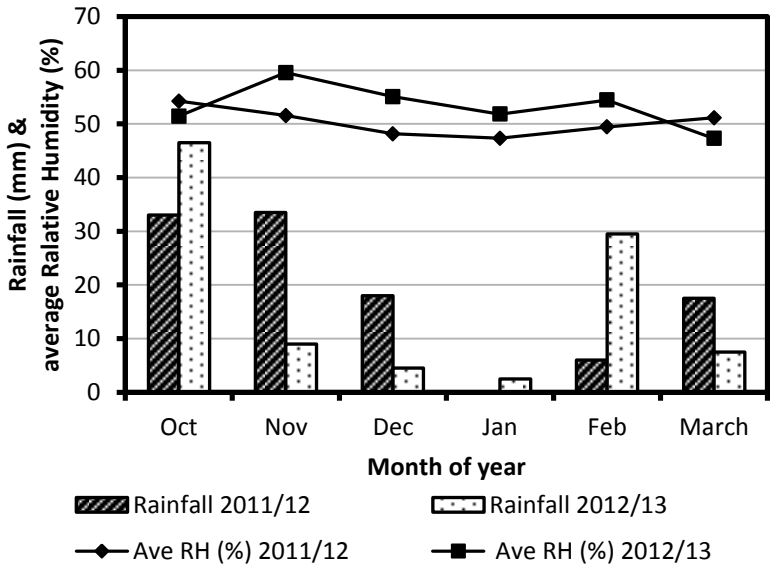

Figure 2. The average monthly rainfall for Paarl, Berg River Valley, for 2011/12 and 2012/13 seasons (Source: ARC-ISCW).

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3.2.2 Experimental design and layout

Three distinct berry sizes were induced by a combination of plant bioregulators. In 2011/12 the three treaments were replicated ten times in a randomised block design. Each replication consisted out of nine vines. Sampling was done weekly for eight weeks. In week eight grapes were harvested for cold storage. In 2012/13 the three treatments were replicated nine times in a randomised block design. Each replication contained 13 vines. Weekly sampling was done for 10 weeks with harvesting occurring in week nine and 10. A different vine was randomly selected per replication each week for sampling.

For two consecutive seasons three distinct berry sizes, namely small (<20 mm), medium (20 to 24 mm) and large (>24 mm) were then selected from the corresponding treated plots and considered as treatments. The three treatments were therefore as follows: 1) Small sized berries - natural berry size smaller than 20 mm. Small size berries were considered as the control, since no treatment was applied; 2) Medium sized berries obtained by standard application of gibberellic acid (GA_3) (dipped with 15 ppm at 8 mm berry size) aimed to obtain berries of average 20 to 24 mm diameter; 3) Large sized berries obtained by a combination of GA_3 and CPPU (forchlorfenuron synthetic cytokinin) application (dipped with 15 ppm GA_3 and 1 ppm CPPU solution at 8 mm berry size) to produce berries larger than 24 mm in diameter. Gibberellic acid and CPPU were applied respectively to all bunches on the nine vines per replicate.

3.2.3 Berry sampling

Berries were sampled weekly from pea size berry i.e. 5 mm berry diameter; stage 31 according to modified E-L scale as described by Coombe (1995), until commercial harvest dates. Every week, four to five bunches were randomly selected per vine from which berries were cut to determine the average berry diameter for each treatment (small, medium and large) using the method described by Joubert (2013). Berries were sized using grids to separate the average berry diameters to correspond to the treatment. A total of 200 berries were selected per diameter size and replication upon which further measurements and analyses were done. This entailed berry mass (g), berry volume (cm^3), total soluble solids (˚Brix), pH, titratable acidity (TA), organic acids (malic acid and tartaric acid), abscisic acid and sugar (glucose and fructose) determination.

3.2.4 Berry analyses

Average berry fresh weight and volume were determined of 50 berries from each replicate of every diameter category as described by Ojeda *et al*. (2001). Thereafter, total soluble solids (^{o}Brix), pH and

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titratable acidity (TA) were determined of the combined pulp of these berries from each replicate of small, medium and large berries respectively. The °Brix was measured by using an electronic hand refractometer, while pH and TA were measured by titrating 50mL juice to end-point of pH=7 using sodium hydroxide (NaOH) with Metrohm, 785 DMP Titrino.

From each berry size (treatment) 50 berries were also peeled. Skins and pulp were stored separately in plastic tubs at -20°C until further analysis. Total phenol concentration was determination of the skins as described by Iland *et al.* (2000). Glucose, fructose, sucrose and organic acid concentrations of the pulp were determined using normal phase HPLC separation with Evaporative Light Scattering Detection (ELSD) according to the method used by Muñoz-Robredo *et al*. (2011). Abscisic acid (ABA) was analysed by UPLC-MS/MS method based on method used by Müller & Munné-Bosch (2011) using the Waters Acquity UPLC/TQD instrument and a water UPLC BEH C-18 1.7µm (2.1 mm x 100 mm) column.

3.2.5 Statistical analyses

Data obtained from both seasons were statistically analysed using SAS statistical software version 9.2 (SAS Institute Inc., Cary, NC, USA). The univariate analysis of variance was performed for each sampling time separately, on all variables accessed, using general linear models (GLM). Observations over time were also combined in a split-plot analysis of variance with week as sub-plot factor (Little, 1972). The Shapiro-Wilk test was performed to test for normality (Shapiro & Wilk, 1965). Student's t-least significant difference was calculated at the 5% level to compare treatment means (Ott, 1998). A probability level of 5% was considered significant for all significance tests.

3.3 RESULTS AND DISCUSSION

Anthesis occurred approximately 14 days prior to pea size berry in both seasons. However, since berry sampling started at pea size berry it will therefore be used as reference point throughout the chapter as days after pea size (DAPS).

Results were expressed in concentration (per volume) and content (per berry) for each component since concentration gave information regarding grapes or juice, while content per berry gave additional information regarding changes occurring throughout development (Coombe, 1992).

Véraison indicates the stage during berry development when berries start to soften, sugars start to accumulate and colour change from green to transparent yellow for white cultivars (Parker *et al*., 2013).

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In this study the start of véraison was represented by the first days of significant sugar accumulation and decrease in acid content as described by Davies *et al*. (2012).

3.3.1 Berry development

Increase in fresh berry weight from pea size berry until harvest for both seasons is indicated in Fig. 3. Berry weight, for all three berry sizes, were lower in season 2011/12 than in 2012/13. Berry weight of medium sized berries treated with GA_3 was lower than large size berries treated with a combination of GA_3 and CPPU. This corresponds with results obtained by Retamales *et al*. (1995). Berry growth plateaued from 42 days after pea size berries (DAPS) in 2011/12, whereas in 2012/13 berry growth continued up to 56 DAPS. Differences in the berry growth rate and development could be ascribed to the differences in temperature between the seasons, since the minimum and maximum temperatures in November and December 2011/12 season were lower compared to 2012/13 season. Higher rainfall also occurred in November and December in 2011/12 than in 2012/13. This corresponds with results obtained from previous studies (Harris *et al*., 1968; Ollat *et al*., 2002) stating that optimal temperature for berry development is average 20°C night temperature and 25°C day temperature. Low minimum and maximum temperatures during berry development in December (14 to 42 DAPS) can reduce berry development rate and finally the berry diameter size.

In both seasons véraison started approximately at 21 DAPS; first in small berries, followed by medium and large berries. Time of véraison can vary within vines since it is influenced by date of anthesis of individual berries (Coombe, 1992). Since all berries reached véraison by 21 DAPS, it was used as reference point for véraison for all three berry sizes.

Increase in berry fresh weight for the three berry sizes did not clearly represent the double sigmoidal curve typical of berry development (Coombe & Hale, 1973; Downton & Loveys, 1978). Berry weight increased rapidly from pea size berry until harvest. If a lag stage did occur before véraison it was too short to be observed with weekly sampling times. Coombe (1980) found that the growth curve of berries from primary bunches without competition (secondary bunches removed) doesn't show a lag stage. The lack of a lag stage could also be ascribed to Prime being an early ripening cultivar with a short transition period from pre- to post véraison (Raath, 2012).

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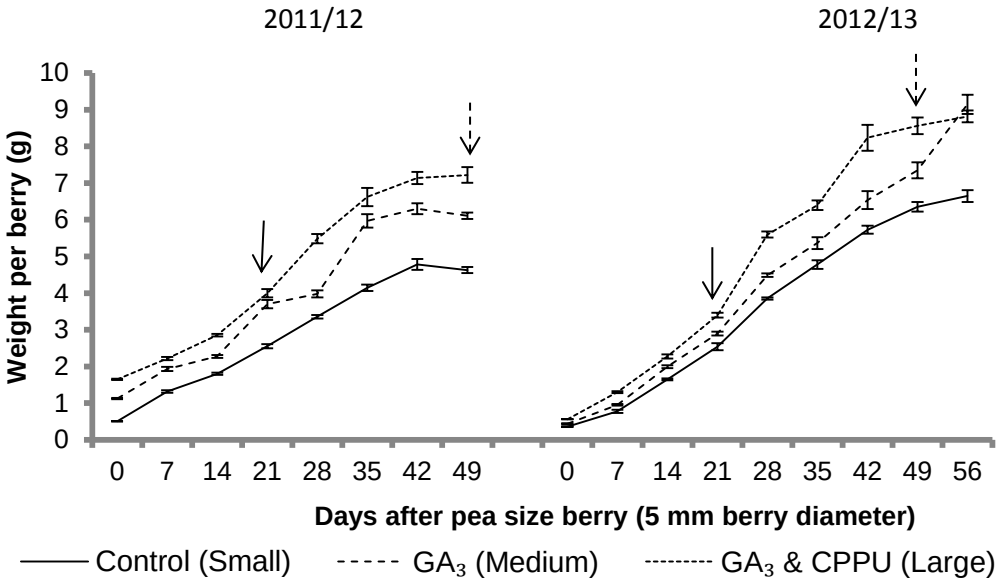

Figure 3. Increase in Prime berry weight for the three berry sizes (small, medium and large) throughout the 2011/12 and 2012/13 seasons. Vertical bars indicate variation for each sampling time ($p \leq 0.05$). The solid vertical arrow indicates start of véraison and broken vertical arrow indicates first harvest date.

3.3.2 Carbohydrates

Glucose and fructose were found to be the main sugars, with sucrose concentration less than 0.5 mg/mL (undetectable) for both seasons in Prime (data not shown). The primary sugars in the in *V. vinifera* cultivars are glucose and fructose with only trace or insignificant amount of sucrose (Nii & Coombe, 1983, Liu *et al*., 2006; Shiraishi *et al*., 2012). Liu *et al*. (2006) found in a study of 98 grape cultivars that the sucrose content of 74 cultivars weren't detectable while the rest contained less than 1 mg/mL sucrose. Liu *et al*. (2006) also found no significant difference between sugar content and composition in successive seasons and concluded that sugar content has a low sensitivity towards climatic change over years. In this study the TSS concentration and berry TSS content also followed similar accumulation patterns for both seasons (Fig. 4 A & B). However, small size berries displayed a faster increase in TSS than medium and large size berries in season 2011/12 (Fig. 4A). Large berries treated with CPPU were expected to have lower TSS concentration (Retamales *et al*., 1995). However, only in 2011/12, the TSS concentration for large berries were lower than small berries but similar to medium size berry concentration, indicating that CPPU treatment did not greatly influence TSS concentration of large berries. In 2012/13 no significant differences in TSS concentrations were found between berry sizes,

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which correspond with results obtained by Walker *et al*. (2005). Total soluble solid concentrations ranged from 13 to 15 ˚Brix for the three berry sizes at first harvest date (49 DAPS) in both seasons, while in the following season TSS concentrations increased to average 16 ˚Brix at second harvest date (56 DAPS) for the three berry sizes (Fig. 4A).

Total soluble solid content (g/berry) showed significant differences in accumulation per berry size in both seasons (Fig. 4B). From 28 DAPS, small berries contained less TSS than medium sized berries, which had less TSS than large berries. From véraison onwards TSS content per berry was higher for the 2012/13 season. This can be ascribed to larger berry sizes obtained for all three treatments (berry size categories) in the 2012/13 season. Lower night and day temperatures during berry development period (November and December) could lead to earlier plateau of TSS content per berry as found in 2011/12 season. However, higher night and day temperatures could result in an increase in berry size and therefore an increase in TSS content per berry as found in 2012/13. Total soluble solid content per berry gradually started to accumulate from pea size berry in both seasons. In 2011/12 TSS rapidly started to accumulate from two weeks before véraison until 42 DAPS, however in 2012/13 TSS started to accumulate a week before véraison (at 14 DAPS) and continued increasing until last harvest date. These results were similar to previous studies stating that TSS accumulation commences at the start of véraison (Coombe, 1992; Robinson & Davies, 2000; Terrier *et al*., 2000; Parker *et al*., 2013).

Berry size seems to affect the concentration of sugar in berries only in sub-optimal conditions, e.g. cool weather and rain. Because berry size has a significant effect on the amount of sugar that accumulates in the berries, i.e. the crop, one might speculate that crop load will affect sugar accumulation in situations where sugar production in the leaves is not optimal. Further work to investigate this is required.

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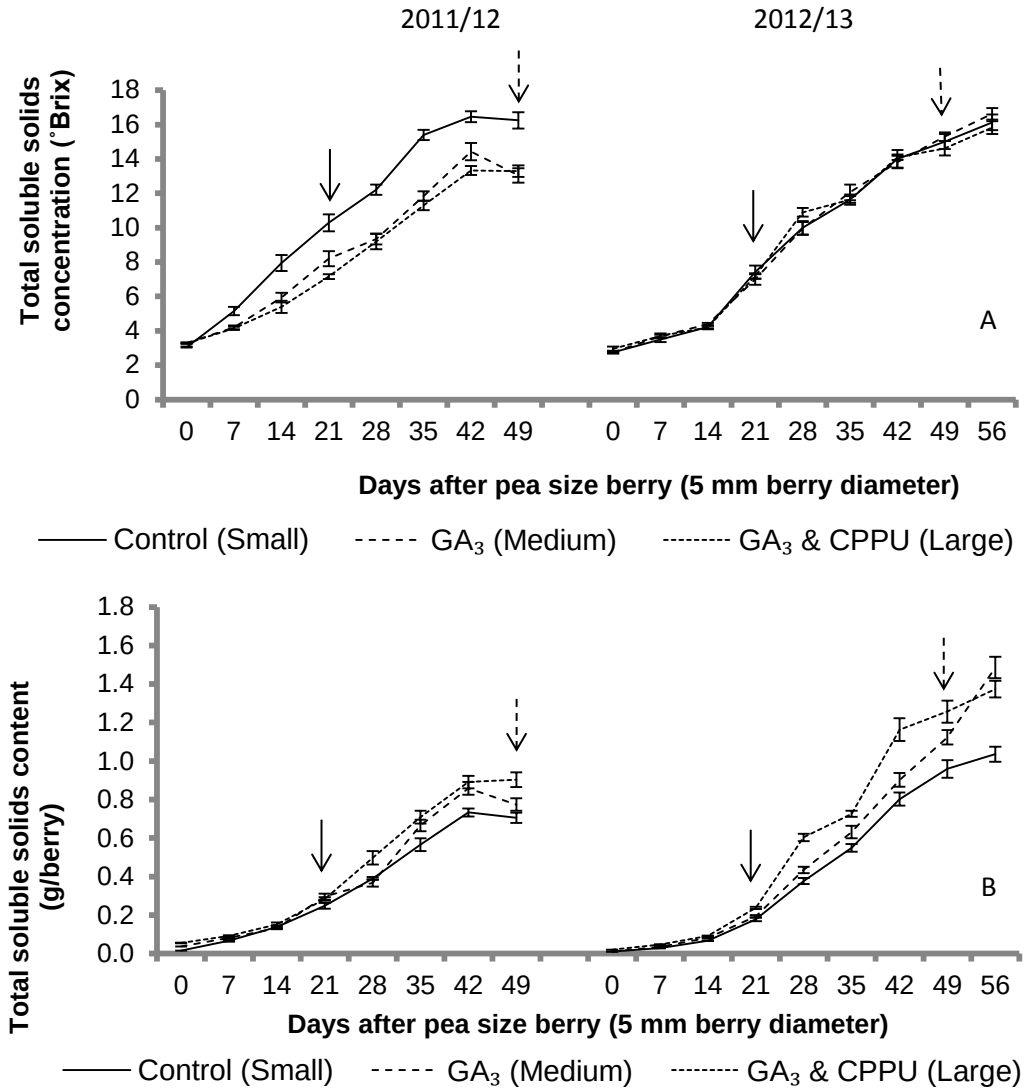

Figures 4A & B. Increase in total soluble solids concentration (°Brix) (A) and content (g/berry) (B) for the three berry sizes (small, medium and large) of Prime, throughout 2011/12 and 2012/13 seasons. Vertical bars indicate variation for each sampling time ($p \leq 0.05$). The solid vertical arrow indicates start of véraison and broken vertical arrow indicates first harvest date.

3.3.2.1 Glucose

Grapes import sucrose which is transformed into glucose and fructose by invertase enzymes (Robinson & Davies, 2000). Glucose concentration started to increase from 14 DAPS for both seasons, one week before start of véraison (Fig. 5A). Small berries had the highest glucose concentration from pea size berry until harvest in 2011/12, however no significant differences in glucose concentration for medium and large berries were found. In 2012/13 glucose concentrations were similar for all three berry sizes (Fig. 5A). Liu *et al*. (2006) found that the average glucose concentrations for *V. vinifera* table grapes

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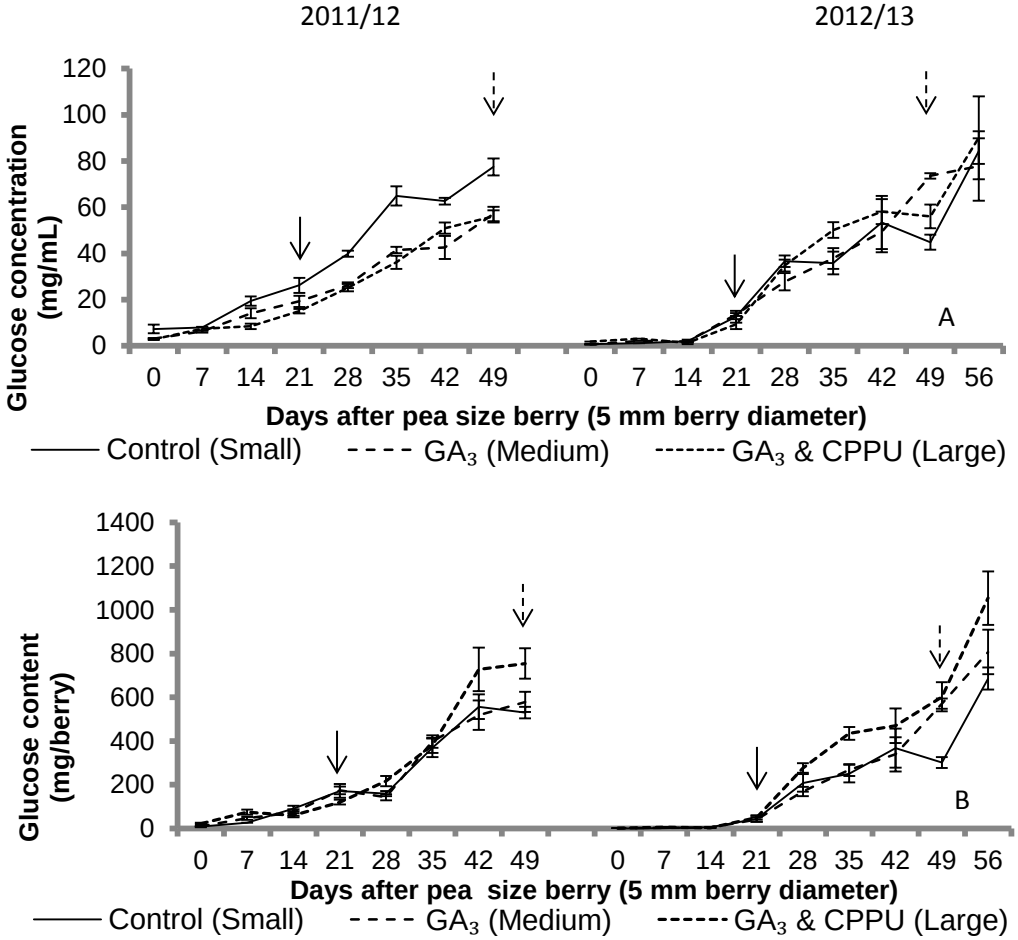
ranged between 67.20 mg/mL to 69.00 mg/mL at harvest which were comparable with the average glucose concentration found for the 2012/13 season in the present study (average 61.22 mg/mL at first harvest date and average 76.78 mg/mL at second harvest). This concentration, however, was only obtained for small berries in the 2011/12 season.

Fig. 5B illustrates the rapid increase in glucose content from the start of véraison for both seasons. In 2011/12 the three berry sizes accumulated similar glucose content per berry until 35 DAPS. The accumulation rate in large berries then increased abruptly before plateauing at 42 DAPS. In 2012/13 glucose content per berry differed in the berry sizes from 21 DAPS. Glucose accumulation never reached a plateau in 2012/13. Large berries accumulated glucose more rapidly, containing highest glucose content at harvest in both seasons (Fig. 5B). Increase in glucose concentration and content can be used to predict start of véraison since glucose concentrations start to increase a week before véraison.



Figures 5A & B. Increase in glucose concentration (mg/mL) and content (mg/berry) for the three berry sizes (small, medium and large) of Prime, throughout 2011/12 and 2012/13 seasons. Vertical bars indicate variation for each sampling time ($p \leq 0.05$). The solid vertical arrow indicates start of véraison and broken vertical arrow indicates first harvest date.

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3.3.2.2 Fructose

Fructose concentration and content per berry followed similar patterns as glucose concentration and content per berry during berry development (Fig 5 & 6). Similar to glucose, accumulation of fructose starts between 10 and 14 days prior to véraison. Small berries had the highest fructose concentration in season 2011/12 but in 2012/13 no significant differences were found between the three berry sizes. Large and medium berries had an average 55.60 mg/mL fructose and small berries 81.52 mg/mL fructose at harvest in 2011/12. At first harvest date in 2012/13, fructose concentration in small berries was 44.43 mg/mL, but it increased to 86.67 mg/mL during the following week. Berry size did not show consistent effect on sugar concentration increase patterns. However, size increase from medium to large berries, did not affect sugar concentration.

Increase in fructose content followed similar patterns as described for glucose content (Fig 5B & 6B). Fructose content increased as véraison commenced. As expected in the 2012/13 season, small berries had the lowest fructose content per berry throughout the season with large berries containing the highest fructose content. Fructose content increased less rapidly than glucose during véraison, but started to accumulate on the same day as glucose. The glucose:fructose ratio decreased from average of two, at pea size berry, to one from véraison for both seasons (data not shown). Coombe (1987) also found that glucose concentrations was double that of fructose in unripe green berries. The glucose: fructose ratio fluctuated around one from véraison until harvest which also corresponds with results obtained from Liu *et al*. (2006) for table grape cultivars. The glucose:fructose ratio might therefore be a reliable indicator of onset of ripening.

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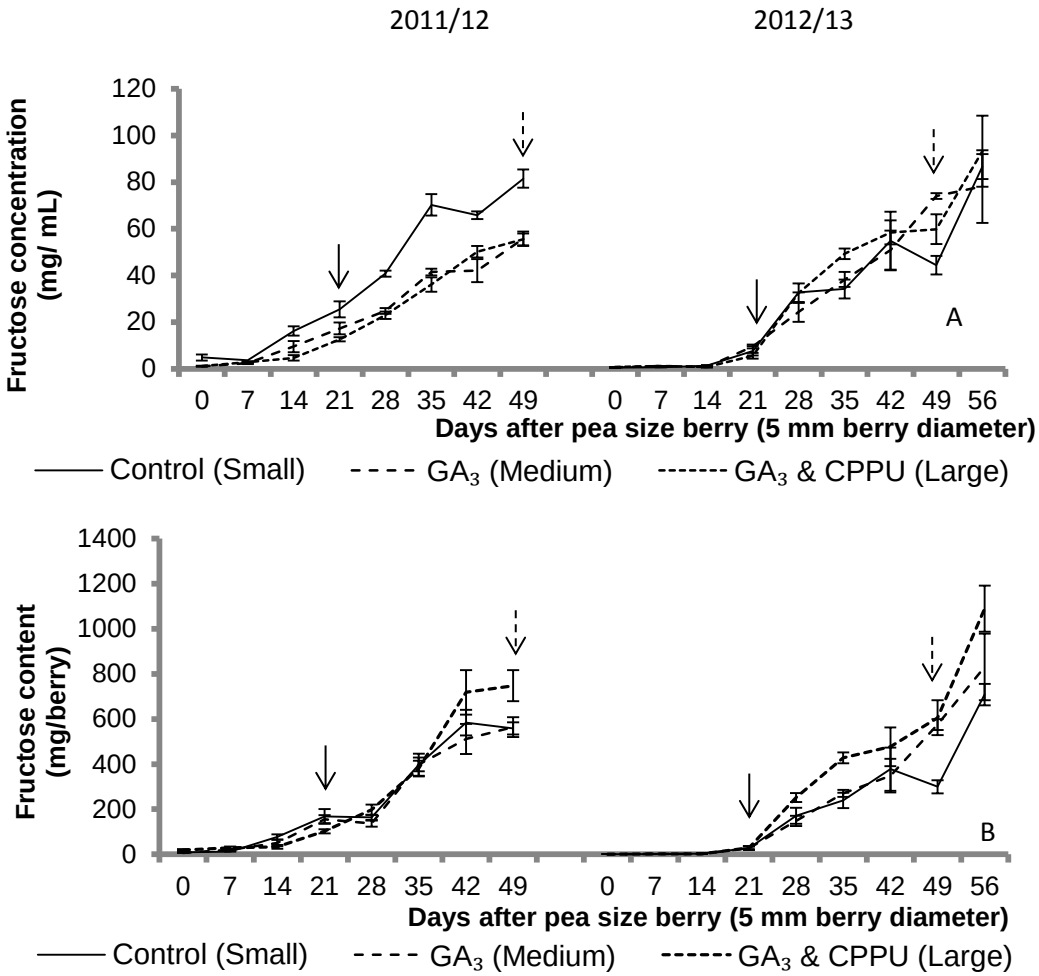

Figure 6A & B. Increase in fructose concentration (mg/mL) and content (mg/berry) for the three berry sizes (small, medium and large) of Prime, throughout 2011/12 and 2012/13 seasons. Vertical bars indicate variation for each sampling time ($p \leq 0.05$). The solid vertical arrow indicates start of véraison and broken vertical arrow indicates first harvest date.

3.3.3 Organic acids

3.3.3.1 Titratable acidity

In both seasons titratable acidity (TA) started at high concentrations that decreased up to harvest (Fig. 7A). This was also described by Liang *et al*. (2005). For both seasons, no significant differences in TA concentration were observed between the three berry sizes (Fig. 7A). At harvest date in 2011/12, the average TA for all three berry sizes was 5.88 g/L which corresponds with 5.8 g/L obtained for table grape cultivars by Liu *et al*. (2006). During the 2012/13 season TA concentrations were lower, i.e. an average TA of 3.89 g/L (at first harvest) and 3.28 g/L (at second harvest) were measured. These differences in TA

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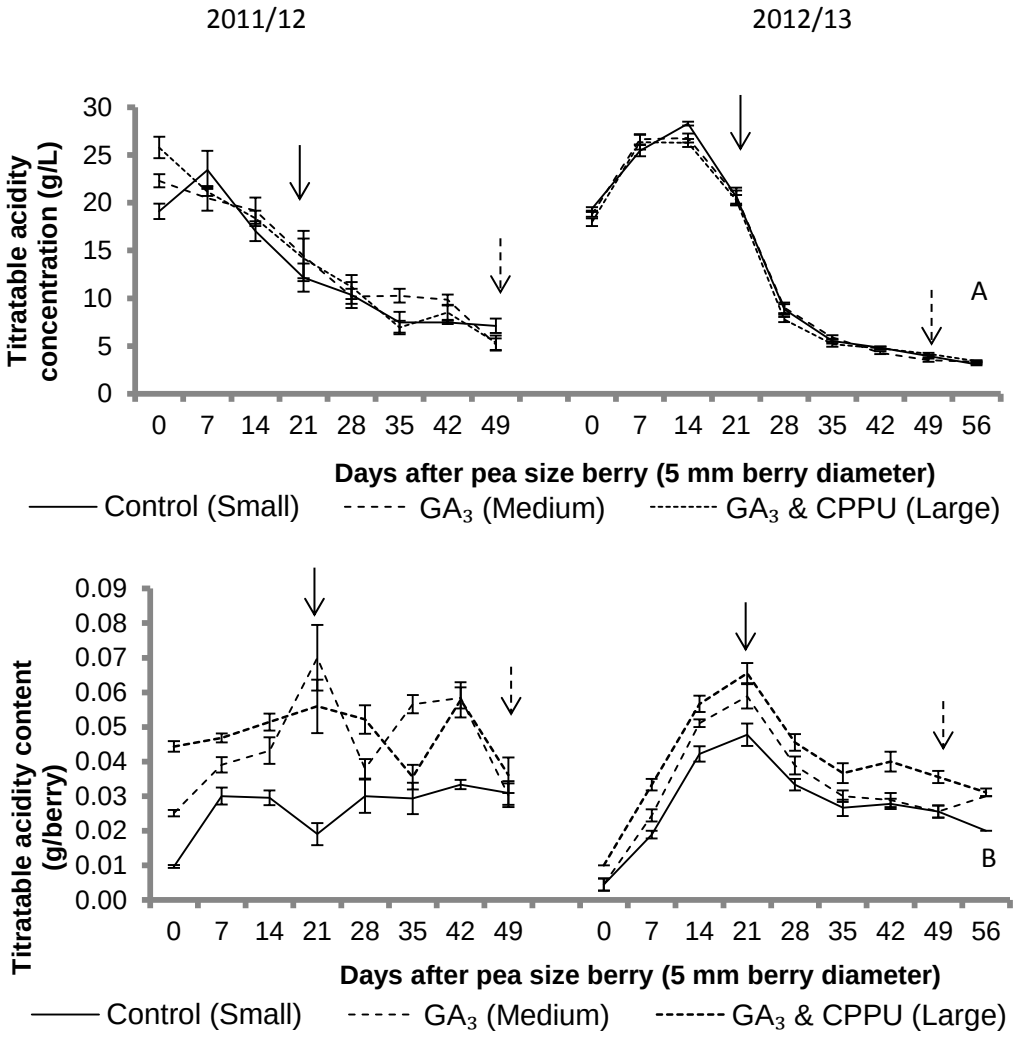
concentrations between seasons are ascribed to higher ambient temperatures during ripening in 2012/13 compared to 2011/12.

The TA content (g/berry) showed a significant difference between berry sizes with small berries containing the lowest TA and large berries containing highest TA content throughout both seasons (Fig. 7B). In 2012/13 TA content (g/berry) increased from pea size berry until start of véraison, from where it decreased rapidly until ripening. Since the trends followed by TA content differed between the seasons, the typical is accepted to be that which was found in 2012/13.



Figures 7A & B. Change in titratable acidity concentration (g/L) and content (g/berry) for the three berry sizes (small, medium and large) of Prime, throughout 2011/12 and 2012/13 seasons. Vertical bars indicate variation for each sampling time ($p \leq 0.05$). The solid vertical arrow indicates start of véraison and broken vertical arrow indicates first harvest date.

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Any TA value above 5.8g/L (rounded to 6g/L) can therefore be regarded as acceptable, while values below this indicate excessive breakdown of malic acid occurred. Furthermore, wine grape pH was considered to stabilise at pH 3.5 (Hrazdina *et al*., 1984). In both seasons, Prime stabilised at an average of pH 4.0 at 49 DAPS for all three berry sizes (data not shown). The date that this pH value is reached might therefore also be regarded as indicative of the last date that harvest for optimal post harvest quality should be conducted.

3.3.3.2 Tartaric acid

In the 2011/12 season tartaric acid started at high concentrations and decreased from seven DAPS until 28 DAPS, after which it remained constant until harvest (Fig. 8A). However, season 2012/13 displayed a different pattern of tartaric acid concentration changes. It increased in the first two weeks after pea size berry and rapidly started to decrease at 14 DAPS. At 28 DAPS tartaric acid concentration increased again and from 35 DAPS remained at a constant concentration until harvest. Hrazdina *et al*., (1984) found that after tartaric acid accumulated, concentration remain constant until véraison, whereafter it declines rapidly. Gutiérrez-Granda & Morrison (1992) found that tartaric acid concentration fluctuated throughout ripening and finished with equal concentrations at harvest than at beginning of véraison. The change in tartaric acid concentration during berry development was not constant for the two seasons and therefore no ideal trend for tartaric acid concentration was obtained. The reason for the different patterns that berry tartaric acid concentration showed between the seasons is not clear.

In accordance with concentration change berry tartaric acid content increased during the first seven DAPS of 2011/12, whereafter it decreased rapidly to véraison (Fig. 8B). This trend is similar to what Iland & Coombe (1988) found in Shiraz grapes. In 2012/13 berry tartaric acid content however followed an inverse trend compared to 2011/12. Tartaric acid continued to increase towards harvest. As expected, large berries contained the highest content of tartaric acid with small berries containing lowest content in both seasons. Differences in night and day temperatures between the seasons could influence the trends.

Both tartaric acid concentration and berry content did not follow similar trends in consecutive seasons and therefore no definite pattern could be established for the change in tartaric acid concentration throughout berry development. Muñoz-Robredo *et al*. (2011) also stated that organic acid trends are different between cultivars, influencing the flavour and harvest date of each cultivar. Total acidity concentration might therefore be a more reliable indicator of berry maturity than tartaric acid.

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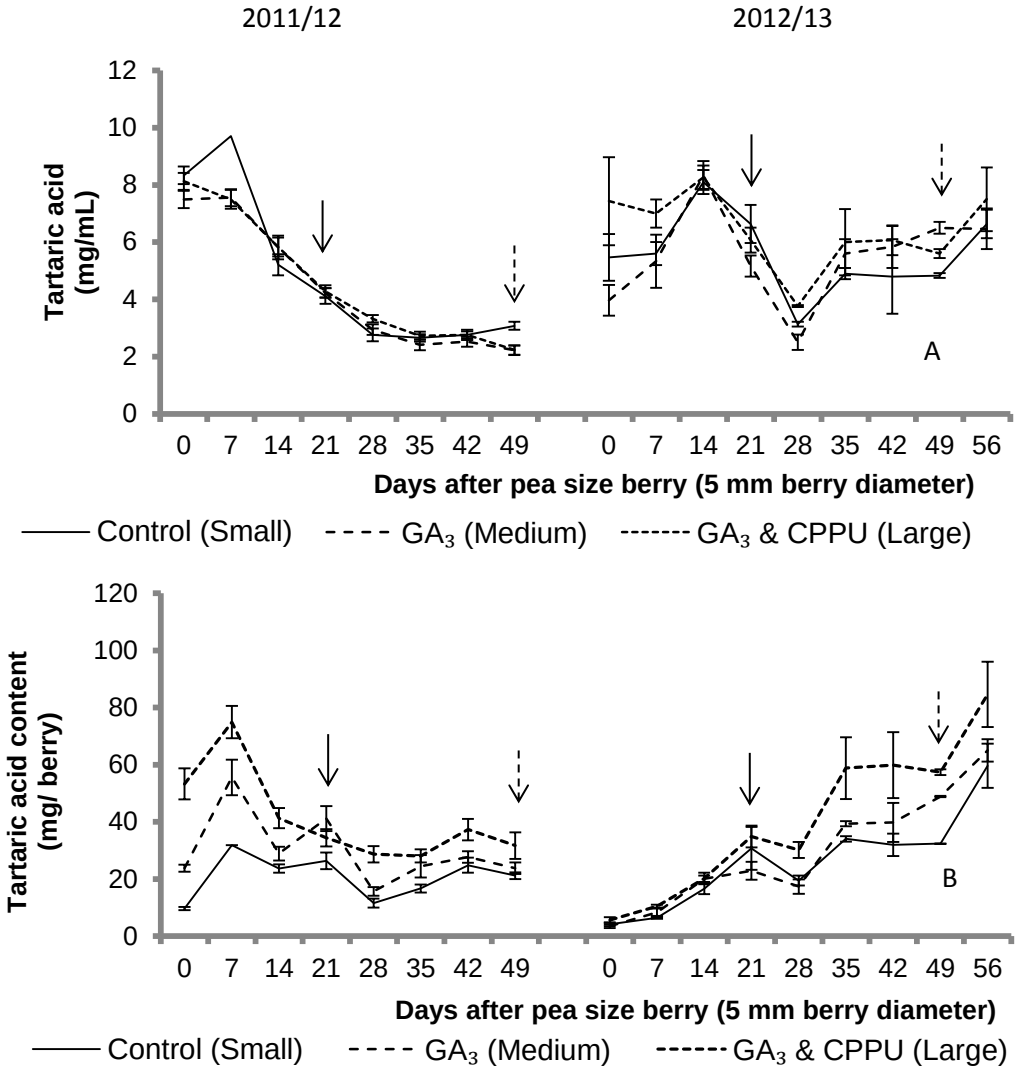

Figures 8A & B. Change in tartaric acid concentration (mg/mL) and content (mg/ berry) for the three berry sizes (small, medium and large) of Prime, throughout 2011/12 and 2012/13 seasons. Vertical bars indicate variation for each sampling time ($p \leq 0.05$). The solid vertical arrow indicates start of véraison and broken vertical arrow indicates first harvest date.

3.3.3.3 Malic acid

The average malic acid concentrations for all three berry sizes were higher in 2011/12 compared to 2012/13 (Fig. 9A). This could be ascribed to the lower temperatures, paired with higher rainfall, during berry development (November and December) in 2011/12 compared to 2012/13 (Figs. 1 & 2). Both seasons followed a general pattern where malic acid concentration increased within the first (2011/12) and second (2012/13) week after pea size berry. This was similar to results obtained by Hrazdina *et al*.

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(1984). In season 2011/12 malic acid concentration started to decrease at one week before véraison, whereas in season 2012/13 malic acid concentration started to decrease as véraison began, which was also found by Gutiérrez-Granda & Morrison (1992). These results correspond with previous studies that found that malic acid concentration increases steadily during the first growing stage and decline rapidly thereafter because it is metabolised, diluted or transformed during ripening (Hrazdina *et al*., 1984; Coombe, 1987). Although similar malic acid concentrations were not obtained before véraison, the final concentrations for both seasons were similar (1 to 2 mg/mL). Liu *et al*. (2006) found that malic acid concentration for *V. vinifera* table grape cultivars range from 2.19 to 2.86 mg/mL at the commercial harvest date for various cultivars. These averages are higher than the results obtained in the present study. This could be ascribed to differences in climatic conditions, cultural practices and vineyard locations.

In line with the malic acid concentrations different trends in malic acid accumulation were obtained for the two seasons (Fig. 9B). In 2011/12 malic acid peaked at seven DAPS and thereafter decreased through véraison up to harvest. As found by Iland & Coombe (1988) malic acid content further decreased throughout ripening. In 2012/13 malic acid steadily increased up to véraison, thereafter started to decrease gradually to lower contents (average 12 mg/ berry) until harvest.

Liu *et al*., (2006) found no correlation between the change in tartaric and malic acid throughout development and at harvest. In this study, however, both tartaric acid and malic acid concentration and content followed the exact same pattern throughout berry development for all three berry sizes. On the other hand, in 2012/13 this correlation was not found. Liu *et al*. (2006) ascribed these differences to organic acids being sensitive to environmental conditions and there is therefore no clear development.

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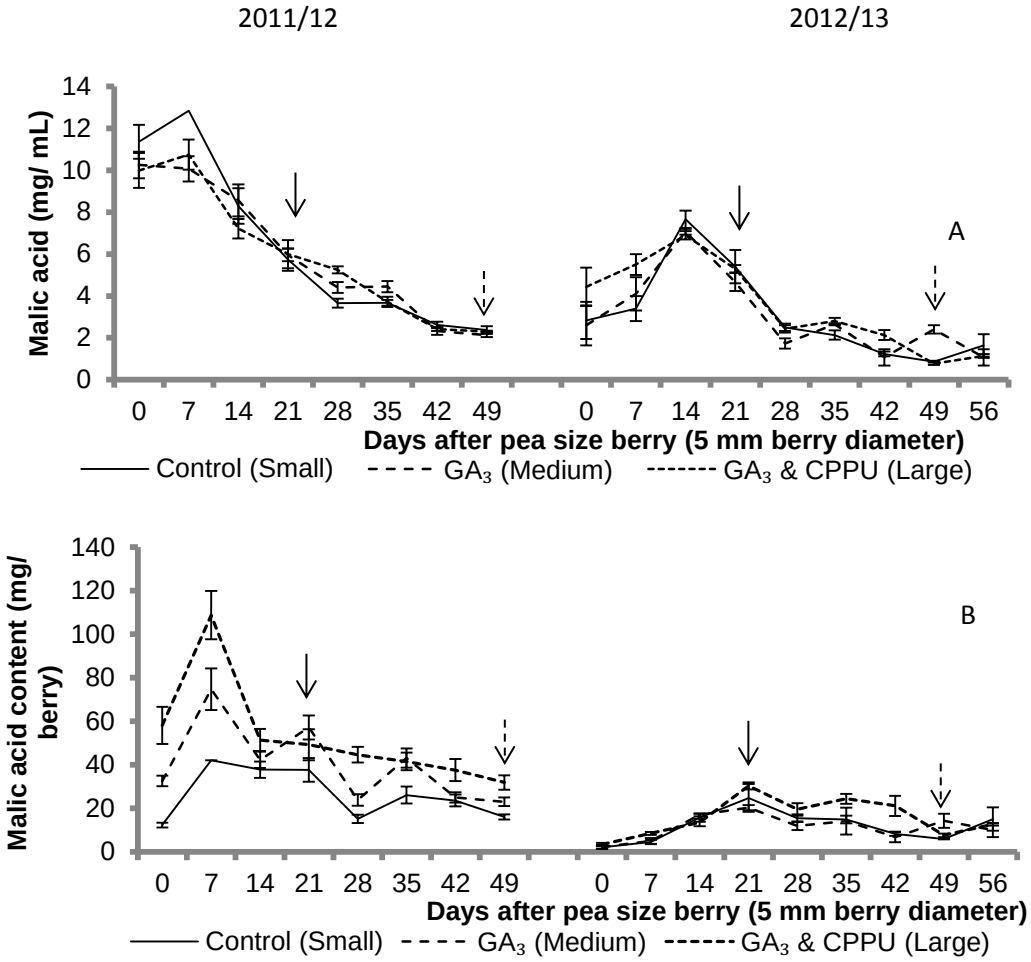

Figures 9A & B. Change in malic acid concentration (mg/mL) and content (mg/berry) for the three berry sizes (small, medium and large) of Prime, throughout 2011/12 and 2012/13 seasons. Vertical bars indicate variation for each sampling time ($p \leq 0.05$). The solid vertical arrow indicates start of véraison and broken vertical arrow indicates first harvest date.

3.3.4 Abscisic acid

Abscisic acid (ABA) concentrations in the berries were low but increased rapidly from 14 DAPS, one week before the start of véraison (Fig. 10A). From one week after véraison ABA concentration stabilised, but showed a slight peak at first harvest (49 DAPS). Concentrations were similar for all three berry sizes throughout the season. The trend followed by ABA concentration in present study roughly corresponded with trend followed by ABA concentration in previous studies (Kataoka, *et al*., 1982; Wheeler *et al*., 2009).

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The ABA content per berry followed similar trends to concentration throughout berry development (Fig. 10B). However, the large berries (GA_3 & CPPU) contained a higher ABA content from 28 DAPS until last day of harvest (56 DAPS), while small berries contained the least amount of ABA until first harvest. At full maturity (56 DAPS) all three berry sizes contained similar amounts of ABA. From 28 DAPS until harvest (49 DAPS) ABA content per berry was therefore influenced by berry size, but at full maturity berry ABA content was similar for all berry sizes.

Previous studies found that ABA content gradually started to increase near the start of véraison, with a rapid increase during véraison for further two weeks reaching a peak, from here on ABA content decreased until harvest (Coombe & Hale, 1973; Gény *et al*., 2005; Wheeler *et al*., 2009). ABA content for Prime followed a similar pattern by increasing at start of véraison and continuing for two weeks. However, ABA content per berry increased again from 49 DAPS. This is contrary to previous studies (Coombe & Hale, 1973; Gény *et al*., 2005; Wheeler *et al*., 2009). The fact that ABA concentration and content increases a week before the observed start of véraison could be used as an indication of the start of ripening, i.e. accumulation of primary and secondary metabolites. The slight peak in ABA concentration around harvest maturity, also possibly indicate to an optimal harvest time.

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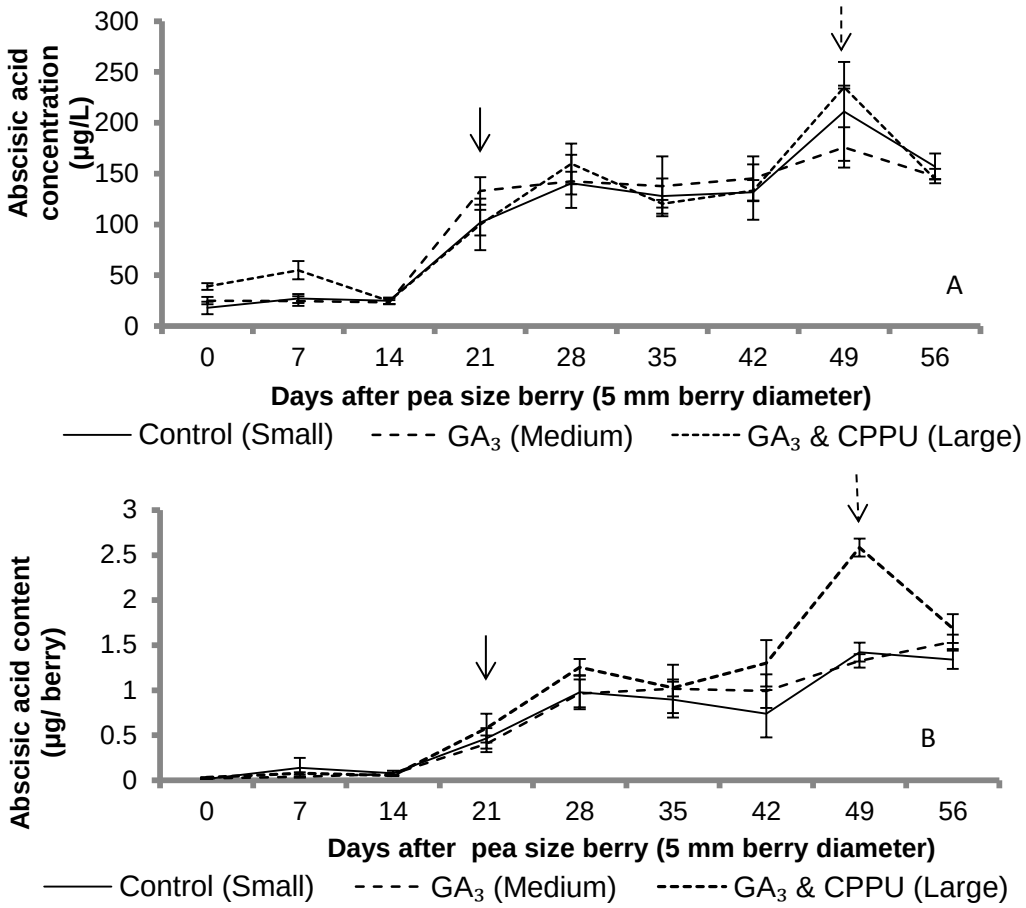

Figures 10A & B. Change in Abscisic acid concentration (µg/mL) and content (µg/berry) for the three berry sizes (small, medium and large) of Prime, throughout 2012/13 season. Vertical bars indicate variation for each sampling time ($p \leq 0.05$). The solid vertical arrow indicates start of véraison and broken vertical arrow indicates first harvest date.

3.3.5 Total phenols

Total phenol concentration of the berry skins followed the same trend in both seasons (Fig. 11A). In 2011/12 total phenols started at higher concentrations, followed by a rapid decrease within a week, compared to 2012/13. During the 2011/12 season a rapid decrease occurred during the first seven days after pea size, while in 2012/13 the decrease was slower and continued up to 28 DAPS. Adams (2006) also found a decrease towards ripening but skin tannin concentrations declined during ripening in proportion to berry expansion. Coombe (1987) also found that phenols decrease during berry development. Total phenol concentration stabilised at low concentrations during ripening for both seasons. These results could suggest that minimum phenol concentration in Prime berry skin is 0.5 mg/g berry.

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Fig. 11B displays the total phenols per berry skin for each of the berry sizes. Season 2011/12 displayed a clear difference in berry size and total phenol content per berry with large berries containing the highest content of total phenols and small berries the least. In 2012/13 total phenol content were similar in the three berry sizes until 35 DAPS, whereafter small berries contained lowest total phenol content with large berries containing highest content of total phenols. Bindon *et al*. (2008) also found that phenolics per berry increase with an increase in Shiraz berry size, since the skin surface area is enlarged. Total phenols per berry did not follow a specific trend and generally remained stable throughout development. Adams (2006) found that tannins (phenols) are produced early in development and decrease prior to véraison. Pirie & Mullins (1980) however, found that total phenols in the skins of Shiraz berries increased one week after TSS (total soluble solids) started to increase. These differences could be ascribed to Shiraz being a red wine grape containing high concentrations of phenolic compounds (Pirie & Mullins, 1980).

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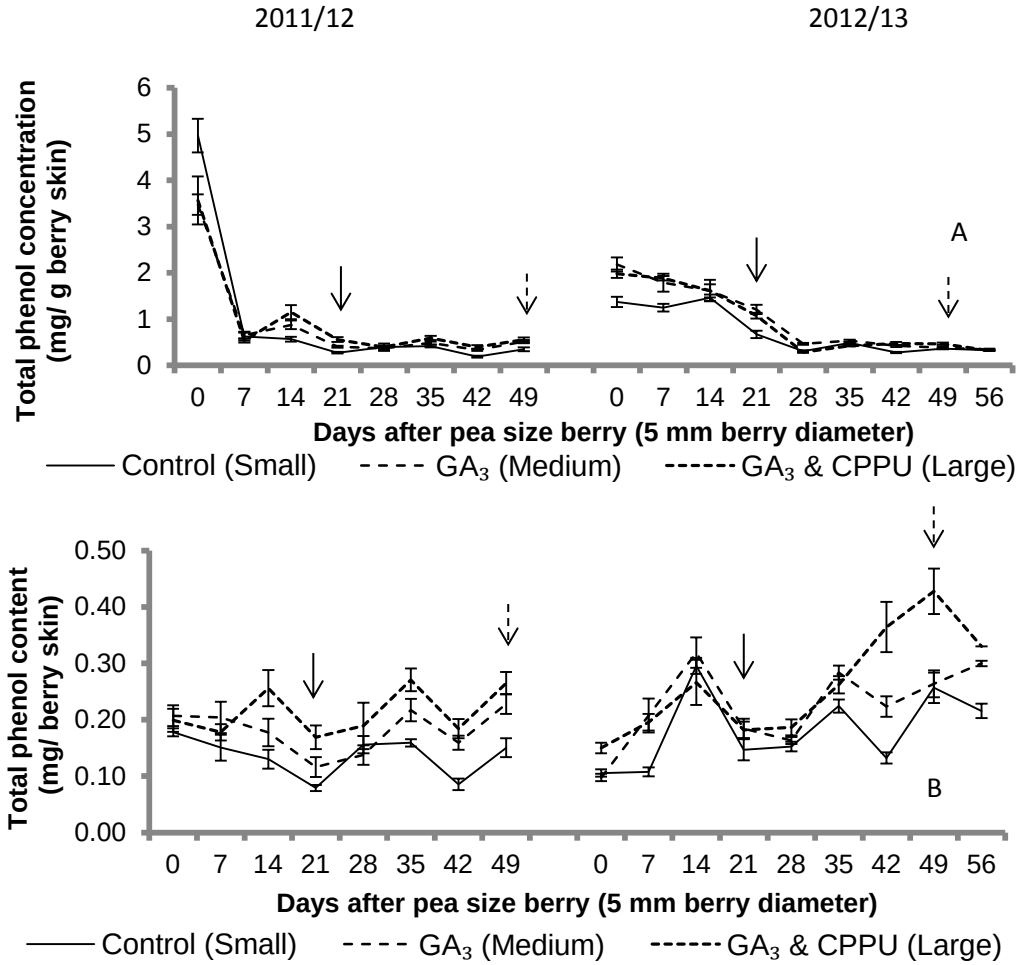

Figures 11A & B. Increase in total phenol concentration (A) and content (B) for the three berry sizes (small, medium and large) of Prime, throughout 2011/12 and 2012/13 seasons. Vertical bars indicate variation for each sampling time ($p \leq 0.05$). The solid vertical arrow indicates start of véraison and broken vertical arrow indicates first harvest date.

3.4 CONCLUSION

Three berry sizes were obtained with significant differences in weight for both seasons, however developing trends for most compounds followed similar patterns between these berry sizes. The trend of changes in compound concentration was therefore not affected by berry size with exception of TTS, and different sugars in 2011/12 season. Berry size also had no effect on the concentration of the compounds discussed in this study. However, some compounds, including TSS, TA, tartaric acid, ABA and total phenol content per berry were influenced by berry size after véraison. Throughout ripening small berries contained lower content per berry with large berries containing highest content for above mentioned components.

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The most important compositional changes occurring in the berry took place at the start of véraison. Total soluble solids, glucose, fructose and ABA concentration increased at the start of véraison while TA, tartaric acid, malic acid and total phenol concentration decreased from around the time véraison commenced. On account of this study, start of véraison was regarded as 21 DAPS. Tartaric acid concentration and content followed different trends between seasons. In 2011/12 trends were similar to what has been found in previous studies with concentrations decreasing from the start of véraison and stabilising at low concentrations until harvest. In 2012/13 an inverse trend was observed. Malic acid concentrations were higher than tartaric acid concentrations at pea size berry in 2011/12 but the reverse was observed in 2012/13. Even though trends differed slightly between seasons, malic acid concentration and content decreased from start of véraison reaching low levels at harvest. Abscisic acid concentration and content followed similar trends as described in previous studies until end of véraison, but contradictory to these studies ABA increased again until harvest.

The final quality of grapes is determined by various components, independently regulated and developed throughout the season but with interchanging functional activities. These components include carbohydrates, organic acids, hormones, phenols and mineral nutrients. Some of these compounds including organic acids are influenced by climatic condition and fluctuate between seasons. Therefore, an ideal development and accumulation model/trend for the various compounds is difficult to describe for Prime. The similar TSS, glucose and fructose accumulation trends that were observed in both seasons, however, suggest that the sugar accumulation rates could probably be used to develop a model for Prime berry development. Further studies focusing on Prime sugar accumulation (TSS, glucose and fructose) in different climatic regions should be considered in order to see whether there is a difference in berry growth and ripening and if an average sugar accumulation trend could be developed. Further studies could also focus on the different components, as in present study, over several years to investigate the effect of climate change on berry development. These trends could aid the producer in scheduling of plant bioregulator appllications to achieve optimal results as well as for harvest program planning.

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Chapter 4

Changes occurring in table grape berry composition during development: (2) Crimson Seedless

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4.1 INTRODUCTION

Crimson Seedless is one of South Africa's most important table grape cultivars, accounting for more than 10% of area planted for table grape production (Anon., 2011). Crimson Seedless is part of the red seedless cultivar group and it is known for its crispy flavour and attractive pink elongated berries (Dokoozlian *et al*., 1995; Anon., 2011). Because the South African Table Grape Industry is reliant on exports, production of good quality grapes with an adequate shelf life is essential. An understanding of berry development and compositional changes that occur throughout growth and ripening will assist to ensure grape quality.

There are numerous compounds in the grape berry that could potentially contribute to the final berry quality. Table grape quality is evaluated according to the colour of berries, their firmness, taste and postharvest shelf life (Muñoz-Robredo *et al*., 2011). Sugar, organic acid and phenols (particularly anthocyanins) are some of the main compounds that influence quality aspects of the grape berry.

Berry development occurs within a period of 90 to 120 days after anthesis (Liang *et al*., 2005) with three main consecutively occurring processes: 1) organic acids increase in the first growth stage (Coombe & McCarthy, 2000; Roubelakis-Angelakis, 2001; Kennedy, 2002); 2) sugar accumulates at berry softening and ripening (Mullins *et al*., 1992; Coombe & McCarthy, 2000; Roubelakis-Angelakis, 2001; Ali *et al*., 2011); 3) anthocyanin start to accumulate one week after sugar accumulation commences (Pirie & Mullins, 1980; Coombe & McCarthy, 2000).

Abscisic acid (ABA) is known to induce berry ripening (Coombe 1992; Wheeler *et al*., 2009). Previous studies found that ABA concentration increases before or at véraison and therefore concluded that ABA control berry ripening (Coombe & Hale, 1973; Downton & Loveys, 1978; Wheeler *et al*., 2009).

Several studies were conducted on wine grape berry development (Coombe, 1976, 1980, 1992; Kennedy, 2002) and compositional changes, but only a few focused on table grape development (László & Saayman, 1990; Cantos *et al*., 2002; Brar *et al*., 2008) and even fewer on seedless cultivars. The present study investigated Crimson Seedless berry development. The aim was to describe the development and accumulation of various compounds in the berries, including total soluble solids (^{o}Brix), pH, titratable acidity (TA), organic acids (tartaric acid and malic acid), carbohydrates (glucose and fructose), total anthocyanins and phenols and abscisic acid. These results might be used in designing a table grape berry growth model for Crimson Seedless. This model could help in decision making regarding postharvest quality of the berries and therefore, the potential markets (international or local).

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4.2 MATERIAL AND METHODS

4.2.1 Experimental vineyards

The experiment was performed on Crimson Seedless (*Vitis vinifera* L.) grafted onto Ramsey rootstock (*Vitis Champinii*). The trial was conducted in two different vineyards, both situated in the Berg River Valley, Paarl, South Africa. During the 2011/12 season a commercial vineyard at the Slot van die Paarl farm (33˚66'S, 18˚91'E) was used and in the 2012/13 season a different block on the Laborans farm (33˚67'S, 18˚94'E). Since organic acid and sugar development are affected by change in environmental conditions (Liu *et al*., 2006), the two vineyards were chosen in the same area, with similar temperature (Fig. 1) and relative humidity, as well as training system, to reduce influences by environmental conditions.

The 11 year old Crimson Seedless vineyard on Slot van die Paarl farm, were spaced 3 m x 2 m while the seven year old Crimson Seedless vineyard situated on the Laborans farm, were spaced 3 m x 1.875 m. Both vineyards were trained on a double gable trellising system with a North-South row orientation. Soil water potential was measured with two tensiometers. When top-soil water potential reached 16 to 18 kPa, irrigation was applied by means of micro-irrigation. During véraison and berry ripening soil water potential was allowed to decrease to 30 kPa. Vines were pruned with 12 evenly spaced long bearers (10 to 12 buds per bearer) with an equal amount of spurs (four to six buds per bearer). Seasonal canopy management included shoot tie-back, suckering, leaf removal and topping. Suckering was done at 30 cm shoot length while shoots were tied back at 60 cm shoot length. After berry set, leaves were removed while topping was done at the start of véraison. No lateral shoots were removed. During crop control the yield was lowered to 26 to 28 bunches per vine. Vines were fertilised during bud break (N and Ca), just after berry set (N, K and Mg) and after harvest (N). Standard cultivation practices were followed as recommended by Greyling (2007).

Fig. 1 displays the average monthly minimum and maximum temperatures, while Fig. 2 shows the average monthly rainfall for the Paarl area in which the experimental farms were situated. Data was only indicated for the periods during which the trials were conducted. Both seasons show similar trends in minimum and maximum temperatures. However, maximum and minimum temperatures were lower in November and December in 2011/12 season than in 2012/13 season. Corresponding to the temperatures, higher rainfall occurred during November and December in 2011/12 compared to 2012/13 season. During 2012/13 harvest month (February), average 29.5 mm rainfall occurred.

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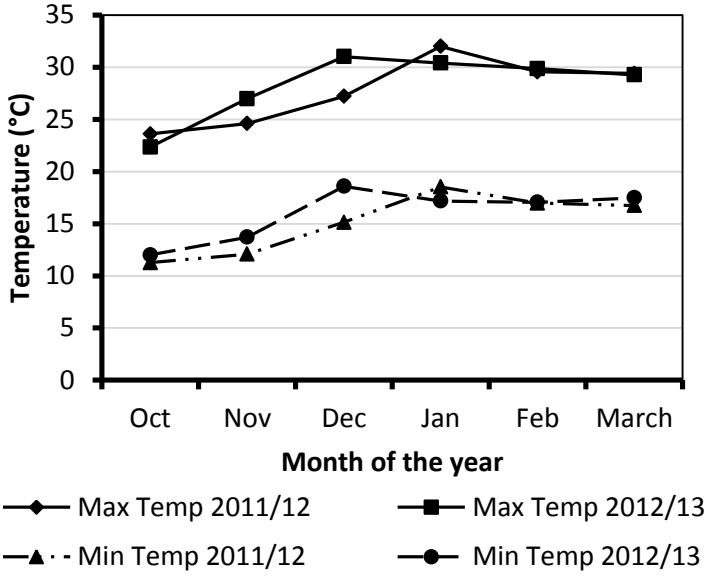

Figure 1. Average minimum and maximum monthly temperatures for Paarl, Berg River Valley, for 2011/12 and 2012/13 seasons (Source: ARC-ISCW).

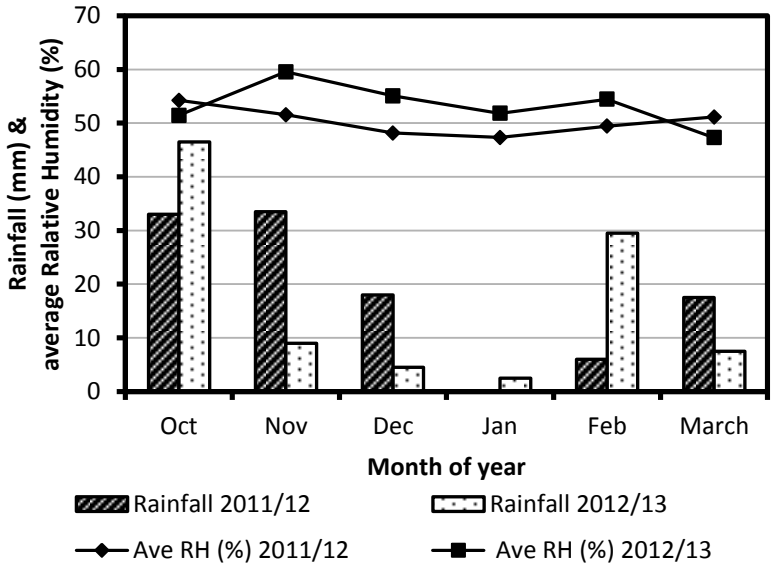

Figure 2. The average monthly rainfall and average relative humidity (RH) (%) for Paarl, Berg River Valley, for 2011/12 and 2012/13 seasons (Source: ARC-ISCW).

4.2.2 Experimental design and layout

Three distinct berry sizes were induced by a combination of plant bioregulators and girdling respectively. Three berry sizes, namely small (<18 mm), medium (18 to 22 mm) and large (>22 mm) were then selected from the corresponding treated plots and considered as treatments. The three berry

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sizes were obtained by the following treatments: 1) Small sized berries - natural berry size without treatment, which acted as the control (<18 mm); 2) Medium sized berries obtained by a standard application of gibberellic acid (GA_3) (dipped with 10 ppm at seven mm berry size) aimed to produce berries of 18 to 22 mm in diameter; 3) Large sized berries obtained by a combination of GA_3 and girdling (dipped with 10 ppm GA_3 at seven mm berry size and knife girdling a week later) to produce berries of >22 mm in diameter. All bunches in each experimental plot of treatment two and three were dipped with GA_3. Girdling was performed on all vine trunks in every experimental plot of treatment three, using a girdling knife.

Throughout the 2011/12 season the experimental layout was not a complete randomized block design. Only subsampling of each treatment was therefore performed. Each treatment block comprised of 18 vines in four rows (72 vines per block). Six vines were randomly selected per sampling time for each treatment. Sampling was done weekly for ten weeks with one harvest date in week ten.

In 2012/13 the three treatments were replicated nine times in a randomised block design on Laborans farm. Each experimental block consisted out of 12 vines. Sampling was done weekly for 12 weeks. In weeks ten, 11 and 12 grapes were harvested. A different vine was selected per experimental block each week for sampling. Experimental plots were separated by a buffer row where no plant bioregulators were applied.

4.2.3 Berry sampling

In both seasons berries were sampled weekly from berry pea size berry i.e. 5 mm berry diameter (stage 31 according to modified E-L scale as described by Coombe (1995), until commercial harvest dates. Every week, four to five bunches were randomly selected from a specific vine in each experimental block. Berries were removed using a scissors and the average berry diameter was determined for each treatment (small, medium and large). A total of 200 berries were selected from each replication for further measurements and analyses, namely berry mass (g), berry volume (cm^3), total soluble solids (˚Brix), pH, titratable acidity (TA), organic acids (malic and tartaric acid), abscisic acid and sugar (glucose and fructose).

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4.2.4 Berry analyses

Average berry fresh weight and volume were determined from 50 berries for each replicate as described by Ojeda *et al*. (2001). Subsequently, total soluble solids (^{o}Brix), pH and titratable acidity (TA) were determined for the combined pulp of the 50 berries. The ^{o}Brix was recorded from the pulp sample using an electronic hand refractometer. The pH and TA were measured by titrating 50mL juice to end-point of pH 7 using sodium hydroxide (NaOH) with a Metrohm, 785 DMP Titrino.

From each berry size (treatment) 50 berries were peeled and skins and pulp were stored separately at -20°C until further analysis. Skins were used for total phenol and anthocyanin determination as described by Iland *et al.* (2000).

Individual anthocyanins were analysed by HPLC with the general phenolics method using a PLRP-S column (Peng *et al*., 2002). Detection was at 520 nm and Malvidin-3-glucoside was used for quantifying all anthocyanins. Each anthocyanin was identified by matching the retention time and UV-Vis library spectra of the respective standard. Glucose, fructose, sucrose and organic acids concentrations of berry pulp from 50 berries were determined using normal phase HPLC separation with Evaporative Light Scattering Detection (ELSD) according to the method used by Muñoz-Robredo *et al*. (2011).

Abscisic acid (ABA) was analysed by UPLC-MS/MS method based on Müller & Munné-Bosch (2011) using the Waters Acquity UPLC/TQD instrument and a Waters UPLC BEH C-18 1.7µm (2.1 mm x 100 mm) column.

Results were expressed in concentration (per volume) and content (per berry) for each component as suggested by Coombe (1992). Berry softening, sugar accumulation and skin colour change from green to red in red cultivars are indicators of véraison (Parker *et al*., 2013). In this study the start of véraison was represented by the first day of significant sugar (glucose) accumulation as described by Davies *et al*. (2012).

4.2.5 Statistical analyses

Data obtained from both seasons was analysed by using SAS statistical software version 9.2 (SAS Institute Inc., Cary, NC, USA). The univariate analysis was performed for each sampling time separately, on all variables, using general linear models (GLM). Observations over time were also combined in a split-plot analysis of variance with week as sub-plot factor (Little, 1972). Shapiro-Wilk test was performed to test for normality (Shapiro & Wilk, 1965). Student's t-least significant difference was

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calculated at the 5% level to compare treatment means (Ott, 1998). A probability level of 5% was considered significant for all significance tests.

4.3 RESULTS AND DISCUSSION

Anthesis occurred approximately 14 days prior to pea size berry (5mm diameter) in both seasons. However, since berry sampling started at pea size berry it was used as reference point, with later stages expressed as days after pea size (DAPS).

Véraison indicates the stage during berry development when berries start to soften, sugars start to accumulate and colour change from green to red (Parker *et al*., 2013). In this study the start of véraison was represented by the first day of significant sugar (TSS, glucose and fructose) accumulation as described by Davies *et al*. (2012).

4.3.1 Berry development

Increase in Crimson Seedless fresh berry weight is shown in Fig. 3. Difference between berry sizes is a result of the treatments. From pea size berry, growth was rapid and almost linear. Start of véraison was at the same time in both seasons, namely 28 days after pea size (DAPS) berry. In the 2011/12 season berry growth terminated at 49 DAPS while in 2012/13 it was at 56 DAPS. In 2012/13 large berries terminated their growth a week later (63 DAPS) compared to the small and medium berries. As found by Brar *et al*. (2008) increase in Crimson Seedless berry weight start to plateau 30 days after véraison, which is similar to this study. The increase in berry fresh weight did not follow the typical double sigmoidal growth curve of many wine grape cultivars (Coombe & Hale, 1973; Downton & Loveys, 1978). No lag stage was detected during berry development at seven day sampling intervals for both seasons, which is in agreement with the results obtained by Raath (2012). Coombe (1980) ascribed berry development without a lag stage to primary bunches without competition (secondary bunches removed) representing typical table grape viticultural practices.

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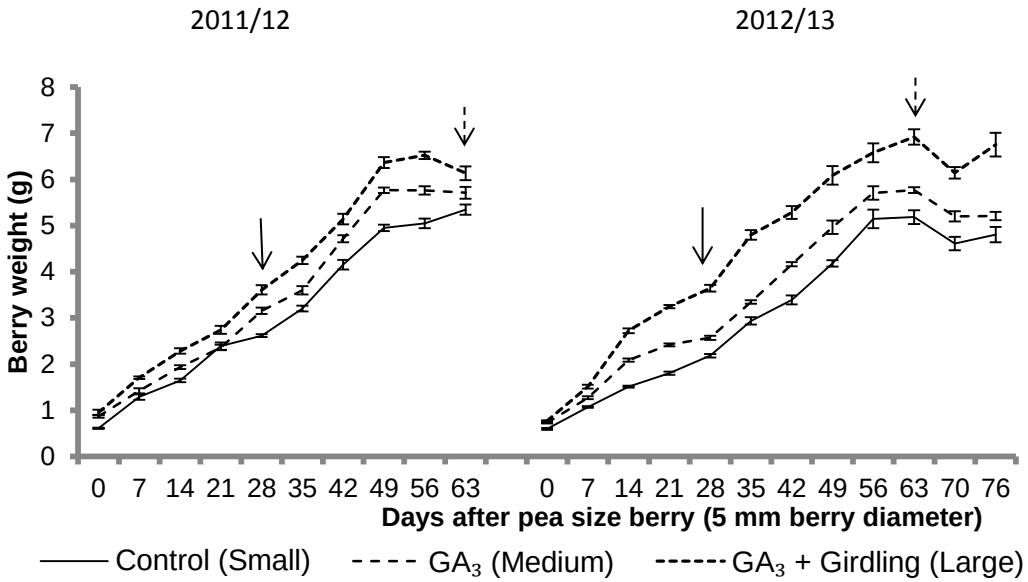

Figure 3. Increase in fresh berry weight (g/berry) for the three induced berry sizes (small, medium and large) for Crimson Seedless throughout 2011/12 and 2012/13 seasons. The solid vertical arrow indicates start of véraison and broken vertical arrow indicates first harvest date.

4.3.2 Carbohydrates

4.3.2.1 Total Soluble Solids

Total soluble solids (TSS) concentration for the three berry sizes followed the same pattern throughout both seasons with no significant differences between berry sizes (Fig. 4A). Concentrations were at average of 3.7˚Brix (2011/12) and 4˚Brix (2012/13) from pea size berry until 14 DAPS for all three berry sizes. As found by Hrazdina *et al*. (1984), TSS started to increase rapidly from three to four weeks after pea size berry until harvest (Fig. 4A). Compared to 2011/12, TSS concentrations in 2012/13 were higher for the three berry sizes at 56 DAPS with a steady increase thereafter. This could be ascribed either to higher night and day temperatures during the berry development period in 2012/13, or that a different vineyard was used.

Similar TSS concentrations trends were followed in both seasons. Based on the trends in TSS accumulation, sugars start to accumulate rapidly a week before first colour development and berry softening are observable. Therefore, TS

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S concentration can be used to predict typical TSS accumulation rates and commercial harvest dates. Night and day temperature during berry development period (December) should be considered when predicting harvest dates.

Total soluble solids (TSS) content per berry followed similar trends to TSS concentration throughout both seasons (Fig. 4B). In 2011/12 smaller differences in berry weight were obtained between berry sizes than in 2012/13 (Fig. 3). This explains why the TSS per berry did not differ between the berry sizes to the same extent in 2011/12 as it did in 2012/13 (Fig. 4B). In both seasons total berry sugar content increased with berry size. In 2012/13 TSS plateaued from 63 DAPS in accordance with the plateau in berry growth that occurred at the same time.

In both seasons, the sucrose content was less than the detectable limit (0.5 mg/mL) for all three berry sizes (data not shown). This is in accordance with Hrazdina *et al*. (1984), Coombe (1992), Liu *et al*. (2006) who found that carbohydrates in grapes consist mainly of glucose and fructose with trace amounts of sucrose.

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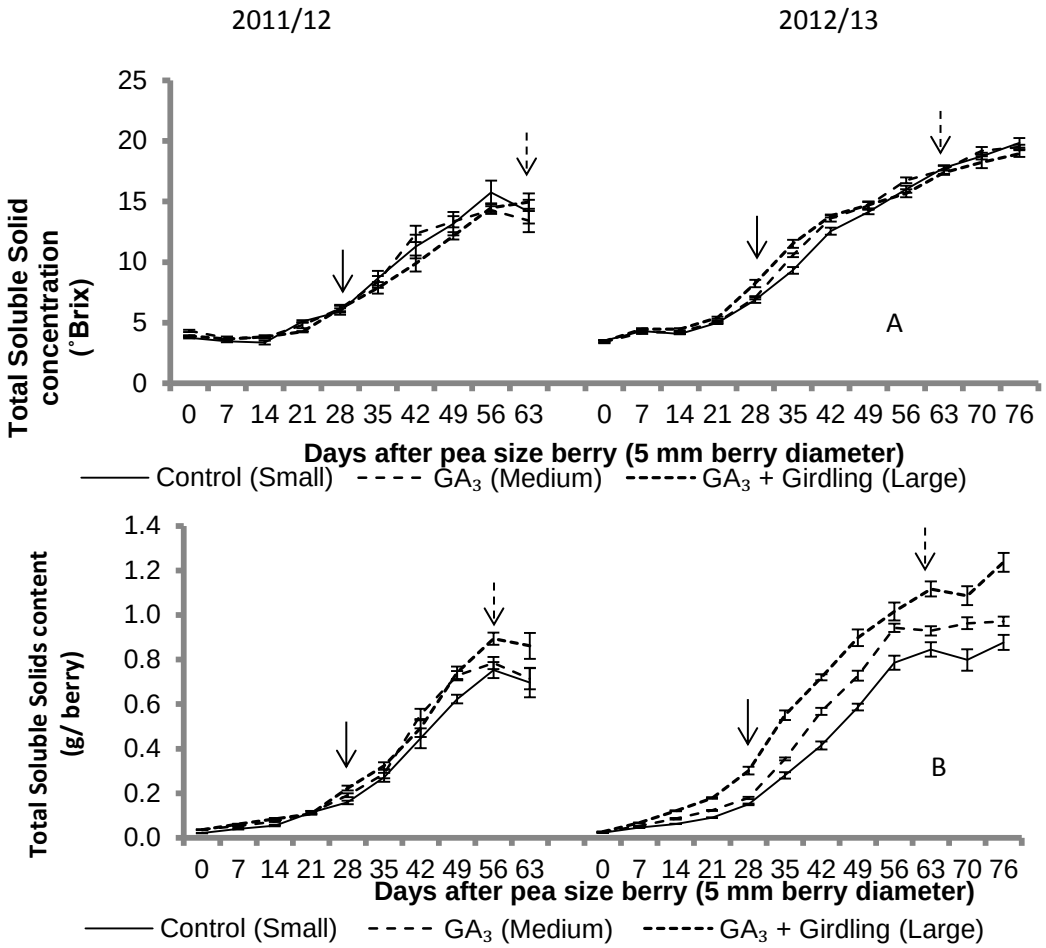

Figures 4A & B. Increase in total soluble solids concentration (°Brix) (A) and content (g/ berry) (B) for the three induced berry sizes (small, medium and large) for Crimson Seedless throughout 2011/12 and 2012/13 seasons. Vertical bars indicate variation for each sampling time ($p \leq 0.05$). The solid vertical arrow indicates start of véraison and broken vertical arrow indicates first harvest date.

4.3.2.2 Glucose

Glucose concentrations (mg/mL) increased throughout berry development in both seasons. No difference occurred between the three berry sizes (Fig. 5A). Average glucose concentrations ranged from 77 to 78 mg/ mL from 56 DAPS for both seasons. Lower concentrations were obtained by Liu *et al*. (2006) for table grapes from various *V. vinifera* species, with glucose concentrations ranging between 67.20 to 69.00 mg/mL. In 2011/12 rapid increase of glucose concentration started from 28 DAPS, but in the 2012/13 season glucose concentrations already started to increase from seven DAPS, though at slower rates (Fig. 5A). The accumulation trends of glucose showed more distinct differences between the seasons than the TSS.

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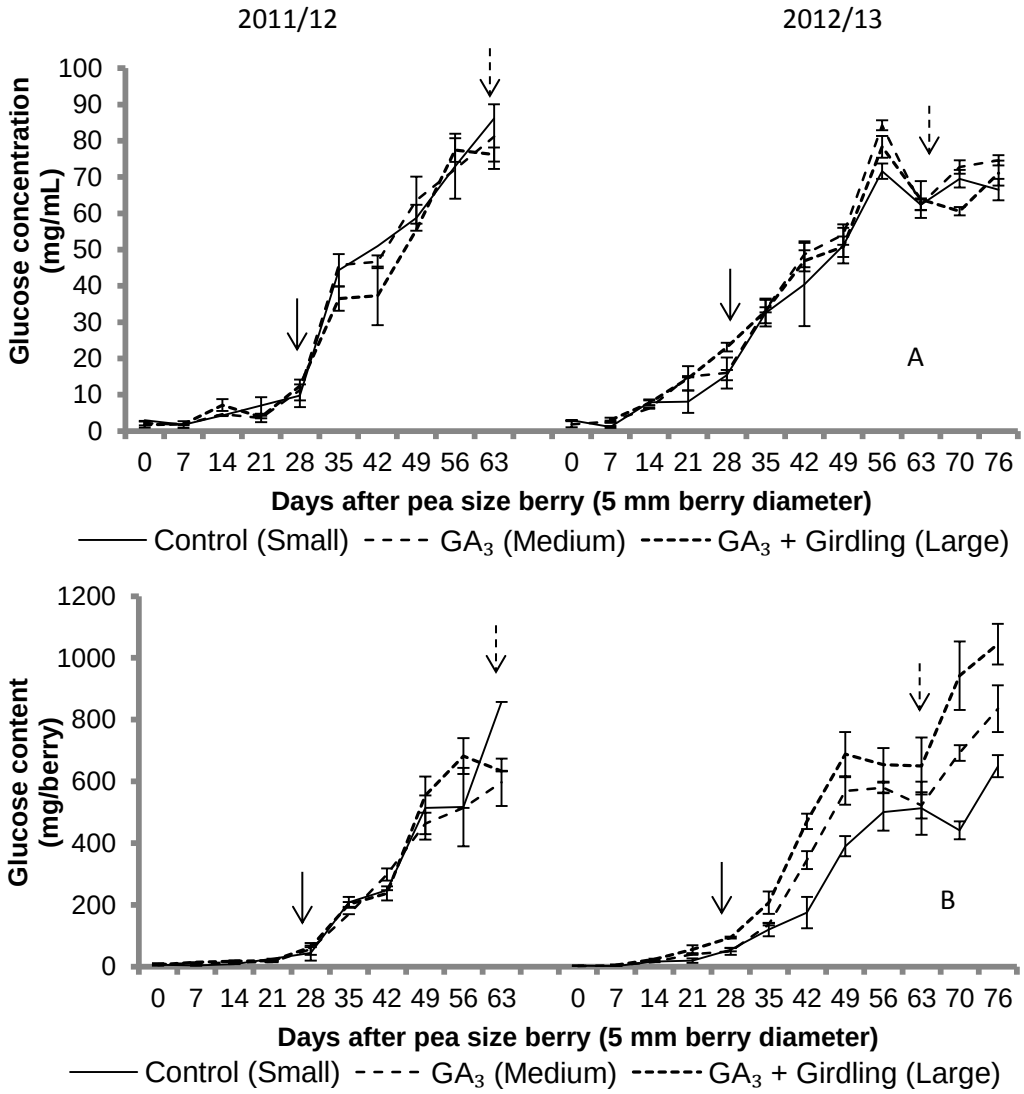
Total glucose content per berry accumulation pattern for the 2011/12 season was similar to the changes in concentration (Fig. 5B). It did not reflect the effect of berry size, probably due to the relatively small differences in berry size obtained in this season. In 2012/13 the glucose accumulation pattern did not reflect the changes in concentration. This is ascribed to the larger influence of berry size. As a result, different patterns were obtained between the seasons. However, for both seasons glucose content increased rapidly from the start of véraison, clearly indicating the onset of ripening. This coincided with the observed date of véraison (28 DAPS).



Figures 5A & B. Increase in glucose concentration (per volume) and content (per berry) for the three induced berry sizes (small, medium and large) for Crimson Seedless throughout 2011/12 and 2012/13 seasons. Vertical bars indicate variation for each sampling time ($p \leq 0.05$). The solid vertical arrow indicates start of véraison and broken vertical arrow indicates first harvest date.

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4.3.2.3 Fructose

Fructose concentrations followed similar trends to glucose throughout both seasons (Fig. 6A). At harvest, no significant differences in fructose concentrations were found between berry sizes for both seasons. Fructose concentrations started at average 0.87 mg/mL and increased to average 66.10 mg/mL at harvest (56 to 76 DAPS) for the three berry sizes. The average fructose concentrations in the present study were slightly lower compared to results from Liu *et al*. (2006) who found an average fructose concentration of 70.34 mg/mL at harvest. In this study, glucose concentrations were found to be higher than fructose concentrations in green berries, but after véraison fructose and glucose accumulation followed an average ratio of 1:1 (data not shown). These results correspond with previous studies (Nii & Coombe, 1983; Coombe 1987; Robinson & Davies, 2000).

Fructose content per berry increased rapidly from 28 DAPS (start of véraison) until harvest for 2011/12 (Fig. 6B). In 2012/13 the fructose content per berry increased significantly from 28 to 49 DAPS after which the rate of accumulation decreased, only to continue increasing from 63 DAPS. In 2011/12 no significant differences were found between berry sizes, while in 2012/13 large berries contained the highest fructose content and small berries the lowest (Fig. 6B). These differences between the three berry sizes suggest that fructose content per berry is affected by berry size.

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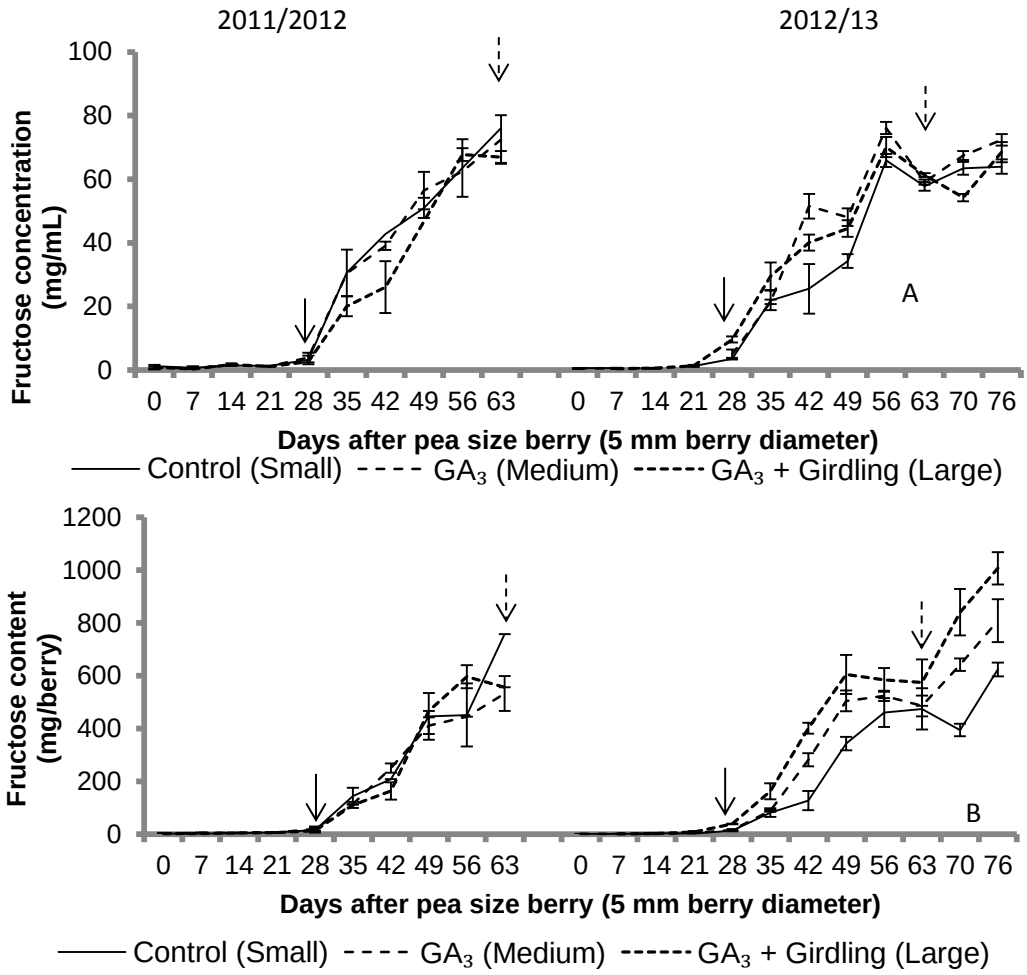

Figures 6A & B. Increase in fructose concentration (per volume) and content (per berry) for the three berry sizes (small, medium and large) for Crimson Seedless throughout 2011/12 and 2012/13 seasons. Vertical bars indicate variation for each sampling time ($p \leq 0.05$). The solid vertical arrow indicates start of véraison and broken vertical arrow indicates first harvest date.

4.3.3 Organic acids

4.3.3.1 Titratable acidity

Titratable acidity (TA) started at higher concentrations in 2011/12 than in 2012/13 (Fig. 7A). In 2011/12 TA reached an average maximum concentration of 42.96 g/L at seven DAPS. In 2012/13 the maximum TA concentration averaged at 28.9 g/L at seven DAPS and remained constant until 28 DAPS, start of véraison. In both seasons, TA concentration decreased rapidly from start of véraison until 63 DAPS. At 63 DAPS TA concentrations were similar in both seasons, at an average of 5.15 g/L. No significant differences were found between berry sizes throughout both seasons (Fig. 7A). The differences in TA

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trends from pea size to 28 DAPS could be ascribed to differences in night and day temperatures as well as rainfall during December (Fig. 1 & 2). In 2011/12 temperatures were cooler with higher rainfall compared to 2012/13. Therefore, the cooler night and day temperatures paired with higher rainfall could lead to higher TA concentrations. Hrazdina *et al*. (1984) found that the TA concentration increases during berry development and decreases during berry ripening.

The TA content per berry showed similar trends in both seasons with significant differences between berry sizes (Fig. 7B). It increased from pea size berry to peak at véraison whereafter it decreased rapidly, reaching minimum content at 63 DAPS (average 0.028 g/berry). Throughout both seasons large berries contained significantly higher TA content per berry than small and medium berries, suggesting that TA content per berry is related to berry size.

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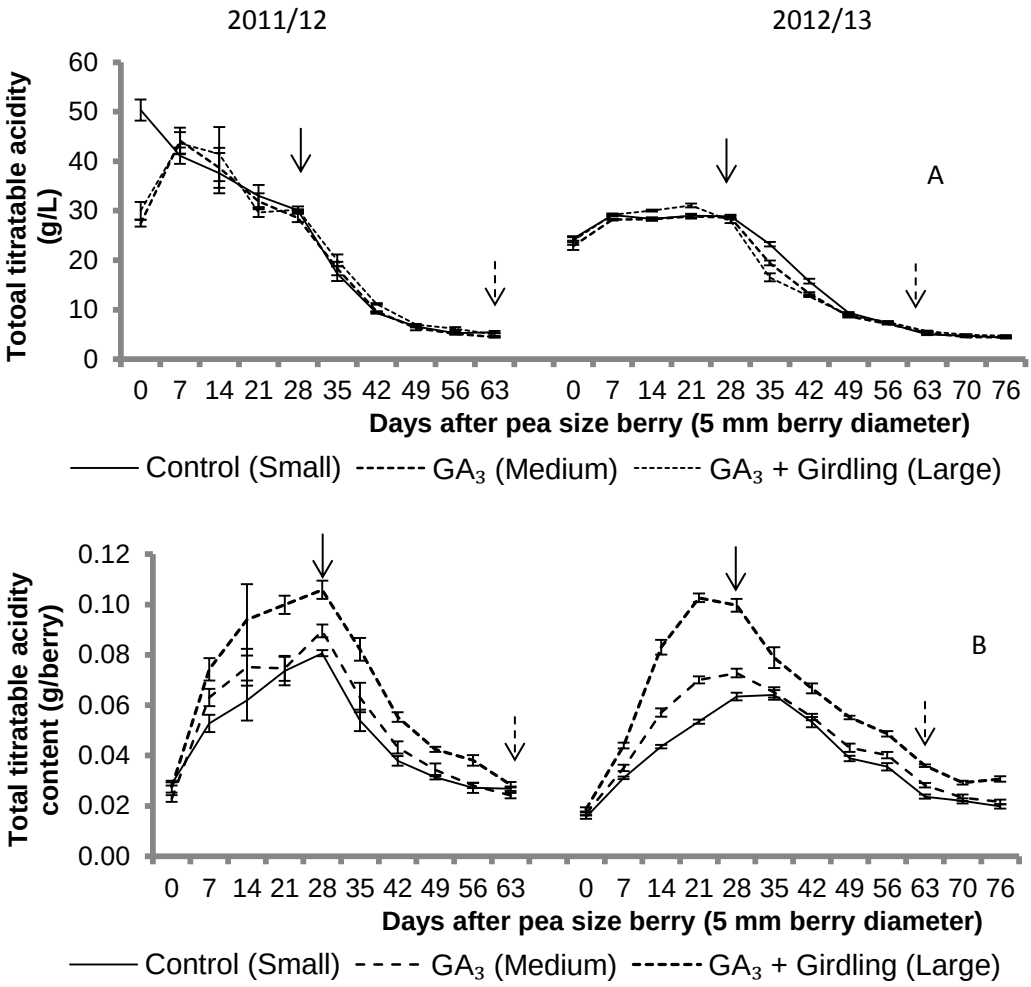

Figures 7A & B. Change in TA concentration (per volume) and content (per berry) for the three berry sizes (small, medium and large) for Crimson Seedless throughout 2011/12 and 2012/13 seasons. Vertical bars indicate variation for each sampling time ($p \leq 0.05$). The solid vertical arrow indicates start of véraison and broken vertical arrow indicates first harvest date.

4.3.3.2 Tartaric acid

In 2011/12 tartaric acid concentrations showed a slow decrease from 14 DAPS, two weeks before véraison started until harvest with no significant differences between the three berry size categories (Fig. 8A). Early tartaric acid concentrations were higher in 2012/13 than in 2011/12 and started to decrease from 28 DAPS, namely the start of véraison. In 2011/12 the final tartaric acid concentration was also lower than in 2012/13. Liu *et al*. (2006) found an average tartaric acid concentration at harvest of 3.72 mg/mL for table grapes from *V. vinifera* species. These results correspond better with the results obtained in 2011/12 (2.0 mg/mL) than 2012/13 (5.7 mg/mL). Differences in tartaric acid concentrations between consecutive years could be ascribed to the differences in the temperature and rainfall of the

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two seasons and change in vineyard location (Fig. 1 & 2). During November and December 2011/12 maximum and minimum temperatures were cooler paired with higher rainfall compared to 2012/13. These cool temperatures could slow down tartaric acid accumulation throughout berry development and therefore, influencing the final tartaric acid concentrations. Nonetheless, the trend followed in 2012/13 corresponds with previous studies in that little change occurred in tartaric acid concentration during ripening (Gutiérrez-Granda & Morrison., 1992). Similar results were obtained in previous studies with tartaric acid decreasing from four weeks after pea size berry with a slight increase before finally decreasing to its lowest concentrations prior to harvest (Hrazdina *et al*., 1984; Iland & Coombe, 1988; Coombe 1992).

Tartaric acid per berry remained at relatively constant levels for all three berry sizes throughout 2011/12 (Fig. 8B). This corresponds with the trend obtained by Iland & Coombe (1988). However, in 2012/13 tartaric acid per berry started at low content per berry at pea size berry, average 8.80 mg/ berry, and increased rapidly after véraison, reaching an average of 71.20 mg/berry (Fig. 8B).

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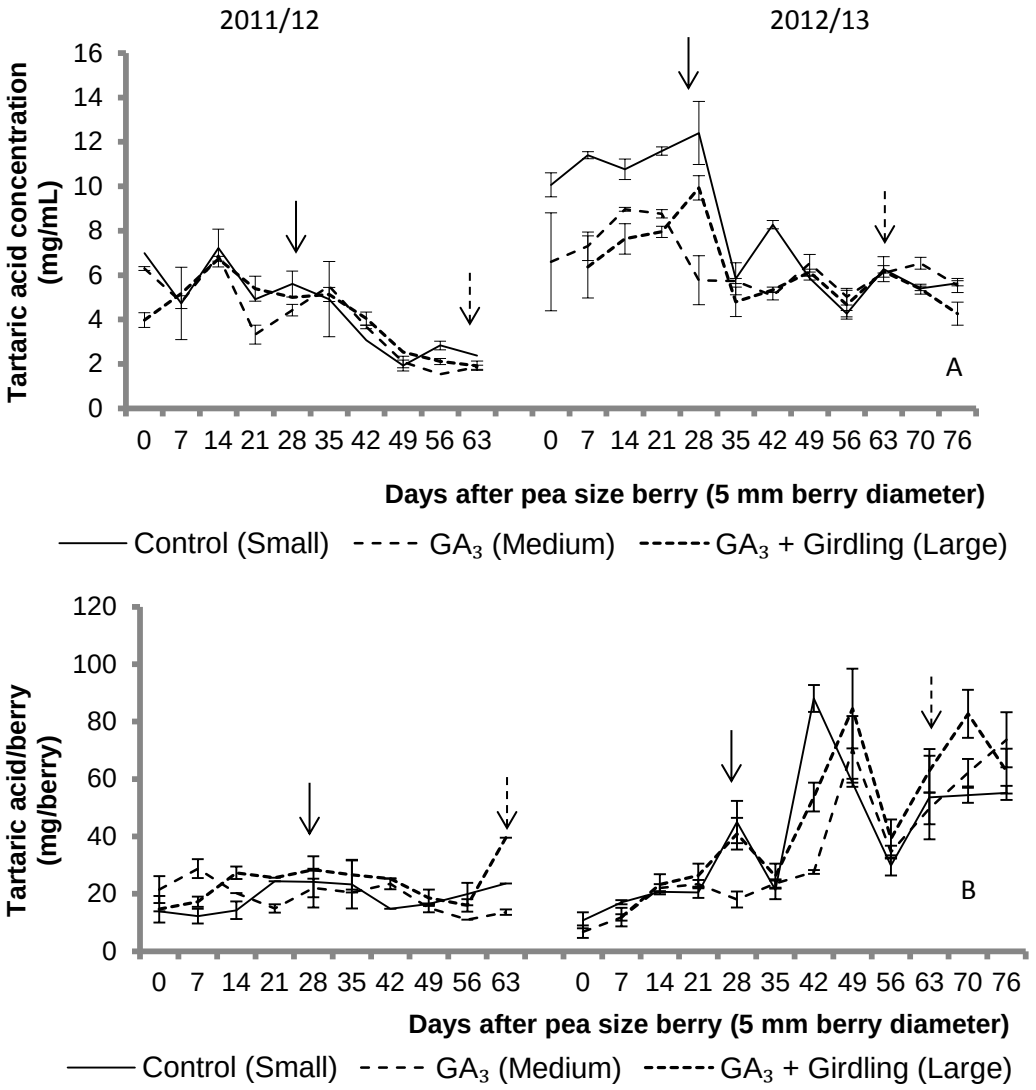

Figures 8A & B. Change in tartaric acid concentration (per volume) and content (per berry) for the three berry sizes (small, medium and large) for Crimson Seedless throughout 2011/12 and 2012/13 seasons. Vertical bars indicate variation for each sampling time ($p \leq 0.05$). The solid vertical arrow indicates start of véraison and broken vertical arrow indicates first harvest date.

4.3.3.3 Malic acid

Up to véraison malic acid concentrations reached higher levels in 2011/12 than in 2012/13 (Fig. 9A). Cool temperatures during berry development (November and December) (Figs. 1 &2) could have induce rapid malic acid accumulation in 2011/12 compared to 2012/13. In both seasons malic acid concentrations increased from pea size berry until véraison and then rapidly declined during ripening reaching minimum concentrations from 56 DAPS (average 2 to 3 mg/mL). These results correspond with previous studies by Hrazdina *et al*. (1984), Coombe (1987), Gutiérrez-Granda & Morrison (1992), Liu *et al*. (2006) and Ali *et al*. (2011) who found that malic acid concentration decreases during ripening.

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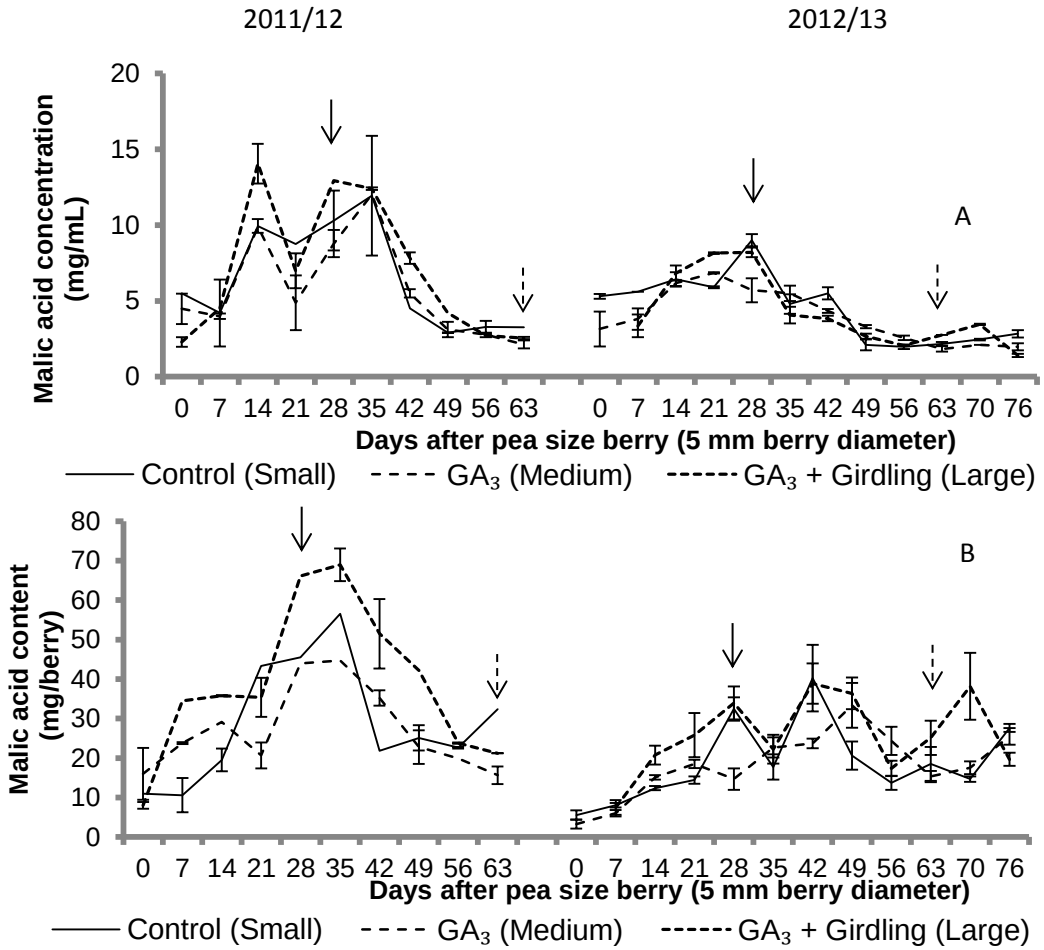
Decrease in malic acid concentration could be ascribed to malic acid metabolism, being converted to acetyl-CoA (used for fatty acid metabolism) or production of secondary metabolites (Hrazdina *et al*., 1984; Terrier *et al*., 2000). Malic acid concentrations did not differ between berry sizes.

Malic acid content per berry increased from pea size berry until 35 DAPS, and subsequently declined rapidly until harvest date in 2011/12 (Fig. 9B). In 2012/13 malic acid per berry increased steadily until 42 DAPS, thereafter decreasing, reaching similar levels at 49 DAPS compared to 2011/12. Cooler temperatures during berry development (December) could lead to an increase in malic acid concentration and therefore an increase in malic acid content per berry. From 28 DAPS (beginning of January) the minimum and maximum temperatures were similar in both seasons and could therefore be the reason for the decrease in malic acid content, reaching similar levels at harvest.



Figures 9A & B. Increase in malic acid concentration (mg/mL) and content (mg/berry) for the three induced berry sizes (small, medium and large) for Crimson Seedless throughout 2011/12 and 2012/13 seasons. Vertical bars indicate variation for each sampling time (p ≤ 0.05). The solid vertical arrow indicates start of véraison and broken vertical arrow indicates first harvest date.

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In both seasons malic acid was the main organic acid, with concentrations double than for tartaric acid from pea size berry until 49 DAPS. From 49 DAPS until harvest tartaric to malic acid concentration followed a 1:1,7 ratio in 2011/12; however in 2012/13 a 1:1 ratio was followed. Contrary to previous findings (Muñoz-Robredo *et al*., 2011) malic acid was the main acid throughout the seasons in this study. These differences in tartaric to malic acid ratio could be ascribed to previous studies being done on wine grapes where higher tartaric acid concentration paired with a lower pH value are required for a cleaner wine fermentation (Liu *et al*., 2006). Differences in organic acid ratios could influence the taste of table grapes (Liu *et al*., 2006; Liu *et al*., 2007).

4.3.4 Total phenols

In 2011/12 total phenol concentration increased rapidly from seven to 14 DAPS and quickly decreased thereafter (Fig. 10A). In 2012/13 total phenol pattern differed from 2011/12 since total phenol concentration started at higher mg/g berry, decreasing from seven DAPS until 35 DAPS. Thereafter total phenol concentration remained at low levels. Coombe (1992) and Ali *et al*. (2011) found that total phenols are higher in green berries, followed by a rapid decline during ripening. From véraison onwards no significant differences were found between berry sizes for both seasons. The results indicate that total phenol concentration in the skin of Crimson Seedless is not affected by berry size.

For both seasons, total phenol content per berry remained fairly constant throughout berry development, but increased in the last phase of ripening (Fig. 10B). In contrast to 2011/12, differences in berry phenol content were obtained during 2012/13 between berry sizes with large berries containing highest total phenol content per berry skin.

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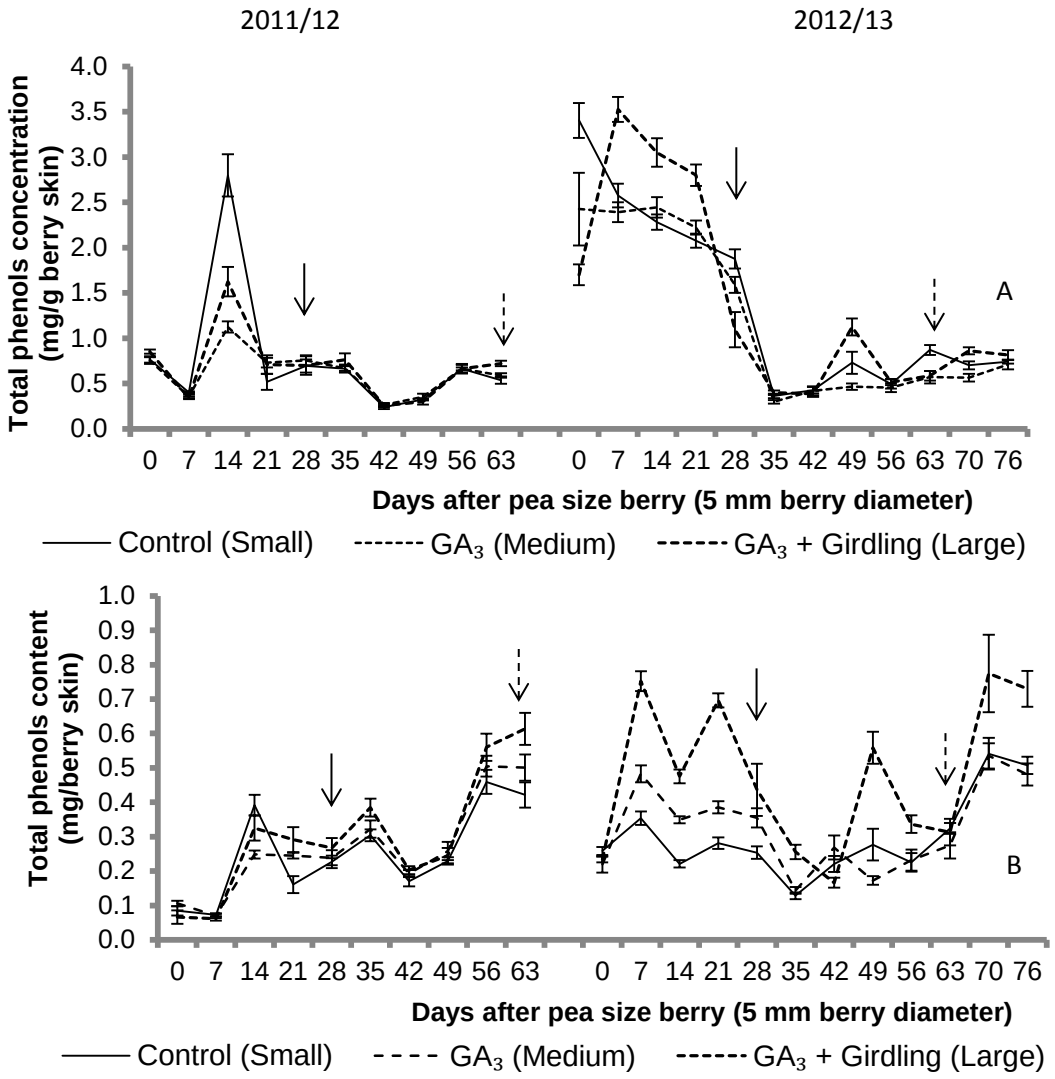

Figures 10A & B. Change in total phenol concentration (mg/g berry skin) and content (mg/ berry skin) for the three induced berry sizes (small, medium and large) for Crimson Seedless throughout 2011/12 and 2012/13 seasons. Vertical bars indicate variation for each sampling time ($p \leq 0.05$). The solid vertical arrow indicates start of véraison and broken vertical arrow indicates first harvest date.

4.3.5 Total anthocyanins

In both seasons, total anthocyanin concentrations started to increase from 35 DAPS, one week after sugars significantly started to accumulate (Fig. 11A). Concentration increase was thereafter rapid, when change in colour was most noticeable (as determined visually in field). Wheeler *et al*. (2009) found that change in skin colour, the start of anthocyanin accumulation, coincides with an increase in TSS (˚Brix). However, Pirie & Mullins (1980) found that total anthocyanins begin to accumulate one week after TSS

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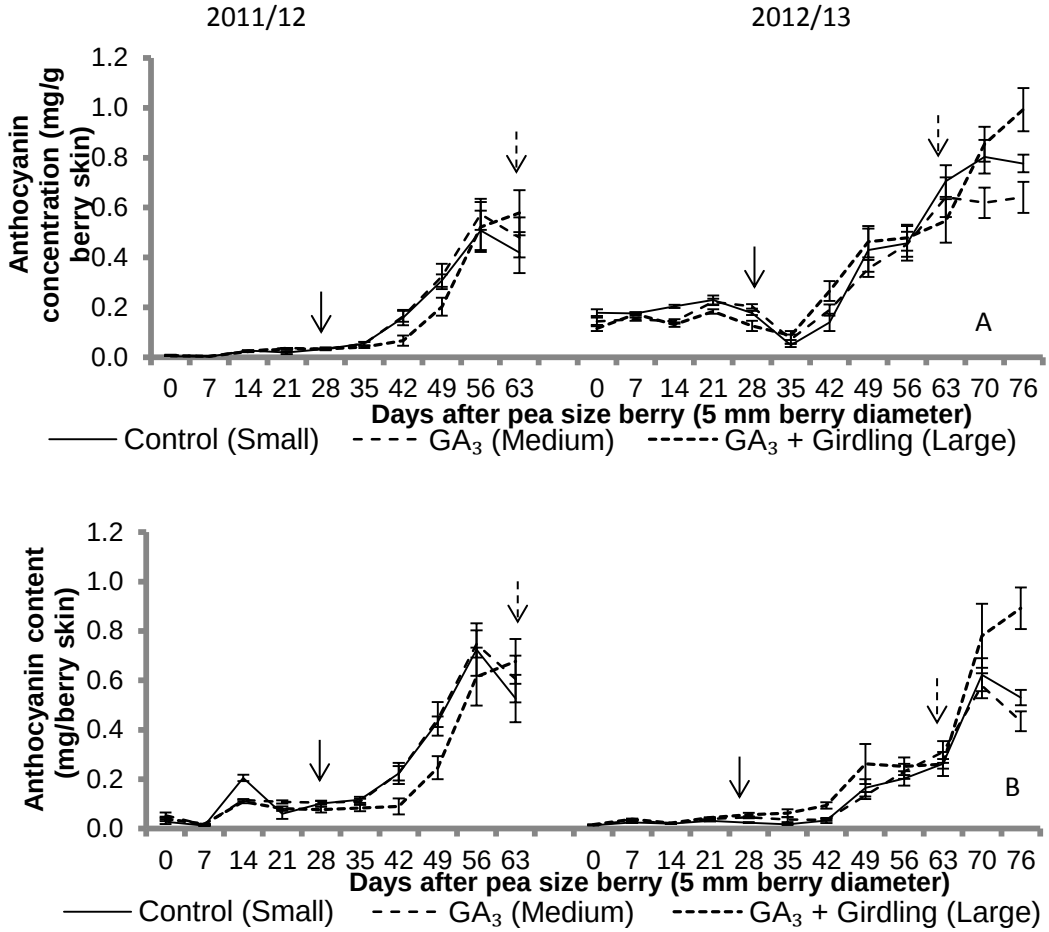
accumulation starts. In 2011/12 total anthocyanin concentration increased until 56 DAPS ending with an average of 0.50 mg/g berry. In 2011/12 anthocyanin concentration and accumulation was delayed in large berries until 42 DAPS (Fig. 11A). Nonetheless, at 63 DAPS no significant differences were found between berry sizes. In 2012/13 differences in anthocyanin concentrations for the three berry size were not observed. Even though total anthocyanin concentration were similar for the three berry sizes, small berries showed first signs of colour change, followed by medium berries and lastly large berries during 35 to 42 DAPS in both seasons (determined visually in the field during sampling).

In both seasons, anthocyanin content per berry were low until 35 DAPS (Fig. 11B). When concentrations rapidly started to increase, content per berry increased to average 0.60 mg/berry in 2011/12 at 63 DAPS and 0.65 mg/berry in 2012/13 at 70 DAPS (Fig. 11B).



Figures 11A & B. Increase in total anthocyanin concentration (mg/g berry skin) and content (mg/berry skin) for the three berry sizes (small, medium and large) for Crimson Seedless throughout 2011/12 and 2012/13 seasons. Vertical bars indicate variation for each sampling time ($p \leq 0.05$). The solid vertical arrow indicates start of véraison and broken vertical arrow indicates first harvest date.

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In the wine grape berry, malvidin-3-glucoside is considered the major anthocyanin pigment (Robinson & Davies, 2000). The most abundant anthocyanin found in all three berry sizes at harvest was peonidin-3-glucoside (data not shown). This corresponds with previous studies since peonidin-3-glucoside was the main anthocyanin found in Crimson Seedless (Cantos *et al*., 2002; Brar *et al*., 2008; Human & Bindon, 2008) followed by cyanidin-3-glucoside and malvidin-3-glucoside (Cantos *et al*., 2002; Human & Bindon, 2008).

4.3.6 Abscisic acid (ABA)

Abscisic acid (ABA) concentration decreased from pea size berry until 21 DAPS. Increase in ABA concentration coincides with the significant increase in sugar (TSS, glucose and fructose) concentration at 28 DAPS, signifying the start of véraison. From 35 DAPS, as anthocyanin concentration started to increase, ABA accumulation rate was lower until 56 DAPS. From here after ABA increased rapidly again until first harvest date (63 DAPS) for small and medium berries and up to 70 DAPS for large berries (Fig. 12A). Significant statistical differences in ABA concentration were not found between the three berry sizes throughout the season. The abscisic acid concentration trend did not agree with previous findings (Downton & Loveys, 1978; Coombe 1992; Wheeler *et al*, 2009). However, Wheeler *et al*. (2009) described a free ABA concentration pattern with a decline in concentrations from anthesis until véraison whereafter free ABA increases, reaching a peak before harvest. The exogenous application of ABA is widely used in the table grape industry in order to increase anthocyanin production in red table grape cultivars (Ban *et al*., 2003; Peppi *et al*., 2006). However, in this study the effect of exogenous ABA application on berry ABA was not measured and should be further investigated in future studies.

Fig. 12B shows ABA content per berry throughout 2012/13. It was low while the berries were small, but significantly started to increased from 28 DAPS, namely the start of véraison. The increase was gradual until first harvest date. Large berries contained the highest amount of ABA per berry from the start of accumulation until harvest. Small and medium berries contained approximately equal amounts throughout the season. Coombe & Hale (1973) found similar accumulation trends in ABA content per berry.

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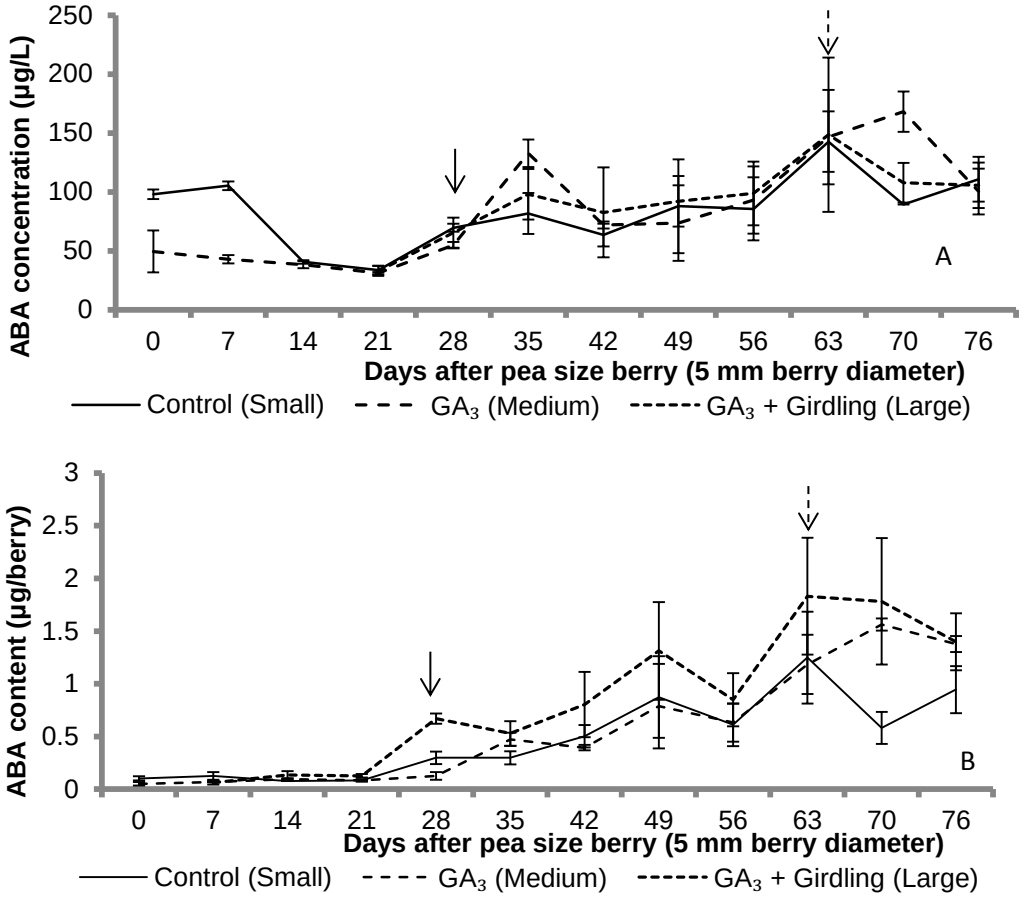

Figures 12A & B. Change in ABA concentration (µg/L) and content (µg/berry) for the three induced berry sizes (small, medium and large) for Crimson Seedless throughout the 2012/13 season. Vertical bars indicate variation for each sampling time ($p \leq 0.05$). The solid vertical arrow indicates start of véraison and broken vertical arrow indicates first harvest date.

4.4 CONCLUSION

Throughout both seasons three significantly different berry sizes were obtained on account of the manipulations. Larger differences in fresh weight per berry occurred in 2012/13 than in 2011/12. This resulted in larger differences in content per berry of TSS, glucose, fructose, TA, total phenols and ABA, suggesting that sufficient production of these compounds occurs and clearly their concentration were not influenced by berry size. Tartaric acid, malic acid and total anthocyanin content per berry however, showed no clear significant differences between berry sizes.

Concentrations of TSS, glucose, fructose and ABA increased significantly from the start of véraison. Total anthocyanin concentrations increased from a week after start of véraison. As véraison started TA, tartaric acid, malic acid and total phenol concentrations decreased. Sugar accumulation was not

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affected by berry size. This suggests that berry size (or crop load) does not affect ripening (in terms of sugar accumulation). This is contrary to what is commonly believed.

Minimum and maximum temperature differences between seasons during berry growth and development (December) seemed to have influenced ripening. With higher temperatures in 2012/13 sugar (TSS, glucose and fructose) and anthocyanin concentrations continued to increase over a longer period, thereby increasing the sugar:acid ratio. In cooler seasons the final sugar:acid ratio is reached earlier and berry physiological processes seems to stop earlier.

Determining berry development and the changes occurring in berry composition can be very challenging since grapes are natural products, influenced by environmental conditions, cultivar genotype, rootstock, soil conditions and canopy management. In the present study an average trend for the different berry sizes for Crimson Seedless were obtained. The study was conducted in the Berg River Valley; berry development could differ in regions with different climatic conditions. An accurate model for berry development was difficult to attain since compounds like organic acids are strongly influenced by climatic conditions which fluctuate between seasons. However, compounds like TSS, glucose and fructose followed similar trends in both season. With further research in different regions and with more data taken over several seasons an average accumulation trend/model for these compounds could be formulated. These trends could help in predicting berry development, start of véraison and therefore start of anthocyanin accumulation for Crimson Seedless and finally optimal harvest dates. Further studies could also focus on the external application of ABA and the effect it has on berry ABA concentration. These studies could aid in decision making regard timing of ABA application and how it influence minimum residue levels (MRL).

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Chapter 5

Influence of sugar:acid ratio and berry size on postharvest quality of table grapes

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5.1 Introduction

Prime and Crimson Seedless are two of the major table grape cultivars produced in South Africa for local and export markets (Anon., 2011). Table grapes are judged by consumers according to colour, firmness, flavour and postharvest shelf life (Muñoz-Robredo *et al*., 2011). Understanding whether berry development affects postharvest shelf life of these cultivars is therefore essential if grapes of acceptable quality need to be produced.

The taste of the berry is mainly determined by the ratio of total soluble solids (TSS) and titratable acidity (TA) and therefore can act as a key harvest indicator (Liang *et al*., 2005; Muñoz-Robredo *et al*., 2011). The TSS:TA ratio can only be taken into account when the berries have reached a certain maturity where typical cultivar colour has developed and TSS concentration is above 16 (Jayasena & Cameron, 2008). The acceptable TSS:TA ratio for Crimson Seedless is between 35 and 40 (Guelfat-Reich & Safran, 1971; Jayasena & Cameron, 2008).

The quality of the final product depends on the ripening stage, from véraison until harvest, since the final size, acidity, sugar, colour and flavour of the berry are determined by the physiology of ripening (Roubelakis-Angelakis, 2001). Producers use plant bioregulators like gibberellic acid (GA_3) and forchlorfenuron (CPPU), synthetic cytokinin, alone or in combination to obtain larger berry sizes adequate for international markets (Retamales *et al*., 1995; Zoffoli *et al*., 2008). Previous chapters of this study focused on compound accumulation throughout berry development and ripening for three different berry sizes obtained by plant bioregulator application; the effect of berry size on postharvest quality could therefore be observed. To date there is no clarity whether the final berry TSS:TA ratio affects postharvest quality. Taking the previous research into consideration, an investigation was conducted to establish whether there is a correlation between TSS:TA ratio and postharvest defects for three different berry sizes (small, medium and large) of Prime and Crimson Seedless.

5.2 Material and Methods

5.2.1 Experimental vineyards

The experiment was performed on two table grape cultivars, Prime and Crimson Seedless (*Vitis vinifera* L.) grafted onto Ramsey (*Vitis Champinii*) respectively. Both vineyards were situated in the Berg River Valley, Paarl.

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The experiment was performed on Prime (*Vitis vinifera* L.) grafted onto Ramsey (*Vitis Champinii* Planch) rootstock. The experimental block was a 15 year old vineyard situated in Slot van die Paarl farm (33˚67'S, 18˚94'E), Berg River Valley, South Africa. The vines were spaced 3 m x 3 m and trained on a flat roof trellis system. Soil water was measured with a tensiometer and drip irrigation was applied as needed during the growing season. Vines were pruned with long bearers, 10 – 12 buds per cane, with an average of ten canes per vine, evenly spaced. Seasonal canopy management included tie-back of shoots, suckering, leaf removal and topping. Leaf removal was done before plant bioregulators were applied as well as during véraison, while topping was done only after the application of plant bioregulators. No lateral shoots were removed. During crop control the yield was reduced to 35 bunches per vine. Standard cultivation practices were followed as recommended by Greyling (2007).

The Crimson Seedless trials were done on two different vineyards, both situated in the Paarl district. During the 2011/12 season the trial took place at the farm, Slot van die Paarl (33˚66'S, 18˚91'E) while during the 2012/13 season the trial was done on the farm, Laborans (33˚67'S, 18˚94'E). The 11 year old Crimson Seedless vineyard on Slot van die Paarl farm, were spaced 3 m x 2 m while the seven year old Crimson Seedless vineyard situated on the Laborans farm, were spaced 3 m x 1.875 m. Both vineyards were trained on a double gable trellising system with a North-South row orientation. Soil water potential was measured with two tensiometers. When top-soil water potential reached 16 to 18 kPa irrigation was applied by means of micro-irrigation. During véraison and berry ripening soil water potential was allowed to decrease to 30 kPa. Vines were pruned with evenly spaced 12 long bearers (10 – 12 buds per bearer) with an equal amount of spurs (4 – 6 buds per bearer). Seasonal canopy management included tie-back of shoots, suckering, leaf removal and topping. Suckering was done at 30 cm shoot length while shoots were tied back at 60 cm shoot length. Leaf removal was done before plant bioregulators were applied as well as during véraison, while topping was performed only after the application of plant bioregulators. No lateral shoots were removed. During crop control the yield was reduced to 35 bunches per vine for Prime and 26 to 28 bunches per vine for Crimson Seedless. Vines were fertilised during bud break (N and Ca), just after berry set (N, K and Mg) and after harvest (N). Standard cultivation practices were followed as recommended by Greyling (2007).

5.2.2 Experimental design and layout

For two consecutive seasons three distinct berry sizes were obtained by combination of plant bioregulator applications. The three berry sizes were obtained by the following treatments: 1) Small sized berries - natural berry size without treatment which acted as the control (<20 mm and <18 mm for

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Prime and Crimson Seedless respectively); 2) Medium sized berries obtained by a standard application of GA_3 (dipped with 15 ppm at 8 mm berry size for Prime and 10 ppm at 7 mm berry size for Crimson Seedless) aimed to obtain berries of 20 to 24 mm diameter and 18 to 22 mm diameter for Prime and Crimson Seedless respectively and 3) Large sized berries obtained by a combination of GA_3 and CPPU application or GA_3 and girdling (dipped with 15 ppm GA_3 and 1 ppm CPPU solution at 8 mm berry size for Prime and 10 ppm GA_3 and knife girdling at 7 mm berry size for Crimson Seedless) to produce berries of >24 mm diameter and >22 mm diameter respectively.

Fig. 1 displays the average monthly minimum and maximum temperatures, while Fig. 2 shows the average monthly rainfall for the Paarl area in which the experimental farms were situated. Data was only given for the periods in which the trials were run. Both seasons show similar trends in minimum and maximum temperatures. However, maximum and minimum temperatures were lower in November and December in 2011/12 season than in 2012/13 season. Correspondingly to the temperatures, greater rainfall occurred during November and December in 2011/12 compared to 2012/13 season. During 2012/13 harvest month (February), average 29.5 mm rainfall occurred.

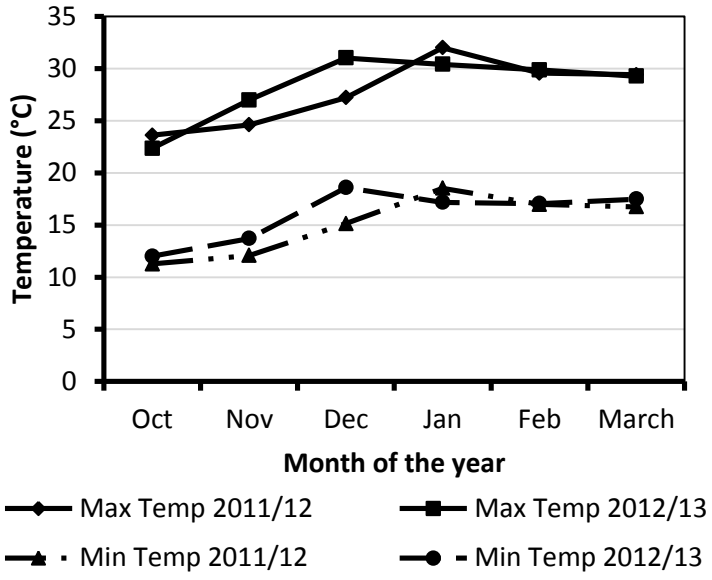

Figure 1. Average minimum and maximum monthly temperatures for Paarl, Berg River Valley, for 2011/12 and 2012/13 seasons (Source: ARC-ISCW).

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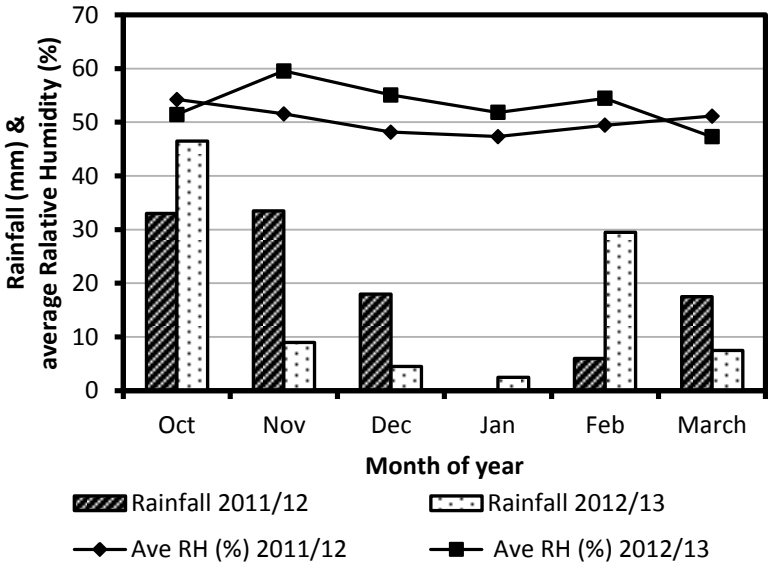

Figure 2. The average monthly rainfall for Paarl, Berg River Valley, for 2011/12 and 2012/13 seasons (Source: ARC-ISCW).

5.2.2.1 Prime

In 2011/12 the three berry treatments were replicated ten times in a randomised block design. Each replication (experimental plot) consisted out of nine vines. Sampling was done weekly for eight consecutive weeks from one randomly chosen vine per replication. In week eight the grapes were also harvested. In 2012/13 the three treatments were replicated nine times in a randomised block design. Each replication contained 13 vines. Weekly sampling was done for 10 consecutive weeks with grapes also being harvested in weeks nine and 10. For sampling a different vine was randomly selected per replication each week. Experimental plots were separated by a buffer row where no plant bioregulators were applied.

5.2.2.2 Crimson Seedless

Throughout the 2011/12 season the experimental layout was not a complete randomized block design and therefore only subsampling of each treatment was performed. The three treatment blocks comprised of four rows of 18 vines per row. Six vines were randomly selected per sampling time from each treatment block. Sampling was done weekly for 10 consecutive weeks with a harvest date in week 10.

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In 2012/13 the three treatments were replicated nine times in a randomised block design. Each replication consisted out of 12 vines. Sampling was done weekly for 12 consecutive weeks. In week 10, 11 and 12 grapes were harvested. A different vine was selected per replication each week for sampling.

5.2.3 Sampling

Sampling commenced from pea size berry (5 to 7 mm) until commercial harvest dates. Every week, four to five bunches were randomly selected per vine from which berries were cut. The average berry diameter was determined for each treatment (small, medium and large). Berries were sized, using grids, according to the average berry diameter for corresponding treatment as described by Joubert (2013). A total of 200 berries were selected from the sized berries for each replication for further measurements and analyses, namely berry mass (g), berry volume (cm^3), total soluble solids (˚Brix), pH and titratable acidity (TA).

5.2.4 Harvest, storage and evaluation

Prime and Crimson Seedless were harvested according to commercial maturity levels and packed in the vineyard. A random selection of four to six bunches per experimental vine for each repetition was sampled. Decayed berries were removed from each bunch before packing in 4.5 kg ribbed carton. Bunches were placed individually in paper carry bags (Prime) or perforated plastic carry bags (Crimson Seedless) inside the perforated liner of the carton. A MAM sheet was placed between the grapes and SO_2-sheet, after which liners were enfolded and cartons sealed. The grapes were transported to the cold storage facility of the ARC Infruitec-Nietvoorbij Research Institute for Fruit, Vine & Wine where both cultivars were stored for five weeks at -0.5^{o}C, followed by another week at 7.5^{o}C. This is the cold storage procedure for export to international markets.

After cold storage, the grapes were subjected to a postharvest defect evaluation. The total weight of each repetition's bunches was measured before evaluation. Each berry cluster was evaluated and berries with defects (loose berries, browning, soft tissue breakdown, berry decay and cracked berries) were removed and weighed individually. The percentage of defects per replication was calculated on a fresh weight/weight basis.

5.2.5 Statistical analyses

Data obtained from both seasons were statistically analysed by using the SAS statistical software version 9.2 (SAS, 2000). The univariate analysis of variance was performed for each sampling time separately, on

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all variables accessed, using general linear models (GLM). Observations over time were also combined in a split-plot analysis of variance with week as sub-plot factor (Little, 1972). Shapiro-Wilk test was performed to test for normality (Shapiro & Wilk, 1965). Student's t-least significant difference was calculated at the 5% level to compare treatment means (Ott, 1998). A probability level of 5% was considered significant.

5.3 Results and discussion

5.3.1 Berry development

As mentioned in the previous chapters, Prime and Crimson Seedless fresh weight per berry increased significantly from pea size berry until harvest without any so-called lag phase. Significant differences in berry weight were obtained between the three berry sizes (small, medium and large) for both cultivars throughout both seasons. Prime, an early ripening cultivar, developed within a shorter time frame (49 days from pea size berry until harvest) than Crimson Seedless, a late ripening cultivar (63 days from pea size berry until harvest).

Average fresh weight per berry for Prime was higher in 2012/13 compared to previous season. At 49 days after pea size (DAPS) the three berries sizes weighed on average one gram more in 2012/13 than in 2011/12. Véraison for Prime occurred at the same time in both seasons, namely at 21 DAPS (Chapter 3).

In both seasons véraison for Crimson Seedless started at 28 DAPS. In 2012/13 differences in the three berry sizes were more pronounced than in 2011/12. However, similar berry fresh weights were reached at 56 DAPS (Chapter 4). In 2012/13 season temperatures were cooler in November and December with higher rainfall during January and February compared to 2011/12 season. These differences in climatic conditions, as well as change in vineyard location, could have led to the differences in berry weight.

5.3.2 Total soluble solid :titratable acidity ratio.

5.3.2.1 Prime

The change in TSS:TA ratio for Prime is shown in Fig. 3. During berry development TSS concentration increased and TA concentration decreased, resulting in an increased in TSS:TA ratio. In 2011/12 small Prime berries had significantly higher TSS:TA ratios from seven to 42 DAPS compared to medium and large berries. At harvest the TSS:TA ratio for medium and large berries exceeded that of small berries. In 2012/13 no significant differences in TSS:TA ratio were found between berry sizes throughout the

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season (Fig. 3). During last mentioned season the TSS:TA ratio started to increase from 21 DAPS at a much more rapid rate than in 2011/12. As a result, higher ratios were obtained for similar phenological stages. It also reached much higher values than in 2011/12.

For Prime, the higher TSS:TA ratios in 2012/13 compared to 2011/12 could be ascribed to an increase in temperature during berry development (November and December) resulting in an increase in berry weight with a greater average TSS concentration and lower average TA concentration in 2012/13 than in 2011/12 (Table 1). László & Saayman (1990) ascribed differences in TSS:TA ratio between seasons to acidity fluctuations between seasons. In 2011/12 small berries had the lowest TSS:TA ratio at harvest, namely 21.82 which, according to Jayasena & Cameron (2008) falls in the lowest ratio category attained for consumer acceptability.

In 2012/13 at 49 DAPS large berries contained the lowest TSS:TA ratio value followed by small berries with medium berries showing the highest TSS:TA ratio (Table 1). At 56 DAPS, TSS:TA ratio for small berries increased more rapidly, exceeding the ratio of medium berries. Zoffoli *et al.* (2008) found that berries treated with GA_3 obtained higher TSS:TA ratios compared to berries treated with CPPU. This findings correlates to the results from the present study since medium berries, treated with GA_3, had at both harvesting dates, higher TSS:TA ratios than large berries treated with combination of GA_3 and CPPU.

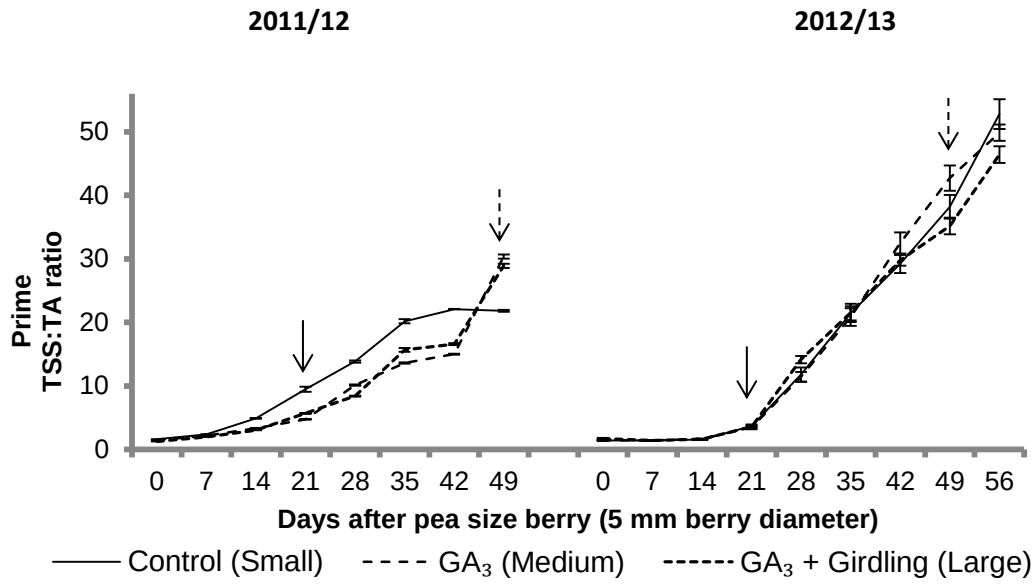

Figure 3. Increase in Prime total soluble solid: titratable acidity (TSS:TA) ratio for the three berry sizes (small, medium and large) throughout 2011/12 and 2012/13 seasons. Vertical bars indicate variance within each sampling time ($p \leq 0.05$). The solid vertical arrow indicates start of véraison and broken vertical arrow indicates first harvest date.

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5.3.2.2 Crimson Seedless

In 2011/12 large Crimson Seedless berries had a lower TSS:TA ratio from 35 to 63 DAPS, indicating a delay in ripening compared to small and medium berries. At harvest (63 DAPS) no significant differences were found between berry sizes (Fig. 4). In 2012/13 large berries had the lowest TSS:TA ratio with small berries showing the highest TSS:TA ratio from 56 DAPS until harvest. Change in the TSS:TA ratio followed by Crimson Seedless was similar for both seasons, and to what Muñoz-Robredo *et al*. (2011) observed.

An average TSS:TA ratio for all the treatments of 31.99 was obtained in 2011/12 at 63 DAPS, and in 2012/13 the average TSS:TA ratio was 33.29. According to Jayasena & Cameron (2008) Crimson Seedless reaches consumer acceptability at TSS:TA ratio from 35 to 40. Applying this rule to the present study, Crimson Seedless reached consumer acceptability after 63 DAPS in both seasons.

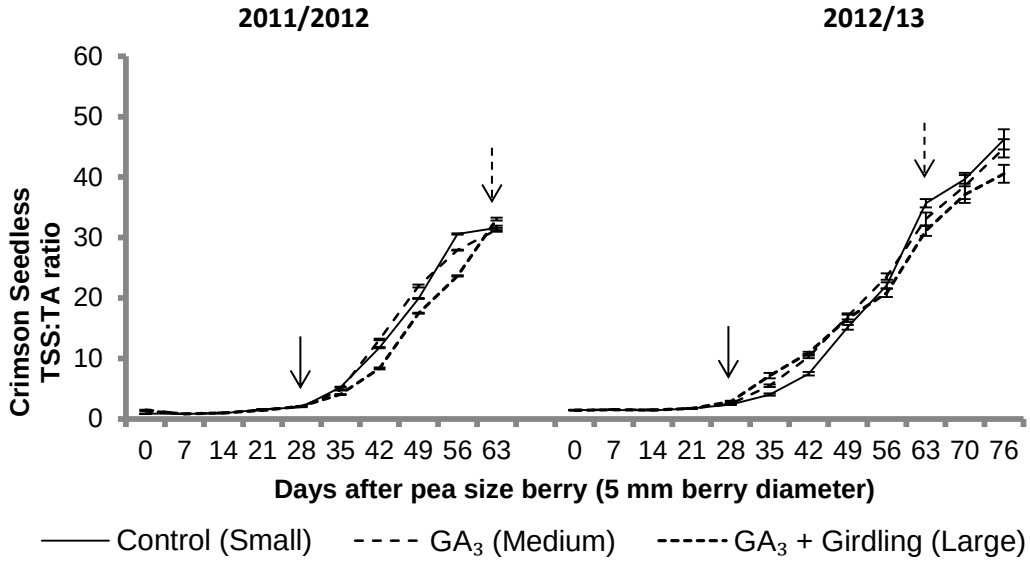

Figure 4. Increase in Crimson Seedless total soluble solid: titratable acidity (TSS:TA) ratio for the three induced berry sizes (small, medium and large) throughout 2011/12 and 2012/13 seasons. Vertical bars indicate variance within each sampling time ($p \leq 0.05$). The solid vertical arrow indicates start of véraison and broken vertical arrow indicates first harvest date.

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5.3.3 Postharvest defects

5.3.3.1 Prime

For the first harvest date, bunches of small berries weighed significantly less than bunches of medium and large berries in 2011/12. However, no significant differences were found between bunch weights for the same time period in 2012/13 (Table 1). At the second harvest date bunches of medium berries weighed significantly morethan bunches of small and large berries (Table 1). These differences in bunch weight could be ascribed to the number of berries per bunch and to bunch size. Bunches from large berries were similar in size to bunches from medium sized berries, since larger berries require more space, thinning was performed more severely leaving less berries per bunch as suggested by Retamales *et al*. (1995).

At first harvest (49 DAPS), Prime TSS concentration in small berries were significantly higher than medium and large berries in 2011/12. In the following season no significant differences for TSS concentration were found between berry sizes at both harvest dates. In 2012/13 large berries treated with GA_3 and CPPU showed a delay in ripening, since TSS concentrations were lower at both harvest dates compared to small and medium berries. Similar results were obtained in previous studies, given that ripening of berries treated with combination of GA_3 and CPPU was delayed between seven to 21 days (Retamales *et al*., 1995; Zoffoli *et al*., 2008). As previously mentioned, the average TSS concentration for the three berry sizes was lower in 2011/12 than in 2012/13. However, the average TA concentrations were higher in 2011/12 than in 2012/13 (Table 1).

Berry quality defects, including browning, loose berries and berry decay increased with the later harvest date in 2012/13. Large berries obtained the lowest percentage browning in 2011/12, but highest percentages were obtained in 2012/13 for both harvest dates. This implies that berry size does not influence the development of browning.

Although not significantly, loose berries were the highest in medium and large berries at first harvest date with medium berries having the highest percentage loose berries at the second harvest date (Table 1). Berries treated with GA_3 are prone to berry drop since the pedicel diameter increases and therefore the flexibility of the pedicel decreases (Ben-tal, 1990; Retamales & Cooper, 1993).

Berry decay was significantly more in small berries in 2011/12 than in medium berries. Even tough percentage decay did not differ significantly in 2012/13 for the three berry sizes, medium berries were

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more prone to decay, followed by large berries for both harvesting dates. As found by Zoffoli *et al*. (2008) berries treated with a combination of CPPU and GA_3 (large size berries) resulted in lower decay percentage than berries treated only with GA_3 (medium size berries). Percentage berry crack were significantly higher in small berries in 2012/13 at first harvest date. However, large berries obtained highest percentage berry crack in 2011/12 and at second harvest date in 2012/13 (Table 1). This could imply that either environmental conditions e.g. rainfall in early January as in 2012/13 season (Fig. 2) or later harvest dates could result in an increase in percentage berry crack for small berries.

5.3.3.2 Crimson Seedless

In 2011/12 no significant differences were found in bunch weight for the three berry sizes. In 2012/13, bunch weight for large berries was significantly higher than medium berries, which again was larger than small berries (Table 2). These last results correspond with other studies where berries treated with GA_3 in conjunction with girdled vines weighed more than berries treated only with GA_3 (Dokoozlian & Peacock, 2001).

Lower average TSS concentrations were obtained in 2011/12 compared to 2012/13 at first harvest date (63 DAPS). However, average TA concentrations did not differ between treatments for first harvest date. At 76 DAPS the average TSS concentration increased with small berries having the highest TSS concentration and large berries the lowest TSS concentration. Average TA concentrations of small berries were the lowest and large berries the highest (Table 2).

In 2011/12 no significant differences in postharvest defects were obtained between the different berry sizes. However, small berries had the highest percentage decay with medium berries showing the highest percentage berry crack and loose berries (Table 2).

In 2012/13 rain one day before the second harvest date possibly enhanced the occurrence of berry defects since the highest percentage berry decay was found for the second harvest date (Table 2). László & Saayman (1993) mentioned that climatic conditions before and during harvesting, and not maturity stage, influence postharvest quality.

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Table 1 Effect of berry size on postharvest quality of Prime at 49 days after pea size (DAPS) berry in both 2011/12 and 2012/13 and also at 56 DAPS in 2012/13.

Harvest date	DAPS	Berry size	Bunch Weight (g)	TSS	TA	TSS:TA ratio	% Crack	% Browning	% Loose berries	% Decay	% Soft tissue breakdown
09/01/2012	49	Small	417.36 b	16.25 a	7.12 a	21.83 b	2.18 a	1.64 a	nd	0.53 a	1.53 ab
	49	Medium	473.97 a	13.05 b	5.16 a	30.36 a	2.72 a	1.26 ab	nd	0.13 b	2.02 a
	49	Large	495.93 a	13.29 b	5.35 a	28.88 a	4.95 a	0.52 b	nd	0.23 ab	1.28 b
LSD (P≤0.05)			47.7	1.26	1.97	8.25	2.98	1.00	nd	0.34	0.74
09/01/2013	49	Small	437.50 a	15.01 a	3.97 a	38.20 b	2.21 a	1.05 a	1.59 a	0.14 a	nd
	49	Medium	524.86 a	15.30 a	3.52 b	42.73 a	0.65 b	0.62 a	1.74 a	0.29 a	nd
	49	Large	478.33 a	14.62 a	4.18 a	35.18 b	1.05 ab	1.22 a	1.81 a	0.20 a	nd
LSD (P≤0.05)			96.54	0.95	0.39	4.37	1.44	0.95	1.52	0.23	nd
16/01/2013	56	Small	455.79 b	16.13 a	3.08 b	52.81 a	1.25 a	1.65 a	4.38 a	0.56 a	nd
	56	Medium	565.74 a	16.62 a	3.35 ab	49.87 ab	0.78 a	1.63 a	5.84 a	0.80 a	nd
	56	Large	527.74 ab	15.83 a	3.43 a	46.41 b	1.72 a	2.10 a	4.05 a	0.76 a	nd
LSD (P≤0.05)			72.10	1.36	0.28	5.67	1.99	1.24	3.10	0.59	nd

DAPS = days after pea size berry (5 mm berry diameter); TSS = total soluble solid, TA= titratable acidity, LSD = lowest significant number; nd = not determined
Letters represent significant differences between berry sizes within each harvest date. Means with the same letter in each column did not differ significantly (P = 0.05).

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In 2012/13, large berries showed the highest percentage berry crack for all three harvest date, while medium berries had the highest percentage loose berries for all the harvest dates. Last mentioned results correspond with previous studies, since berries treated with GA_3 are susceptible to loose berries (Ben-tal, 1990; Retamales & Cooper, 1993; Zoffoli *et al*., 2008). Decay, however, did not show a consistent pattern with berry size.

5.4 Conclusion

Prime showed low-overall levels of decay, soft tissue breakdown and loose berries. Berry size also did not seem to affect the occurrence of these defects. Similar results were found for Crimson Seedless.

The overall decay was low for both cultivars with low percentage differences between berry sizes. This could be attributed to harvesting and packaging in the vineyard with a maximum period of three hours from harvest to loading into the cold storage.

Rain caused a higher percentage of decay at the second harvest date for Crimson Seedless in 2012/13. It is therefore considered necessary to wait more than 24 hours after rain before harvesting commences again to ensure that the berries have completely dried.

According to results obtained from present study, postharvest defects were therefore not specifically affected by berry size or TSS:TA ratio, but rather by harvest time (berry maturity stage). Environmental conditions before and during harvest date/time can also play a significant role in postharvest quality.

Producers should not only rely on TSS:TA ratios in order to predict harvest dates and postharvest quality, but rather keep climatic conditions in mind as well as maturity of berries. Care should be taken to reduce the time from picking of bunches in the vineyard until packaging and storage. As found by Crisosto *et al*. (2001) cooling delays should be minimised to avoid stem water loss. Crisosto *et al.* (2001) also found that packaging material (box material and cluster bags) plays an important role in postharvest quality.

Individual compounds affecting TSS:TA ratio like tartaric acid, malic acid, glucose and fructose might individually or in combination affect postharvest quality. Therefore it is necessary to investigate whether the concentration of these compounds influence postharvest defects.

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Table 2. The effect berry size has on postharvest quality of Crimson Seedless at 63, 71 and 77 days after pea size (DAPS) berry in 2011/12 and 2012/13.

Harvest date	DAPS	Treatments	Bunch Weight (g)	TSS	TA	TSS:TA ratio	% Crack	% Loose berries	% Decay	% Soft tissue breakdown
04/02/2012	63	Small	541.96 a	14.17 a	5.48 a	31.62 a	0.038 a	0.55 a	0.68 a	0.04 a
	63	Medium	540.99 a	13.43 a	4.50 b	31.32 a	0.072 a	1.19 a	0.30 a	0 a
	63	Large	572.31 a	14.95 a	4.98 ab	33.04 a	0.0 a	0.61 a	0.23 a	0 a
LSD (P≤0.05)			31.56	2.98	0.72	8.69	0.15	0.71	0.45	0.07
04/02/2013	63	Small	517.68 b	17.79 a	5.00 b	35.68 a	0.14 a	0.22 b	0.00 a	nd
	63	Medium	501.23 b	17.61 a	5.35 ab	33.08 b	0.27 a	0.75 a	0.00 a	nd
	63	Large	585.99 a	17.39 a	5.61 a	31.11 b	0.41 a	0.53 ab	0.013 a	nd
LSD (P≤0.05)			47.51	0.80	0.40	2.59	0.31	0.42	0.021	nd
12/02/2013	70	Small	486.26 b	18.74 a	4.74 a	39.63 a	1.44 b	0.63 b	0.40 b	nd
	70	Medium	505.77 b	19.19 a	4.46 b	38.52 ab	2.72 ab	1.78 a	0.66 ab	nd
	70	Large	574.79 a	18.24 a	4.94 a	37.12 b	3.77 a	1.69 a	1.20 a	nd
LSD (P≤0.05)			49.29	1.09	0.24	2.36	1.82	0.82	0.54	nd
18/02/2013	76	Small	501.71 b	19.84 a	4.32 a	46.24 a	0.53 b	0.66 b	0.54 a	nd
	76	Medium	540.03 ab	19.50 a	4.39 a	44.78 ab	0.53 b	1.70 a	0.19 b	nd
	76	Large	578.81 a	18.96 a	4.72 a	40.55 b	1.83 b	0.88 b	0.33 ab	nd
LSD (P≤0.05)			68.08	0.98	0.41	4.96	0.79	0.63	0.33	nd

DAPS = days after pea size berry (5 mm berry diameter)
TSS = total soluble solid, TA= titratable acidity, nd = not determined
Letters represent significant differences between berry sizes within each harvest date. Means with the same letter in each column did not differ significantly (P = 0.05)

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Chapter 6

General discussion and conclusions

The final berry quality that reaches the market is important for the producer, exporter and consumer. It is therefore necessary to understand how to obtain good berry quality throughout berry development, transportation and cold storage. Quality berry production starts in the vineyard. By understanding berry development, the various compositional changes taking place in the berry can be manipulated, favouring a good postharvest shelf life. Table grape berry quality is determined by berry size, firmness, sugar concentration, titratable acidity (TA), colour and lack of postharvest defects. Numerous studies have been done on wine grape compositional changes throughout berry development, but little research focused on table grape development.

This study was done on two cultivars, Prime and Crimson Seedless. Three berry sizes (small, medium and large) were obtained for both cultivars by application of plant bioregulators such as gibberellic acid (GA_3) and synthetic cytokinin (CPPU) or girdling. Changes in berry composition during development were observed for all three berry sizes. Berry components such as sugars (total soluble sugars, glucose and fructose), titratable acidity (TA), organic acids (tartaric and malic acid), abscisic acid (ABA), total phenols and anthocyanins (for Crimson Seedless) were measured throughout development. Furthermore, postharvest defects were measured for the three berry sizes.

The aim of this study was: 1) to determine compositional changes taking place throughout growth and ripening for three different size berries and 2) establishing the relationship between sugar: acid ratio and postharvest shelf life.

Significant differences were observed between berry sizes for both cultivars in both seasons. Prime, an early ripening cultivar, developed within 49 DAPS compared to Crimson Seedless, a late ripening cultivar, that matured at 63 DAPS. Véraison started around 21 DAPS for Prime and 28 DAPS for Crimson Seedless. No lag stage was observed in both cultivars. Except for malic acid concentration and content per berry, trends followed by compounds were similar for both cultivars. In 2011/12 TSS content per berry was lower than in 2012/13 for both cultivars.

The TSS concentration of both cultivars started to increase before véraison was observed – in Prime sugar increased from pea size berry while for Crimson Seedless it started to increase from 14 DAPS.

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Significant changes in berry composition were observed as véraison commenced. In both cultivars the following component concentrations increased significantly at the start of véraison: glucose, fructose and ABA. In Crimson Seedless total anthocyanin concentration started to increase one week after véraison. The main anthocyanin found in Crimson Seedless was peonidin-3-glucoside followed by cyanidin-3-glucoside and malvidin-3-glucoside.

The following component concentrations decreased at the onset of véraison: TA, tartaric acid, malic acid and total phenols. Compounds that were correlated to berry size include: TSS, glucose, fructose and TA content per berry. In Prime, tartaric acid content per berry was correlated to berry size from véraison onwards.

General observations to also take note of are:

- No extended lag stage was observed during seedless table grape development.
- Total soluble solid concentrations start to increase before véraison (berry softening and colour development) is visually observed.
- Malic acid was the main organic acid in Crimson Seedless, however tartaric acid was the main organic acid in Prime.
- Peonidin-3-glucoside was the main anthocyanin in Crimson Seedless.

Low percentages of postharvest decay were obtained for both cultivars. Rain was a possible cause for higher percentage defects at second harvest date of Crimson Seedless. Postharvest decay cannot totally be ascribed to differences in TSS:TA ratio or berry size.

In this study typical trends of compositional changes throughout development are presented. Further research is required on the development of different table grape cultivars, focusing on compositional changes occurring within the berry in various environmental conditions. Future research could focus on different packaging material and packaging procedures (mainly on how to minimise cooling delay) and the effect it has on postharvest quality.