Cellular factors that affect table grape berry firmness

by

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DECLARATION

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SUMMARY

The South African table grape industry is under great pressure to produce table grapes of the best quality for the export markets. Quality defects such as poor colour, inadequate berry firmness, browning and soft tissue breakdown cause great losses in export. The firmness of table grapes is one of the major factors determining the eating quality of grapes. Consumers prefer grapes with a firmer flesh above those with soft flesh. Firmer berries are commonly accepted to have better eating quality and longer cold storage capacity. Factors that promote and maintain berry firmness are only speculated about; therefore producers cannot effectively control the development of firmer berries by managerial practises or by applying specific sprays.

The study was done on Redglobe and two Waltham Cross clones (the firmer Clone 8 and softer Clone 13). The aim of this study was two-fold. Firstly the cellular and ultracellular differences between the tissues of firm and soft berries were determined. The effect of gibberellic acid (GA_3), synthetic cytokinin (CPPU) and bunch applied calcium sprays on the cellular and ultracellular structure of berry tissues were also under investigation. Secondly, the effects of GA_3, CPPU and bunch directed calcium sprays on berry firmness, eating quality and storage capacity were determined.

To determine the cellular and ultracelular structure of berry tissues, light microscope (LM) and transmission electron (TEM) studies were done. In order to investigate the effect of different sprays on berry firmness, 20 mg/L GA_3 (GA_3 treatment) was applied at 10mm average berry size; 20 mg/L GA_3 plus 3 mg/L CPPU (CPPU treatment) was applied at 10 mm average berry size; and a mixture of 8 L/ha Stopit® and 5 L/ha Caltrac® (calcium treatment) was applied directly to the bunches every two weeks from berry set till veraison for the calcium treatments. The control received no plant bioregulators (PBR's). The treatments were the same for both cultivars.

Grapes were stored three weeks at 0 °C and one week at 10 °C after which it was evaluated for loose berries, botrytis infections, rachis browning and berry split. Afterwards it was tasted by an independent tasting panel.

Firm berries were found to have an opaque coloured flesh while soft berries had a gel-like translucent flesh. For berries with normal firmness, the opaque flesh is limited to the outer mesocarp of the berry. Extremely firm berries' whole mesocarp consisted of the opaque coloured flesh while soft berries' mesocarp consisted of mostly the gel-like translucent flesh with, in some cases, a very thin layer of opaque flesh just under the skin.

Berry firmness was not related to cell size as the cell size of the tissues in the firm and soft berries were identical. Cell shape seems to play an important role in berry firmness. The cells in the opaque coloured flesh of the outer mesocarp are more turgid and oval than those in the gel-like flesh of the inner mesocarp. Berry firmness is therefore determined by the thickness of the outer mesocarp with the opaque coloured flesh that contains turgid cells. The thickness of cell walls between the different tissues

did not differ. There was however a difference between the cell contents and the plasmalemmas of the inner and outer mesocarp. The plasmalemma and tonoplast of the outer mesocarp cells was more intact than those of the inner mesocarp. The membranes in the inner mesocarp are more subtracted form the cell wall than in the outer mesocarp.

Both the PBR's and calcium treatments cause a delay in sugar accumulation in the case of Redglobe and Waltham Cross. The CPPU treatment results in significantly bigger and firmer berries for both cultivars. In the case of Redglobe, this treatment cause bigger cells in the outer mesocarp suggesting a correlation between berry firmness and cell size. In the case of Waltham Cross, however, cell size did not play a role in berry size and firmness; instead the rate of cell division earlier in berry development. The CPPU treatment was the only treatment that maintains berry firmness during cold storage for Redglobe while GA_3 and CPPU did so in the case of Waltham Cross.

PBR's seems to have no effect on cell wall thickness. In the case of Redglobe, the calcium treatments resulted in significantly thinner cell walls, but this can not be explained.

Calcium and GA_3 treatments had a negative effect on grape quality after cold storage of both Redglobe and Waltham Cross. The Waltham Cross CPPU treatment results in better taste and colour as observed by the tasting panel, while in the case of Redglobe, the tasting panel preferred the control.

It is found that the use of CPPU in combination with GA_3 had the best effect on the eating quality, storage capacity, berry size and firmness. When a producer decides to use the CPPU treatment in order to improve berry firmness, he must realize that it can cause delayed ripening which can affect the export of the fruit.

OPSOMMING

Die Suid-Afrikaanse tafeldruifbedryf moet tafeldruiwe van die hoogste gehalte vir die uitvoermarkte produseer. Gehaltedefekte soos swak kleur, onaanvaarbare korrelfermheid, verbruining en sagteweefsel verval veroorsaak dat produsente baie geld op uitvoere verloor. Fermheid van tafeldruiwe is een van die belangrikste faktore wat die eetgehalte van druiwe bepaal. Verbruikers verkies druiwe met fermer vleis bo die met sagte vleis. Daar word algemeen aanvaar dat fermer druiwe beter eetgehalte en langer opbergingsvermoë het. Verder word nog net gespekuleer oor die faktore wat korrelfermheid kan verbeter en tydens koelopberging kan onderhou. Produsente kan dus nie die ontwikkeling van fermer druiwe effektief deur bestuurspraktyke of deur spesifieke spuitmiddels verseker nie.

Hierdie studie was op Redglobe en twee Waltham Cross klone (die fermer Kloon 8 en sagter Kloon 13) uitgevoer. Die doel was tweeledig. Eerstens is die sellulêre en ultrasellulêre verskille tussen die weefsels van ferm en sagte korrels bepaal. Die effek van gibbereliensuur (GA_3), sintetiese sitokinien (CPPU) en trosgerigte kalsiumspuite op die sellulêre en ultrasellulêre struktuur van die korrelweefsels is ook ondersoek. Tweedens is die effek van GA_3, CPPU en trosgerigte kalsiumspuite op korrelfermheid, eetgehalte en opbergingsvermoë van die druiwe ondersoek.

Ligmikroskoop (LM) en transmissie elektronmikroskoop (TEM) studies is gedoen om die sellulêre en ultrasellulêre struktuur van die korrelweefsels te bepaal. Om die effek van die verkillende behandelings op korrelfermheid te bepaal, is 20 mg/L GA_3 (GA_3 behandeling) toegedien tydens 10 mm gemiddelde korrelgrootte; 20 mg/L GA_3 plus 3 mg/L CPPU (CPPU behandeling) toegedien op 10 mm gemiddelde korrelgrootte; en ‘n mengsel van 8 L/ha Stopit® plus 5 L/ha Caltrac® (kalsiumbehandeling) is direk op die trosse toegedien, elke twee weke vanaf korrelset tot deurslaan, ‘n total van drie spuite. Die kontrole het geen plantgroeireguleerders (PBR’s) of kalsiumbehandeling ontvang nie. Dieselfde behandelings is op beide kultivars toegepas.

Die druiwe is vir drie weke by 0˚C gestoor en daarna vir een week by 10˚C, waarna dit vir loskorrels, botrytis-infeksie, trosstingelverbruining en korrelbars geëvalueer is. ‘n Onafhanklike proepaneel het die druiwe daarna geëvalueer.

Ferm korrels het ‘n wit, ondeurskynende vleis terwyl sagte korrels ‘n deurskynende jellie-agtige vleis het. Korrels met normale fermheid se wit vleis is beperk tot die buitenste deel van die mesokarp. Uiters ferm korrels se hele mesokarp bestaan uit hierdie wit vleis, terwyl die sagte korrels se hele mesokarp, en in sommige gevalle was daar ‘n dun lagie van die wit weefsel net onder die skil, wat uit die deurskynende jellie-agtige weefsel bestaan.

Korrelfermheid word nie deur selgrootte bepaal nie, want die selle in die weefsel van die ferm en sagte korrels was ewe groot. Selvorm speel ‘n rol in korrelfermheid. Die selle in die wit, ferm vleis in die buitenste deel van die mesokarp is meer turgied en ovaal as dié in die deurskynende jellie-agtige vleis. Korrelfermheid word dus bepaal

deur die dikte van die ferm, wit gekleurde weefsel wat die turgiede selle bevat. Die selwanddikte tussen die verkillende weefsels het nie verskil nie. Daar was wel ‘n verskil tussen die sel-inhoud en plasmamembrane van die binneste en buitenste deel van die mesokarp. Die plasmamembraan en tonoplast van die selle in die buitenste deel van die mesokarp was meer intakt as die van die selle in die binneste deel van die mesokarp. Die membrane in die binneste deel van die mesokarp was verder weggetrek van die selwand as in die buitenste deel.

Die PBR-behandelings, sowel as die kalsiumbehandeling, het vertraagde rypwording van die Redglobe en Waltham Cross druiwe. Die CPPU behandeling het betekenisvol groter en fermer korrels by albei die kultivars tot gevolg gehad. In die geval van Redglobe, het die behandeling tot groter selle in die buitenste deel van die mesokarp aanleiding gegee, wat kan beteken dat daar ‘n verhouding kan wees tussen korrelfermheid en selgrootte. In die geval van Waltham Cross, het die selgrootte nie ‘n rol in korrelgrootte of fermheid gespeel nie, maar die tempo van selverdeling vroeër in die ontwikkeling van die korrel wel. Die CPPU behandeling was die enigste behandeling waarvan die korrelfermheid van Redglobe behoue gebly het tydens koelopberging terwyl dit die geval was vir die CPPU- en GA_3-behandelings by Waltham Cross.

PBR’s het geen effek op selwanddikte gehad nie. By Redglobe, het die kalsiumbehandeling betekenisvol dunner selwande tot gevolg gehad. Daar is nie ‘n verduideliking vir hierdie verskynsel nie.

Die kalsium- en GA_3-behandelings het ‘n negatiewe effek op druifgehalte na koelopberging vir beide Redglobe en Waltham Cross gehad. Hierdie behandlings het die voorkoms van Botrytis-infeksie, loskorrels en korrelbars verhoog. Die proepaneel het die kontrole in die geval van Redglobe en die kalsium en CPPU behandelings in die geval van Waltham Cross verkies, op grond van die beste smaak en kleur.

Die gebruik van CPPU in kombinasie met GA_3 het die beste effek op eetgehalte, gehalte na opberging, korrelgrootte en –fermheid van die druiwe gehad. Indien ‘n produsent besluit om die CPPU behandeling vir die verbetering van korrelfermheid toe te pas moet hy in gedagte hou dat dit vertraagde rypwording tot gevolg kan hê wat die uitvoer van die druiwe kan benadeel.

This thesis is dedicated to my parents Jan and Ousus du Plessis who loved, supported and motivated me throughout my academic career. It is also dedicated to my brothers, Francois and Pieter-Jan and grandmother, Beatie van Zyl.

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BIOGRAPHICAL SKETCH

Trix du Plessis was born in Clanwilliam on 1 February 1983. She grew up on the farm, Aggenbagskraal in the Clanwilliam region. She matriculated in 2001 at Augsburg Agricultural Gymnasium and enrolled in 2002 at the University of Stellenbosch and obtained the BScAgric (Viticulture and Oenology) in 2005. In 2006 she enrolled for the MScAgric (Viticulture) degree at the University of Stellenbosch.

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PREFACE

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

Chapter 1	**General Introduction and Project Aims**
Chapter 2	**Literature Review** Factors that affect table grape berry firmness
Chapter 3	**Research Results** Cellular and ultracellular differences between soft and firm table grapes
Chapter 4	**Research Results** Utilisation of gibberellic acid (GA_3), CPPU and bunch applied calcium sprays to increase berry firmness of table grapes: (I) Redglobe
Chapter 5	**Research Results** Utilisation of gibberellic acid (GA_3), CPPU and bunch applied calcium sprays to increase berry firmness of table grapes: (II) Waltham Cross
Chapter 6	**General Discussion and Conclusions**

CONTENTS

Chapter 1

INTRODUCTION AND PROJECT AIMS

GENERAL INTRODUCTION AND PROJECT AIMS

The South African table grape industry is constantly under pressure to produce grapes of the best quality for consumers worldwide. This industry suffers losses in export programs due to quality defects such as browning, SO_2 damage, soft tissue breakdown and poor colour. These defects reduce the eating quality of the grapes.

The firmness of table grapes is one of the main factors that determine its eating quality. Consumers prefer grapes with firmer flesh above those with soft flesh. Ideally table grape berries will develop white flesh with high consistency towards ripening. It is commonly accepted that these berries have a higher cold storage capacity and eating quality than berries with the soft watery flesh (Personal communication: G. van der Merwe). Factors that promote the development of firm berry flesh are only speculated about. As a result, producers have no proven managerial practices whereby the development of firm berries can be ensured.

Very little research has been done on the factors that may play a role in the development of firm grape berries with crispy flesh. Gibberellic acid (GA_3) applications at vèraison were found to increase the firmness of table grape berries (Ben-Arie *et al.*, 1997 & Singh *et al.,* 1978). Synthetic cytokinin (CPPU) applications also improved berry firmness. Ben-Arie *et al*. (1997) however proposed that greater firmness brought on by CPPU applications can be the effect of delayed fruit maturation while Coombe & Hale (1973) ascribed it to modifications in the anatomy of the berry.

The exoskeleton of the plant cell is the cell wall. The cell wall determines the shape and turgor pressure of the cell (Taiz & Zeiger, 1998). According to Nunan et al. (1997) the cell wall structure appears to play a role in the firmness of table grapes. Calcium determines the structure of the cell wall (Grant et al., 1973). It acts as a binding agent in the middle lamella (Dey & Brinson, 1984) and increases the cohesion of the cell walls (Demarty et al., 1984). Most research on the effect of calcium on fruit tissue cell walls was done on apples. It increases fruit quality by promoting cell wall cohesion. Whether the same effect on cell walls of grape berries occurs is not elucidated.

Table grape producers require guidelines to successfully cultivate grapes with hard, crispy flesh. It was therefore necessary to investigate cellular and ultracellular differences between tissues of firm and soft berries, and whether treatments can be exerted to improve berry firmness.

The aims of this study were twofold. Firstly, the difference between the tissues of firm and soft berries was determined on cellular and ultracellular level. The effect of gibberellic acid (GA_3), synthetic cytokinin (CPPU) and calcium sprays on the cellular and ultracellular structure of berry tissues was also investigated. Secondly, the effects of GA_3, CPPU and calcium sprays on the firmness, eating quality and storage capacity was determined.

In order to achieve the abovementioned aims, the following approaches were followed:

1. The choice of relevant vineyards with a history of respectively producing soft and firm berries;
2. Application of GA_3, CPPU and calcium sprays at the appropriate time during development of the berries;
3. Sectioning of grape berries for light and electron microscope studies;
4. Measuring of berry firmness at the appropriate times during berry development;
5. Evaluate storage capacity of grapes after cold storage;
6. Evaluate eating quality of grapes after cold storage.

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Chapter 2

LITERATURE REVIEW

Factors that affect table grape berry firmness

LITERATURE REVIEW

2.1 INTRODUCTION

The firmness of table grapes is one of the main factors that determine the eating quality of grapes. Consumers prefer grapes that have a high aesthetic value – grapes must have the right colour, size and firmness. Other factors that will determine the eating quality of grapes after cold storage are browning, SO_2 damage and soft tissue breakdown.

Very little research has been done on table grapes regarding the factors that affect berry firmness. Therefore no managerial practises, supported by scientific research, can be prescribed to enhance the firmness of berries. Calcium applications to the bunches and calcium fertilisation, as well as gibberellic acid (GA_3) and synthetic cytokinin (CPPU) applications, are performed by South African table grape producers to enhance berry firmness. The value of none of these practices has been elucidated.

The developmental stage of the berry determines the time of application of growth regulators as well as calcium fertilisation. Growth regulators must be applied at the correct time of berry development to have the desired effect. Gibberellic acid has the biggest effect during the first growth phase of the berry (Iwahori *et al.,* 1968). Sachs & Weaver (1968) found that GA_3 enhances the division and expansion of the parenchyma cells in the pericarp of the berry. The enlargement of the cells results in a decrease of cell density (Ben-Arie *et al*., 1997). Ben-Arie *et al*. (1997) and Singh *et al.* (1978) found that when grapes are treated with GA_3 at vèraison its firmness increases. CPPU increases the density of the cells because it promotes cell division (Ben-Arie *et al*., 1997). Ben-Arie *et al.* (1997) proposed that the increased firmness of berries brought on by CPPU applications can be the effect of delayed fruit maturation while Coombe & Hale (1973) ascribed it to modifications in the anatomy of the berry.

The cell wall is the exoskeleton of the plant cell. The shape and the turgor pressure in the cell are determined by its cell wall (Taiz & Zeiger, 1998). The structure of the cell wall appears to play a role in the firmness of the table grape berry (Nunan *et al.,* 1997). Whether the thickness of the cell wall, and therefore conditions during berry development that determines its thickness, will affect the firmness of the grape berry, however, remains unclear.

Calcium plays a major role in the structure of the cell wall (Grant *et al*., 1973). It acts as a binding agent in the middle lamella (Dey & Brinson, 1984) and

calcium ions increase the cohesion of the cell walls (Demarty *et al*., 1984). Most research on the effect of calcium on cell walls was done on apples. Whether calcium treatments will have the same effect on the cell walls of table grapes has not yet been clarified. If so, calcium sprays and fertilization should also be applied at the correct time during berry development. Fertilization should be done during the period when the grape berry can still accumulate calcium, taken up by the roots.

2.2 BERRY DEVELOPMENT

Development of the berry may play an important role determining its firmness. Managerial practises that might increase the firmness of table grape berries must be applied at the correct time during its development to obtain the desired effect. A thorough understanding of the berry's development will therefore provide insight required to utilize and evaluate these practises properly.

The berry is divided into different tissues. The pericarp is the ovary wall that surrounds the seed cavities. It is divided into two sections – the exocarp or skin (consisting of the cuticle, epidermis and hypodermis) and the mesocarp or the pulp (Fig. 1). The exocarp consists of 6 to 8 cell layers, while the mesocarp has between 25 and 30 layers of cells (Dokoozlian, 2002). The outer wall of the epidermis is protected by a cuticle made out of layers of wax (Casado & Heredia, 2001). The exocarp consists of compact collenchymatic cells while the mesocarp has a spongy texture caused by a loose connection between cells (Esau, 1960).

The growth of the grape berry follows a double sigmoid curve which consists of three development stages as shown in Fig. 2 (Coombe, 1992). These stages of development are characterized by different levels of division and enlargement of cells in the pericarp (Nakagawa & Nanjo, 1965). A detailed discussion follows below.

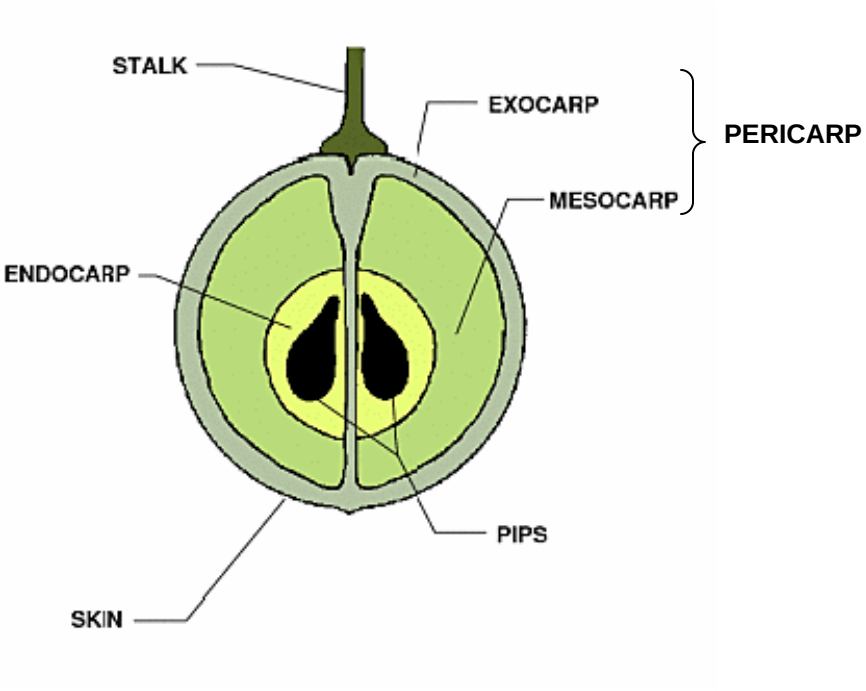

Figure 1 Cross section of a grape berry (Anonymous, 2002).

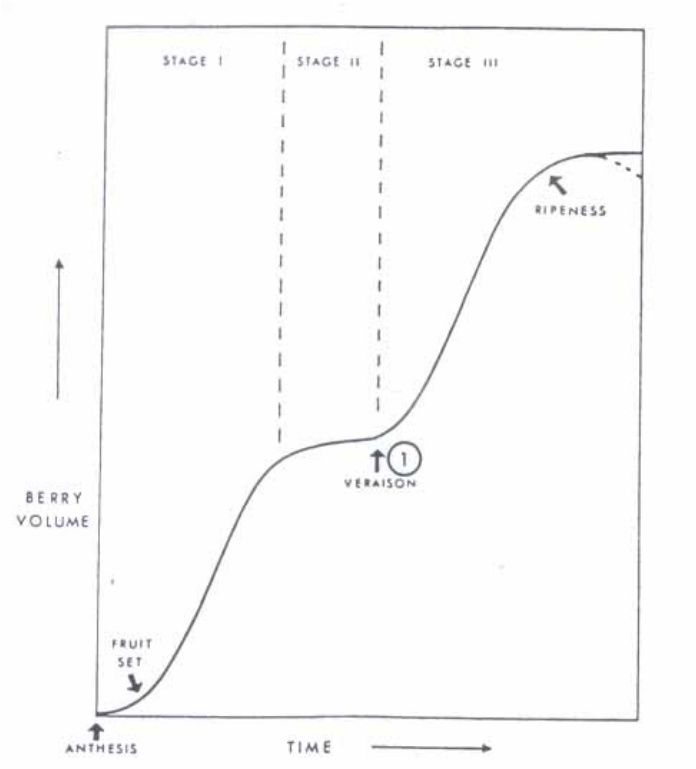

Figure 2 The development stages of a grape berry (Coombe, 1992).

2.2.1 DEVELOPMENTAL PHASE 1: RAPID GROWTH PHASE

Division of cells and development of the seeds occur in the first growth period (Pratt, 1971). According to Harris *et al.* (1968) the duration of this phase is from bloom up to 60 days thereafter. According to Dokoozlian (2002) it lasts between two to three weeks for raisin varieties in the San Joaquin Valley. Ojeda *et al*. (1999) divides phase 1 of berry development into three periods. The first period is from anthesis up until five days thereafter. It is characterised by intense mitosis while none of the cells enlarge significantly. Thus during this time, berry enlargement is based on cell division, not cell enlargement. The second period is from five days until 35 days after anthesis. This period shows a reduced rate of cell division and the induction of cell enlargement. The third period represents the last week of phase 1 of berry development. During this week cell division stops completely while cell enlargement continues. Last mentioned is now the only factor that influences berry growth.

According to Coombe (1960) the majority of cell division takes place during the first five to ten days after bloom. No further cell division occurs in the berry after this stage (Dokoozlian, 2002). At the end of this phase, the total number of cells in the berry has therefore been established (Harris *et al*., 1968). Thus, cell division during this stage determines the cell number in the berry for the rest of its development (Dokoozlian, 2002). After termination of cell division, berries increase in volume because of accumulation of solutes (Possner & Kliewer, 1985) and the import of water (Keller, undated). During this stage water enters the berry through the xylem (Keller, undated). Division of cells ceases in all tissues during this growth phase, the biggest contribution to berry growth is therefore cell enlargement (Nakagawa & Nanjo, 1965).

During this phase the hypodermis consists of a collection of small, isodiametric thin walled cells (Hardie, *et al.,* 1996) that expand tangentially (Nii & Coombe, 1983). They are also similar in size than the underlying mesocarp cells (Considine & Knox, 1981) which are also isodiametric (Ollat *et al*., 2002). Phenolic compounds typically occur against the vacuoles of pericarp cells; these compounds resemble membraneless globules (Nii & Coombe, 1983).

There are fewer layers of cells in the sub epidermis of seedless berries than in seeded berries. The formation of cell layers in the sub epidermis of both seeded and seedless berries ceased at the same time (Shiozaki *et al.,* 1997).

Phase 1 of berry development is very sensitive to temperature and light. The optimum temperature for cell division and enlargement is between 20 and 25˚C while temperatures above 35˚C will reduce the growth rate and size of the berry

at the end of its development (Dokoozlian, 2002). Temperatures lower than the optimal will not result in smaller berries but it will affect the length of the growth period by delaying the onset of ripening (Hale & Buttrose, 1974), that starts with the transition to phase 3 of berry development.

Berries grown under heavily shaded conditions are significantly smaller than well exposed berries – cell division and/or cell enlargement are therefore stimulated by light during this developmental stage (Dokoozlian, 2002). The size of the cells in the mesocarp are negatively affected by limited supply of assimilates such as sugar early in development (phase I) of the berry (Ollat & Gaudillere, 1998). The same authors also established that the pericarp cells of grapes on vines subjected to leaf removal were significantly smaller than grapes on vines with no leaves removed.

Vines subjected to water stress during growth phase I also produce smaller berries – this effect cannot be overturned by subsequent watering due to a permanent decrease of cell size (Dokoozlian, 2002). A limited water supply does not affect cell division but it decreases cell volume (Ojeda *et al.,* 1999; Ojeda *et al.,* 2001).

Throughout the whole of phase 1, the berry has a firm texture and a green colour because of the presence of chlorophyll. Sugar content in the berry remains low while organic acids accumulate (Dokoozlian, 2002). The size of the berry at the end of this stage will determine the potential size to which the berry might enlarge during ripening (Ollat *et al*., 2002).

2.2.2 DEVELOPMENTAL PHASE 2: THE LAG PHASE

The second growth period, which follows on the first, is called the slow growth period (Pratt, 1971) also known as the lag phase. This phase lasts about two to three weeks depending on the variety and season (Dokoozlian, 2002). Researchers differ regarding the stages in which development of the berry is divided. Coombe & McCarthy (2000) suggest three phases – berry formation (from set to vèraison) and berry ripening (from vèraison to 20˚B) and the last phase from 20˚B to harvest. The lag phase as described by Dokoozlian (2002) is included in the first phase of Coombe & McCarthy (2000). Winkler *et al.* (1974) divides the development into two phases – the green stage (from set to the beginning of ripening) and the ripening stage (from the beginning of ripening until full ripeness). The lag phase is included in the ripening stage as the turning point from the green stage to the ripening stage.

This phase is characterised by a drastic decrease in the growth rate of berries as well as a decreased concentration of growth substances (Nitsch *et al.,* 1960). The berries remain firm while their organic acids reach its highest level. At the end of this phase, chlorophyll begins to break down (Dokoozlian, 2002).

2.2.3 DEVELOPMENTAL PHASE 3: THE RIPENING PERIOD

The third growth period is characterized by a second phase of rapid berry growth. This is due to the enlargement of the cells (Pratt, 1971). The berries grow fast at the beginning of ripening and slow down towards maturity (Ollat *et al.*, 2002; Keller, undated).

This phase begins with vèraison (Kennedy, 2002) which is described by Coombe (1973) as the point when an acceleration of growth; an increase in deformability resulting in softening of the berry; an accumulation of sugars – glucose and fructose – in the pericarp; a decrease in the concentration of organic acids; loss of chlorophyll and an accumulation of anthocyanins occur. Furthermore, it is also characterised by an increase in enzyme activity (invertase, sucrose phosphate synthetase, sucrose synthetase and hexokinase) (Hawker, 1969).

At the onset of vèraison sugar, in the form of sucrose, is transported into the berry. This occurs throughout the whole period of fruit ripening (Kennedy, 2002). Increase of sugar and softening of the berry starts at the same time (Coombe, 1992). While sugar concentration in the berry increases, the concentration of organic acids declines (Dokoozlian, 2002), resulting in an increase in the pH of the berry (Keller, undated). Aroma compounds also begin to accumulate in the berry (Dokoozlian, 2002).

Berry deformibility increase at the early stages of the second period of berry expansion while elasticity of the skin tissue decreases. Loosening of the cell walls in the mesocarp allows the accumulation of sugar. The loosening of cell walls takes place first in the mesocarp and then in the exocarp. Cell wall loosening is followed by the expansion of the berry (Huang & Huang, 2001) due to cell expansion.

The cell wall and the middle lamella contain pectic substances. The middle lamella consists primarily of these pectic substances. When the pectic substances are removed from the middle lamella the cells will fall apart. Individual cells, however, will retain their shape because of the other material still present in the cell wall (Jensen, 1973). The cell wall itself consists of microfibrils – made up of polysaccharide chains – embedded in a matrix. The pectin content

of the grape change during ripening as the result of cell wall metabolism which is associated with berry softening. Silacci & Morrison (1990) found a strong correlation between the accumulation of sugar and the increase of water soluble pectin. There is a decrease of cell wall bound pectin in the berry with ripening while the water soluble pectin increased. Berry expansion also dilutes the pectin concentration. According to Nunan *et al*. (1998) there are no major changes in the composition of cell wall polysaccharides during the softening and ripening of berries. They did, however, observe significant changes to specific polysaccharide components and the protein composition of the berry. Silacci & Morrison (1990), on the other hand, found that the concentration of pectic polysaccharides in the berry decreases after vèraison. This concentration continues to show a decline after harvest.

A decrease of polysaccharides in skin cells of the berry is accompanied by a decrease in pectic-bound calcium and the acidification of the apoplast. The cells in the berry skin therefore loose a part of its structural polysaccharides while incorporating structural proteins. These contribute to the strength of the skin tissue to maintain the integrity of the berry (Huang *et al*., 2005).

In the case of the cultivar Pinot noir, the increase in deformability of the berry precedes the development of colour and expansion of the berry (Creasy *et al*., 1993). Softening of the berry happens on average of six days before the expansion of the berry (Coombe, 1992; Coombe & Bishop, 1980) some varieties, however, expands before it softens – this indicates that berry softening and expansion are two different events (Coombe & Bishop, 1980). Increased cell elasticity causes deformability of the berry while increased cell wall plasticity allows berry expansion (Coombe, 1992).

The berry's size can double from the start of véraison to harvest (Kennedy, 2002). The enlargement of cells is the only factor playing a role in berry expansion during this phase (Dokoozlian, 2002). Cell enlargement happens mostly due to the import of water and sugar into the berry. Modification of the cell wall makes this expansion of the cells possible (Nunan *et al*., 1998). The potential expansion of cells is influenced by 1) cell wall behaviour, this include plasticity of the cell wall, the deposition of the cell wall material and the degree of secondary cell wall development; 2) turgor which is affected by water influx and the osmotic pressure gradient between the inside and the outside of the cell; and 3) the limitations on the expansion of the flesh such as the extensibility of the skin and the outer cell layers (Cleland, 1971).

During this stage the outer layers of the hypodermis cells elongate tangentially while the cells in the inner layers expand in an irregular shape

(Sachs & Weaver, 1968 & Ollat *et al*., 2002). The cell walls of the epidermal cells are thin and it looks similar to the mesocarp cells (Ollat *et al*., 2002).

One week after vèraison, the walls of some of the cells in the skin thicken while the rest of the cell walls stay thin (Nii & Coombe, 1983). The mesocarp cells increase considerably in size. This expansion of the cells can be correlated with expansion of the central vacuole. The vacuole represent up to 99% of the cell's volume (Diakou & Carde, 2001). An osmotic gradient develop in the vacuole of the cells due to the accumulation of sugar. This attracts water into the vacuole and causes the vacuole to expand further (Coombe, 1960). The expansion of cells therefore requires the following factors: a turgor pressure to set cell wall pressure above a critical value; the permeability of the cell walls must allow the required influx of water; the initiation of the building of cell walls and the maintenance of cell walls which are in the process to be broken down (Preston, 1974; Ray *et al.*, 1972). The cell walls of the mesocarp are thin and have a different composition than at the beginning of the berry's development (Nunan *et al*., 2001). According to Nunan *et al.* (1998) the thickness of these cell walls does not change during the expansion of the cells in the ripening process. The phenolic compounds disappear from the mesocarp and are converted to a thin layer in the cells of the skin (Nii & Coombe, 1983). Dissociation of cells may occur further into the ripening process (Esau, 1960).

At this stage water is no longer imported by the xylem into the berry but by the phloem (Keller, undated). Xylem discontinuities happen during the softening of the berry. This discontinuity of xylem is ascribed to (i) the rapid expansion of the berry that leads to the stretching and eventually the breaking of the xylem tracheids in the brush region of the berry, making the xylem vessels non-functional (Creasy *et al.,* 1993); (ii) the loss of cell membrane integrity (compartmentation breakdown) in the berry (Lang & Düring, 1991). Due to the fact that berry softening occurs before berry expansion (Coombe, 1992; Coombe & Bishop, 1980) and xylem discontinuity occurs at berry softening, xylem breakage may actually be a result of a mechanism other than berry expansion (Creasy *et al.,* 1993). Bondada *et al*. (2005) suggested that there is not a loss in the xylem function during and after vèraison, rather a loss of the hydrostatic gradient in the apoplast of the berry which act as the driving force of water uptake. This is caused by the changes in the solute partitioning pattern between the apoplast and the symplast of the berry.

The hydraulic conductance of the berry decreases ten-fold from vèraison to ripeness, this is caused by changes in the above mentioned anatomy of the xylem as well as the role that aquaporines, situated in the membranes, play in

the regulation of the hydraulic conductance (Tyerman *et al*., 2004). One of the key factors that influence the firmness of berries is their ability to absorb water. With the lower water potential in the berry towards ripening, more water can be taken up and the berry develops a firmer texture. The resumption of berry growth during phase three was accompanied by a decrease in water potential (Matthews, *et al*., 1987) leading to an uptake of water into the berry which result in a berry with a firmer texture. Coombe & Bishop (1980) found that the berries' diameter decreases and its deformability increases during daytime while recovering at night time. This determines the best time to pick table grapes. The berries are firmer early in the morning than in the evening.

This third phase of ripening lasts between six and eight weeks (Dokoozlian, 2002). The development of the berry's cuticle is nearly complete at the end of this stage. Its thickness remains the same during the maturation process (Casado & Heredia, 2001).

Seedless berries have a less distinctive growth pattern than seeded berries (Pratt, 1971). The cells in the pericarp of seeded berries follow a different path of development than those in the seedless berries. Seedless berries have fewer cell-layers than seeded berries, while their cell size is much larger. This is ascribed to GA_3 applications on the seedless berries (Shiozaki *et al.,* 1997).

All the cells in the grape berry are formed in the first developmental phase. This developmental phase is thus the optimal time to apply growth regulators that will enhance cell division. During the third developmental phase, the berry enlarges by cell enlargement only. Growth regulators that will have a positive effect on cell enlargement will therefore have to be applied during phase I. Cabanne & Donéche (2003) found that the berry accumulates the majority of its calcium during the first developmental phase. From vèraison up until ripeness its concentration decreases in the pericarp and increase in the seeds and skin. The optimal time to apply calcium will therefore probably be before vèraison.

2.3. FACTORS THAT MAY AFFECT BERRY FIRMNESS

2.3.1 NUTRIENTS

2.3.1.1 Calcium

Application of calcium (Ca^{2+}) fertilizer and bunch directed calcium sprays are common practices applied by South African table grape producers. It is believed

that calcium will enhance berry firmness and storage life. Very little research, however, has been done to establish the effect of calcium applications on table grapes.

2.3.1.1.1 Physiological role of calcium in fruit

Calcium plays a role in the ripening and storage life of fruit such as apples. When a high concentration of calcium is maintained in the fruit tissue during its development, the fruit will have a slower ripening rate. This is caused by lower respiration rates and lower ethylene production. The softening of the fruit will also be slowed down (Ferguson, 1984). Sams & Conway (1984) found that calcium delays the softening of apples, because it slows down the degradation of cell wall polymers. Apples infiltrated with $CaCl_2$ also showed a lower ethylene production and were firmer before and after cold storage. Apples treated with calcium retain their firmness and cell-to-cell contact which plays an important role in the firmness of fruit tissue. Electron microscope studies showed that for calcium treated apples, cell walls were well preserved and the middle lamella undergone very little degradation when compared to untreated apples. Last mentioned, softens more rapidly as the cell walls in the tissue swelled and separated (Poovaiah *et al.*, 1988). For long periods after harvest, apples continue to respond to calcium treatments, remaining firmer than the untreated control (Sams & Conway, 1984). The effect of calcium to delay the ripening and senescing processes is therefore primarily extra cellular. It affects the cell walls and the external surface of the plasmalemma (Ferguson, 1984).

2.3.1.1.2 Biochemical function of Ca^{2+}

The highest concentration of calcium is in the inner- (near the pip) and outermost (just under the skin) part of the berry, i.e. tissues where there are a large number of cells and therefore a high amount of cell walls (Possner & Kliewer, 1985). The main function of calcium in the cell wall is the structural role it plays. Calcium forms cross links between pectic polymers - to give rise to the so-called ‘egg-box’ model (Grant *et al.,* 1973) - primarily in the middle lamella. Galacturan chains are the most prominent pectic components in the cell wall. Some of the carbonyl groups in these chains are methylated. The methylated groups are available for bonding with Ca^{2+}. The degree of methyl esterfication will therefore determine the degree of Ca^{2+} bonding in the cell wall (Ferguson, 1984). Calcium acts as a binding agent in the middle lamella to stabilize the pectin-protein complexes (Dey & Brinson, 1984). Calcium ions therefore increase the cohesion of the cell walls (Demarty *et al.,* 1984). Huang *et al.* (2005) found

that the cell wall bound calcium concentration in the skin decreases significantly after vèraison. The cross linking of the polysaccharides in the cell wall by calcium may require displacement or release of the Ca^{2+} to make cellular growth possible (Cleland & Rayle, 1977). Effects of calcium during ripening are seen in the structure and functioning of the cell wall and plasmamembrane (Ferguson, 1984). Calcium contributes to the rigidity of the cell wall (Dey & Brinson, 1984). Low concentrations of Ca^{2+} will make the cell wall more elastic and it can easily rupture while higher concentration will make it more rigid and reduce its flexibility (Hepler, 2005). Calcium also helps to resist external osmotic pressure (Saxton, 2002).

Cells need only low concentrations of free calcium in the cytoplasma to function normally (Ferguson, 1984). Calcium movement between the cytoplasma and other cellular compartments takes place via channel proteins. Increased Ca^{2+} concentration in the cytoplasma causes the channels to close. Calcium-ATPases move the Ca^{2+} back from the intracellular stores and into the cell wall (Trewavas, 1999). When shortage in calcium occurs, it is taken out of the cell walls, leaving them with reduced resistance to enzyme attack and fungal infections (Doneche & Chardonnet, 1992).

Calcium plays a role in the maintenance and control of the plasmamembrane structure and function (Poovaiah & Leopold, 1973). Ben-Arie *et al.* (1982) found that Ca^{2+} keeps the membrane lipids in the plasmamembrane in a more fluid state. Low concentrations of Ca^{2+} increase the permeability of the plasmamembrane (Hepler, 2005). Marinos (1962) showed that calcium deficiencies in plants cause membranes to loose their integrity. The rise in the K^+/Ca^{2+} ratio during ripening can contribute to the increase of cell permeability (Sacher, 1973). Hanson (1984) cultured cells in a medium low in Ca^{2+} in the presence of the cation chelator, EDTA. These cells leaked ions and metabolites. This experiment also showed that when Ca^{2+} is removed from cell walls, it becomes more permeable for ions and metabolites.

Calcium may have a direct effect on the activity of cell wall degrading enzymes (Poovaiah *et al.,* 1988 & Demarty *et al*., 1984). The higher cell walls are in calcium concentration, the less susceptible it is to enzymatic digestion (Chardonnet & Doneche, 1995). Polygalactorunase is an enzyme that breaks down the pectin structure of the cell wall. Calcium inhibits this enzyme thus increased levels of calcium may delay cell wall degradation resulting in firmer berries (K. Bindon: Personal communication, 2005).

2.3.1.1.3 Ca^{2+} transport

Calcium is delivered to different parts of a plant by xylem via transpiration (Saxton, 2002). According to Rogiers *et al*. (2000), Ca^{2+} content shows a linear increase during development and ripening of the berry while Possner & Kliewer (1985) indicated that the accumulation of calcium stops at vèraison. Calcium accumulation is the fastest during the first developmental phase of the grape berry. Its concentration decreases from véraison in the pericarp while it increases in the seeds. It is furthermore transported from the pericarp to the skin during ripening, leading to a decreased concentration in the pericarp (Cabanne & Donéche, 2003). The cell walls of the skin cells therefore contain high amounts of calcium at maturity (Donéche & Chardonnet, 1992). The K/Ca ratio in the berry increases suddenly at vèraison. This shows that the phloem inflow increased relatively to the xylem inflow (Rogiers *et al.,* 2000). Xylem discontinuities happen during the softening of the berry. There is still to some degree a xylem connection between the vine and the grape berry after véraison which can supply the berry with water and Ca^{2+} (Creasy *et al*., 1993). Calcium is phloem immobile (Hanger, 1979; Saxton, 2002), and therefore the lowered transport of calcium via the xylem will lead to a lower accumulation thereof in the berries.

Chardonnet *et al.* (1997) and Alcaraz-López *et al.* (2005) found that when calcium is applied externally to grape berries; it accumulates in the outer cell layers of the skin. Research done by Alcaraz-López *et al.* (2005) showed that berries sprayed with calcium in combination with titanium (Ti) are firmer, larger and loose less weight during storage than untreated berries. Poovaiah (1988) found that fruit treated with calcium remain firm during storage. The cells of this fruit have a dense middle lamella and very good cell-to-cell adhesion. This indicates the protection role calcium plays in the cell wall against normal breakdown that is related to maturation.

2.3.1.2 Potassium

Potassium (K^+) is the most abundant cation in plant tissues. Clarkson & Hanson (1980) state that the physiological-biochemical roles of K^+ in the plant are: enzyme activation, membrane transport processes, anion neutralization and osmotic potential regulation. Since cell membranes are highly permeable to this ion (Mpelasoka *et al*., 2003), it plays the most important role in the water status of a plant (Mengel & Kirkby, 1982). The active uptake of K^+ by plant cells has an impact on the turgor of the cell because it causes uptake of water into the cell via the lowering of the osmotic (Läuchli & Pflüger, 1978; Saxton, 2002).

Grape berries are a large sink for K^+ (Storey, 1987) and is present in high concentrations in all parts of the berry (Possner & Kliewer, 1985). Flesh cells contain high amounts of K^+ (Donéche & Chardonnet, 1992) but up to 50% of the accumulated K^+ is directed to the skin where it is stored as potassium salts (Iland, 1988). The K^+ content in grape berries increase during the ripening process (Rojas-Lara & Morrison, 1989). Ripe berries have 15 times more K^+ than green berries (Donéche & Chardonnet, 1992). Accumulation is slow during the pre-vèraison phase but the rate increase significantly after vèraison (Rogiers *et al*., 2000).

Potassium therefore seems to play a very important role in the water status of the cells in the berry. As a result it might affect berry firmness - the more water there is in the berry, the firmer the berry will be. There, however, was no reference to this aspect found in the literature. Furthermore, as discussed above, the K/Ca ratio plays an important role in the permeability of the cell membrane. With berry ripening the K/Ca ratio increases which raise the permeability of the cell membrane (Sacher, 1973). This results in a higher uptake of ions which will lower the water potential of the cell. Uptake of water is therefore facilitated.

2.3.2 THE ROLE THAT CELL WALLS PLAY IN BERRY FIRMNESS

The cell wall serves as an exoskeleton to the plant cell, as a result of this it determines the shape of the cells and allows high turgor pressures to develop. The cell wall is necessary for normal water relations in the plant, because of its influence on the turgor pressure (Taiz & Zeiger, 1998). All plant cells have a primary cell wall while some cells have a secondary cell wall (Jensen, 1973). A cell wall is seen as primary during all the cell development stages. It only becomes secondary when cell growth stops and the wall begin to thicken (Hall *et al*., 1974). The young cell is surrounded by a primary cell wall – it is thin, elastic and expands as the cell grows. A secondary wall forms between the cytoplasm and the primary cell wall when cell growth ceases (Jensen, 1973). The integrity and texture of tissues are largely determined by the primary cell wall (Doco *et al*., 2003). Substances such as lignin are deposited in the cell wall. When secondary walls become lignified, the primary cell walls also become lignified (Cutter, 1978). The lignified walls are thick, rigid and give the cell great tensile strength (Jensen, 1973).

2.3.2.1 Cell wall composition

The cell wall is a cross-linked polymer system (Ray & Ruesink, 1962) that consists of a series of layers. Cell wall material consists of strands called microfibrils surrounded by a matrix. The matrix is made up of hemicellulose (Jensen, 1973), pectins, water and a bit of structural protein (Taiz & Zeiger, 1998). Between 60% and 70% of the cell wall's weight is water (Hall *et al*., 1974). About 80% of the water in the primary cell wall is located in the matrix (Taiz & Zeiger, 1998). The proportion between different types of polymers in the cell wall can control the amount of water in the matrix. Pectic polysaccharides can bind much more water than hemicelluloses (Cook & Stoddart, 1973). The amount of water in the matrix plays an important role in the physical properties of the wall. The removal of water from the matrix will make the wall more rigid and reduce its extensibility (Taiz & Zeiger, 1998).

The microfibrils consist of bundles of cellulose molecules. These cellulose molecules are composed of long chains of glucose molecules. Cellulose plays an important role as a structural element in the cell wall and contributes most to the strength of the plant cell (Jensen, 1973). It forms the framework around which all the other components of the wall are positioned (Clowes & Juniper, 1968). Polysaccharide chains in the microfibrils are strongly bound to each other (Preston, 1974) or to xyloglucans (Hayashi, 1989). This makes it relatively inaccessible to enzyme attack. Cellulose is therefore a very stable molecule (Preston, 1974). It will only break down during late development stages such as abscission and senescence (Taiz & Zeiger, 1998). The amount of cellulose and polysaccharides in the cell wall and their stage of breakdown will determine the strength and rigidity of the cell wall. This may affect the firmness of the grape berry.

Large molecules that also play a role in the strength of the cell wall are proteins and nucleic acids. Transfer of information between cells is essential for the general functioning of the cell. These molecules also play a role in this information transfer (Jensen, 1973). There is uncertainty regarding the protein content of cell walls. Only small amounts of protein are recovered from primary cell walls. It is suggested that glycoprotein forms bonds with specific polysaccharides (Preston, 1974) such as carbohydrates (Hall *et al.,* 1974; Preston, 1974; Preston, 1979). Cell walls also contain enzymes, phenolic polymers and other materials that modify their physical and chemical characteristics (Taiz & Zeiger, 1998). Other important but less common

components are fats, waxes, tannins, pigments, terpenoids, gums and mucilages (Clowes & Juniper, 1968).

Primary cell walls are connected to each other by the middle lamella. Pectic substances are a chemical component both of the primary cell wall, as well as the middle lamella (Jensen, 1973). The pectins form a gel phase in which the cellulose and hemicellulose are embedded to prevent the aggregation and collapse of the cellulose network. The pectins also determine the cell wall's permeability to large molecules (Taiz & Zeiger, 1998), such as hexuronic acids - a derivative of hexose sugars. When the pectic substances of the middle lamella are removed, cells fall apart. The cell wall however stays intact because of structural materials still present therein. The cells will therefore retain their shape (Jensen, 1973).

2.3.2.2 The functions of cell walls

The cell wall however limits the size of the plant cell (Cleland, 1971). The plasmamembrane surrounds the cytoplasma to separate it from the cell wall. It is very thin and flexible and is composed of proteins and lipids. This membrane regulates the movement of substances in and out of the cell. The membrane functions as a differentially permeable barrier that prevents the leakage of organic materials such as sugar and protein out of the cell and allows water and salts to enter the cell. Water passes through it by osmosis. This results in the build up of pressure inside the cytoplasma which force the cytoplasma and the plasmalemma against the cell wall (Jensen, 1973). Cells can therefore only enlarge due to turgor pressure if the area of the cell wall increases (Cleland, 1971) i.e. the expansion of cell walls (Taiz & Zeiger, 1998). The degree to which the cell wall expands does not exceed its ability to function as an unbroken envelope (Preston, 1974).

2.3.2.3 The role of cell walls in cell enlargement

Cell enlargement is an active process and needs energy provided by respiration (Cleland, 1971). The age and type of cell is some of the factors that may influence the expansion rate of cell walls (Taiz & Zeiger, 1998). Cell enlargement therefore requires not only the stretching of the existing wall, but also production of new wall material; this requires continuous synthesis of RNA and protein (Cleland, 1971). Loosening of cell walls (stress relaxation) precedes its expansion. This occurs when the cross links between the polymers breaks (Lockhart, 1967). There are many theories regarding cell wall loosening. One of the theories is cell wall acidification, when protons are extruded over the

plasmalemma (Taiz & Zeiger, 1998). This action reduces the pH of the cell wall. The acid-liable crosslinks in the wall is cleaved to allow wall loosening. These bonds are then rapidly reformed (Cleland, 1971). Other theories involve auxin, which causes the breakdown of bonds between the components of the cell wall. It also promotes the synthesis of new wall material to be incorporated into the wall, which results in the increase of its surface area (Hall *et al*., 1974). Plant cells selectively loosen bonding between cell wall polymers and secrete cellulose in the direction of cell expansion. The loosening enables the cell wall polymers to glide past each other to increase the surface area of the cell wall and reduce the physical stress in the wall (Taiz & Zeiger, 1998). It is thought that in the case of collenchyma cells – which have high water content – the water enable the microfibrils to easily slip past one another in a dilute pectin matrix. This seems to explain the high extensibility of collenchyma cells (Preston, 1974). Turgor pressure is proposed to be the driving force behind cell enlargement (Cleland, 1971). When the stress in the wall is reduced, the turgor and the water potential in the cell are also reduced. This enables the cell to absorb more water which leads to expansion (Taiz & Zeiger, 1998). Although the cell wall can expand extremely, its thickness stays the same due to new wall material laid down on the inside of the cell wall surface (Preston, 1979). Contrary to this, Thomas & Doyle (1976) found that the cell wall may become thinner. Considine & Knox (1979) found a swelling in the cell walls of grape berries at the beginning of véraison, this happens at the same time as the berries' plasticity increases (Coombe, 1973). During softening of the berry, pectins and xyloglucan molecules depolymerize and the cellulose and hemicellulose content decreases (Yakushiji *et al.*, 2000). The thickness of the cell walls, as well as the changes it undergo during development of grape berries, may have an effect on the firmness of berries.

2.3.2.4 The effect of fruit ripening on cell walls

The polysaccharides in the cell wall undergo certain modifications during the ripening process of the grape berry such as: a change in molecular weight, degree of solubility and the degree of substitution of individual polysaccharides (Silacci & Morrison, 1990). The solubility of the pectins increases when Ca^{2+}, which act as the cross link between pectic polymers, is removed (Jona *et al.*, 1983). Nunan *et al.* (1998) found that the cell wall polysaccharide composition does not undergo major changes during the softening of the berry, but there was a significant change in a specific polysaccharide component. The cell wall

polysaccharide level decreases in the berry flesh but not in the skin (Chardonnet *et al*., 1994).

Extensive cell separation happens during fruit ripening; this is due to changes in the middle lamella. The driving force of cell separation is turgor pressure. Cell separation is followed by structural changes of the primary cell wall and middle lamella (Bartley & Knee, 1982). The cohesion of the middle lamella depends on ionic bonds with divalent cations of which Ca^{2+} is the major one (Ginsburg, 1961). Calcium ions increase the cohesion of cell walls (Demarty *et al.*, 1984). The Ca^{2+} ions form cross links between the pectic polymers in the cell wall (Grant *et al.*, 1973). Low Ca^{2+} concentration in the cell wall will make it more elastic and it is more at risk of rupture while high concentrations makes it more rigid and less flexible (Hepler, 2005).

2.3.2.5 Cell walls and berry firmness

Very little research on cell walls of grape berries and its effect on berry firmness have previously been done. According to Nunan *et al.* (1997), however, cell wall structure appears to have an effect on the firmness of grape berries. Cell walls of the mesocarp of the berries of a firmer table grape cultivar, Ohanez, have significantly higher cellulose content than a softer cultivar, Gordo. The xyloglucans is also higher in Ohanez than in Gordo. Xyloglucans is closely associated with microfibrils (Carpita & Gibeaut, 1993). The softer Gordo berries have cell walls enriched with galacturonans. These cell walls therefore have a more extensive pectic matrix phase than the firmer Ohanez berries (Nunan *et al.,* 1997). As seen above, the firmness of the berry is determined by the composition of the cell walls. Questions regarding the role of the thickness of the cell wall to determine firmness of the berry still exist. Likewise, possible changes in cell wall thickness throughout berry development have not been elucidated.

2.3.3 THE USE OF PLANT BIOREGULATORS (PBR'S) TO IMPROVE BERRY FIRMNESS

2.3.3.1 Gibberellic acid

Synthetic gibberellic acid (GA_3) is commonly used to increase grape berry size (Morris, 1987). Gibberellic acid enhances the division and expansion of the parenchyma cells in the pericarp which leads to an increase in berry size (Hashim, undated; Sachs & Weaver, 1968). By enhancing the enlargement of cells, GA_3 causes a decrease in cell density (Ben-Arie *et al*., 1997).

Weaver & Pool (1971) suggested that GA_3 applications at bloom influence the cell division stage during berry development, while applications at fruit set affect cell enlargement. Ben-Arie *et al.* (1997) found that gibberellic acid is only effective for a short period during phase I of berry development. Iwahori *et al*. (1968) suggests that the application of GA_3 has the biggest effect on the first rapid growth phase (phase I) of the development of the seedless berry. An increase in GA_3 concentration will gradually increase the weight of the treated berries, but too high concentrations can cause corkiness of the stems and the berries as well as berry crack which will lead to decay (Jawanda *et al*., 1974). Thus, it is important to apply the lowest concentration of GA_3 to get the best effect (Wolf *et al*., 1991). It also has a bigger effect on the enlargement of the distal parenchyma cells than on the proximal parenchyma cells, this can explain the indentation that develops on the apical end of Thompson Seedless berries that were treated with GA_3 (Sachs & Weaver, 1968).

Gibberellic acid applications do not increase berry size of seeded grapes as significantly as it does seedless grapes. Berries with fewer seeds have a better response to GA_3, but only to a limited extent – GA_3 seems to compensate for the lack of seeds, but when the berry have a certain number of seeds, the GA_3 application has no effect (Weaver & McCune, 1959; Lavee, 1960). Considine & Coombe (1972) classified grapes into three categories according to their response to GA_3 applications. The categories are: 1) Parthenocarpic berries that respond well to applications at anthesis by enlarging several-fold; 2) Stenospermocarpic berries that respond the best to applications one to two weeks after anthesis - these berries do not enlarge as much as the parthenocarpic berries; 3) Seeded berries where a very small, if any, response to GA_3 applications is obtained.

Application of GA_3 increases berry weight and yield but decreases the development of seeds (Roller, 2003). Shiozaki *et al.* (1997) found that GA_3 has a bigger effect on cell enlargement than on cell division in the case of seedless berries. Since seeds are an endogenous source of GA_3, it appears as if application of exogenous GA_3 compensate for the lack of seeds (Hashim, undated). The cells in the pericarp of seedless berries follows a different path of development than those in the seeded berries, this can be the result of the different hormonal equilibrium induced by GA_3 applications than by the seeds (Shiozaki *et al.,* 1997). Berries in which seedlessness was induced by GA_3 applications tend to have shorter development stages than untreated berries (Inaba *et al.,* 1976).

Little research has been done to establish the effect of gibberellic acid on the firmness of table grape berries. Application of optimal concentration of GA_3 on grapes results in bigger berries with better storage life (Pool *et al.*, 1972). Furthermore, Singh *et al.* (1978) and Ben-Arie *et al.* (1997) demonstrated that the firmness of berries can be increased when it is treated with GA_3. In the case of only a single application, berries treated at véraison, were the firmest.

2.3.3.2 Cytokinin

Cytokinins are plant bioregulators that stimulate cell division and cell expansion while it delays senescence (Reynolds *et al.*, 1992). Ogata *et al*. (1989) however stated that cytokinins only promote cell division, while gibberellins promote cell division and cell enlargement. The effect of cytokinin on cell divisions early in the season affects the growth of the berry later in the season (Thomas & Blakesley, 1987). Fruit treated with cytokinins, has a lower total soluble solids concentration and pH, at harvest while red and black cultivars have a lighter skin colour (Reynolds *et al.,* 1992).

N-(2-chloro-4-pyridyl)-N'-phenylurea or forchlorfenuron (CPPU) is a synthetic cytokinin that is commercially used to promote cell division, therefore increasing berry size (Dokoozlian, 2001). It increases cluster weight and the size of the berries of several seedless grape cultivars (Reynolds *et al.,* 1992). Although application of CPPU on Flame Seedless, results in increased berry size, it reduces the development of colour. Ebisuda & Dokoozlian (2003) found that the number of harvestable clusters decreased linearly to the increase of applied cytokinin. CPPU delays fruit ripening by delaying sugar accumulation, colour accumulation and organic acid respiration. When it is applied to sensitive cultivars such as Redglobe and Flame Seedless at high dosages, it also alters berry flavour and texture (Dokoozlian, 2001; Nickell, 1986).

Very little research has been done on the application of CPPU on table grapes to improve their firmness. Ebisuda & Dokoozlian (2003) found that the firmness of Flame Seedless berries increases linearly with increased concentrations of applied CPPU. Ben-Arie *et al*. (1997) stated that the increased firmness of berries can be the effect of delayed maturation induced by CPPU, although it can also be related to modifications in the anatomy of the berry (Coombe & Hale, 1973). CPPU applications promote cell division; it will therefore theoretically increase the density of cells (Ben-Arie *et al.,* 1997). The use of CPPU increases the thickness of berry the skin. Ben-Arie *et al.* (1997) thought this to be the cause of improved berry firmness.

Retamales *et al.* (1995) found that the berry diameter increased when CPPU was used on its own, but the best results were found when it was used in addition with GA_3. Avenant & Avenant (2006) also found that CPPU used in combination with GA3 increase berry diameter as well as firmness. Reynolds *et al*. (1992) however found no synergistic effect when CPPU was used in combination with GA_3.

There are many questions in the South African table grape industry regarding the use of CPPU to improve the firmness of berries. The fact that it reduces the colour development and storage life of the grape makes the use of CPPU for this purpose risky.

2.3.4 ENZYME ACTIVITY

Structural changes occur in the middle lamellae and cell wall which leads to cell separation and fruit softening (Jackman & Stanley, 1995; Bartley & Knee, 1982). This is caused by the combined reaction of cell wall hydrolytic enzymes produced by fruit, i.e. polygalactorunase (PG), pectinesterase (PE), β-galactosidase (β-GAL), pectate lyase (PL) and cellulase. These hydrolytic enzymes break down the polysaccharides in the cell wall (Hall *et al*., 1974) which changes the cell wall structure and composition (Brummel & Harpster, 2001). Fruit softening is more due to cell wall loosening and loss of cell cohesion than to cell wall degradation (Ferguson, 1984) The transfer of PG to the middle lamellae initiates fruit softening (Soll & Bottger, 1981). The enzymes which breakdown polysaccharides are highly specific for particular glycosidic bonds in the cell wall (Hall *et al*., 1974). Cellulase which breaks down cellulose is always found in fruit, while its activity increases greatly during fruit ripening (Huber, 1983). It is not as evident as as polygalacturonases (Pesis *et al*., 1978).

The activity of PG increases with grape ripening (Cabanne & Doneche, 2001). There are exo- and endo-PG, where the former is a terminal cleavage enzyme and the latter a random cleaving enzyme (Bartley *et al.*, 1982). Difference in firmness between tomato cultivars are established early in ripening (Brady *et al*., 1985). Firm tomato cultivars in general had less PG activity than soft cultivars (Sobotka & Watada, 1970). Fruit softening is accompanied by the increase of soluble pectic polysaccharides (Huber, 1983). PG action on cell walls is limited by calcium ions and the level of substrate methylation (Buescher & Hobson, 1982), therefore high levels of calcium may delay cell wall degradation resulting in firmer berries (K. Bindon: Personal Communication, 2005). Calcium ions inhibit the activity of PG (Cabanne & Doneche, 2001) and therefore cell wall

hydrolysis (Buescher & Hobson, 1982). As calcium levels decrease during fruit ripening it may enhance the solubilisation of pectin by PG (Cabanne & Doneche, 2001). The flesh of the berry softens more than the skin, as the calcium content in the skin is higher than in the flesh (Doneche & Cabanne, 1992). Studies done on tomatoes showed that gibberellic acid reduced galacturonase activity while it had less effect on cellulose activity and softening (Babbit *et al*., 1973). PG activity shows two patterns in different cultivars. In some it is more active at the beginning of colour change and in other its activity increase with berry ripening (Deytieux *et al*., 2005).

2.3.5 PLANT WATER RELATIONS AND BERRY FIRMNESS

Water deficits between anthesis and véraison (Phase I) of berry development may lead to smaller berries. This occurs due to cell size reduction which can often not be reversed by subsequent watering (Dokoozlian, 2002; Hardie & Considine, 1976; Matthews *et al*., 1987; McCarthy, 1997). Water deficits between anthesis and véraison do not affect cell division (Ojeda *et al*., 2001).

Water stress, nutrient deficiencies and other disorders that may have an effect on photosynthesis during berry developmental Phase II and III can also lead to cell volume reduction. This reduction is due to a decreased supply of sugars to the fruit (Dookoozlian, 2002). Cell enlargement during Phase III of berry development is mostly due to the import of water and sugar into the berry (Nunan *et al*., 1998).). Low turgor pressure in the cell, after véraison, promotes the uptake of solutes into the berry (Tyler *et al*., 2006). Solute accumulation, mainly in the form of glucose and fructose, decrease the osmotic potential of grape berries (Matthews *et al*., 1987). The low osmotic potential allows low water potential to develop which lead to the uptake of water and fruit growth (Grange & Andrews, 1994). Water move from inside the cells, which have a high solute concentration, to the cell wall, which have a lower solute concentration. The water movement causes turgor pressure which makes the berries firmer. When there is not enough water in the cell for turgor pressure to develop, the berries will be softer (Liang *et al*., undated). If water is available during developmental Phase III (ripening), berry size of stressed berries can recover partially or totally (McCarthy, 1997).

Potassium and its role in water relations of the berry were discussed in section 2.3.1.2.

2.4 CONCLUSIONS

Berry firmness has an effect on the eating quality and the storing capacity of the grapes. Apples treated with Ca^{2+} are firmer than untreated fruit. The cell walls in the tissues of the untreated fruit swell and separate. Calcium increases the cohesion of cell walls – this promotes cell-to-cell contact. The integrity and texture of tissues are determined by the cell walls in the tissue. Cell wall structure plays a role in the firmness of the berry. Since Ca^{2+} seem to have a major role in the cell wall structure its value to enhance grape berry firmness should be elucidated.

The role that synthetic growth hormones like CPPU and GA_3 play in promoting improved berry firmness is still in question. The phenological stage at which treatment such as growth regulators and Ca^{2+} sprays are applied may also play a role in its efficiency to promote berry firmness. Researchers found that when GA_3 is used to enhance berry firmness, it is best to apply only one application at vèraison. Most plant bioregulators are only effective in the first developmental stage of berry development. It will therefore be wise to apply plant bioregulators during the first developmental phase as it will then have the best effect.

The South African table grape industry commonly use plant bioregulators with the expectation of obtaining firmer grapes while there is no scientific study that proofs this fact. The same goes for the use of Ca^{2+} sprays and Ca^{2+} fertilisation. The purpose of this study is twofold. Firstly, to determine whether Ca^{2+} fertilisation or sprays, GA_3 and CPPU have an effect on berry firmness, and secondly, to determine the effect of these treatments on the cellular and ultracellular structure of the grape berry.

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Chapter 3

RESEARCH RESULTS

Cellular and ultracellular differences between soft and firm table grape berries

CELLULAR AND ULTRACELLULAR DIFFERENCES BETWEEN SOFT AND FIRM TABLE GRAPE BERRIES

3.1 INTRODUCTION

The table grape industry needs to produce grapes of the highest quality to meet the standards of the export market and obtain best prices. Table grapes is an aesthetic product that has to look, feel and taste good to meet consumers' expectations. It should therefore have the required berry colour and firmness. Little research has been done to relate firmness of table grapes to berry anatomy and, in particular, cell structure of berry tissues.

The grape berry is divided into different tissues. The pericarp surrounds the seed cavities. It consists of an exocarp or skin and a mesocarp or the pulp (Dokoozlian, 2002). The exocarp is made up of compact collenchymatic cells while the mesocarp has a spongy texture because of a loose connection between cells (Esau, 1960).

The first developmental stage of the grape berry is characterized by intense mitosis and very little enlargement of cells (Ojeda *et al*., 1999). The cells formed during this stage determine the amount of cells in the berry for the rest of its development (Harris *et al*., 1968 & Dokoozlian, 2002). The third growth phase is the second period of rapid growth of the berry. During this stage the enlargement of the berry is caused by cell enlargement (Pratt, 1971) which happens due to the import of water and sugar into the berry (Nunan *et al*., 1998). The expansion of cells is correlated with the expansion of the vacuole which represents 99% of the cell's volume at the end of berry development (Diakou & Carde, 2001). As a result of water uptake the turgor pressure inside the vacuole increases, this forces the cytoplasma and plasmalemma against the cell wall (Jensen, 1973). Cells can only enlarge due to turgor pressure and when the area of the cell walls increases (Cleland, 1971). The cell walls have to be modified to make the expansion of cells possible (Nunan *et al*., 1998). Ollat *et al*. (2002) found that the cells of the hypodermis expand tangentially and the cells of the mesocarp expand in an irregular shape. At this stage the walls of the epidermal and mesocarp cells are thin and similar looking. Thomas & Doyle (1976) found that the cell walls may become thinner as the cells enlarge. Contrary to this, Nunan *et al*. (1998) found that thickness of the mesocarp cell walls do not change during the expansion of the cells, although its composition changes during berry

development (Nunan et al., 2001). According to Preston (1979), the thickness of the cell wall stays the same due to new wall material laid down on the inside of the cell wall surface.

The cell wall is the exoskeleton of the plant cell; it determines the shape and the turgor pressure in the cell (Taiz & Zaiger, 1998). Nunan *et al*. (1997) found that the structure of the cell wall appears to play a role in the firmness of grape berries. The composition of the cell wall may also play a role in the firmness of the berry. Cell walls of firmer table grape berries have a higher cellulose and xyloglucan content than softer berries while softer berries have higher galacturonan contents (Nunan *et al.,* 1997).

Whether the thickness of the cell wall plays a role in the firmness of table grapes has not yet been properly investigated. The role of berry development on the thickness of the cell wall has also not yet been elucidated. The focus of this study was to investigate which cellular characteristics, e.g. cell size or cell wall thickness, determine the firmness of table grape berries' flesh.

The hypothesis is that there is a difference in cell wall thickness as well as cell size and shape between the tissues of soft and firm berries, i.e. the mesocarp cells of firmer berries have thicker cell walls and smaller cells than that of soft berries.

3.2 MATERIALS AND METHODS

3.2.1 EXPERIMENTAL VINEYARDS

The experiment was conducted on two micro irrigated nine year old *Vitis vinefera* L. cv. Redglobe vineyards grafted on Richter 110 and a ten year old *Vitis vinefera* L. cv. Waltham Cross grafted on Ramsey vineyard. Both Redglobe vineyards are situated on the farm De Hoop in Paarl. The Redglobe vines were trained on a double gable trellis system while the Waltham Cross vines were trained on a factory roof trellising system. One of the Redglobe blocks (block A) has a history of consistently producing firm berries while the other vineyard (block B) consistently produces soft berries. The soil texture of the blocks differs significantly, i.e. block A has a higher clay content (15% clay) than block B (5% clay). Spacing between the vines of both these blocks is 3 m x 2 m. The irrigation of both blocks was scheduled using tensiometers. Viticultural practises of both vineyards were identical.

The Waltham Cross vineyard is situated on the farm Non Pareil in the Hex River Valley, De Doorns. The block is divided in two sections, i.e. 30 rows of

Waltham Cross Clone 13 and 5 rows of clone 8. Clone 8 consistently produces firmer berries than clone 13. The vines are spaced 3 m x 2 m. Irrigation of this micro irrigated block is also done using tensiometers.

3.2.2 BERRY SAMPLING

Berries were sampled for transmission electron microscope (TEM) and light microscope (LM) studies at five stages of development (pea size, 15 mm diameter, vèraison, 14 days past vèraison and harvest). Four berries were randomly selected, every time from the same experimental vines. The berries were always sampled from the middle part of the bunch. From each berry one radial section was made for both electron and light microscope studies.

3.2.3 LIGHT AND ELECTRON MICROSCOPY

The procedure followed for the preparation of TEM and LM samples were adapted from that described by Diakou & Carde (2001). Radial sections (1 mm^2) of the fresh berries were made in the vineyard with a razor blade and immediately fixed and kept overnight (16 hours) in 2.5 % glutaraldehyde (in 0.1 M phosphate buffer, pH 7.4). Sections included the epidermis and the mesocarp. After fixation they were then washed two times (5 minutes each wash) in 0.1 M phosphate buffer, pH 7.4 and stained with a 1 % tannic acid (in 0.1 M phosphate buffer, pH 7.4) solution for 30 minutes. The samples were then washed twice (5 minutes each wash) in 0.1 M phosphate buffer, pH 7.4, after which it was placed in 1 % osmium tetroxide (in 0.1 M phosphate buffer, pH 7.4) for an hour. Again it was washed two times (5 minutes each wash) in 0.1 M phosphate buffer, pH 7.4, after which it was rinsed twice (5 minutes each wash) in distilled water. The samples then underwent a dehydration process using ethanol (1 x 10 minutes in 50 % ethanol; 1 x 10 minutes in 70 % ethanol; 1 x 10 minutes in 90 % ethanol; 1 x 10 minutes in 95 % ethanol; 2 x 10 minutes in 100 % ethanol (EM grade) and 2 x 10 minutes in acetone) and subjected to a process of infiltration and embedding: The samples were placed overnight in a 50/50 solution of acetone/Spurr's resin, followed by 75:25 resin:acetone for 8 hours and then 100 % resin overnight. The samples were then put in fresh 100 % resin in the morning for two hours. They were then orientated into a mould and resin added. The mould with the samples was then placed in a 60˚C oven for 24 hours.

The mould contained a transverse section which included the exocarp and mesocarp. From each mould two sections were made to represent the outer mesocarp (directly below the hypodermal cell layers) and the inner mesocarp

(directly above the endocarp) respectively. For the light microscope studies, 1 µm sections were cut with a Reichert ultracut S ultramicrotome. These sections were stained with 1 % (w/v) toluidine blue and then washed under water. For the TEM studies, 120 nm ultrathin sections were obtained using the same ultamicrotome. These sections were collected on 200 mesh copper grids and stained with 2 % uranyl acetate and Reynolds lead citrate. Samples were studied with a Leo Omega 912 transmission electron microscope. The electronmicroscope photos were taken with a Proscan CCD camera mounted on the LEO 912 TEM. The images were viewed and analysed using the EasiVision Pro software which was developed by Soft Imaging System GmbH. Mean cell wall thickness could be measured using this software and was determined by measuring two nearby cells' cell walls including the middle lamellae. This measurement was divided in two to determine the mean thickness of one cell wall. Cell size was determined by counting the amount of cells in a 1 mm^2 area.

3.3 RESULTS AND DISCUSSION

3.3.1 RED GLOBE

3.3.1.1 General appearance

When sliced diagonally in the middle with a blade, grape berries harvested from block A, which has a history of consistently producing firm berries, developed an opaque flesh towards ripening (Fig. 1A). Such berries are known to have a better keeping quality as well as superior eating quality (G. van der Merwe: Personal communication, 2005). Berries harvested from block B, which consistently produces soft berries, developed a translucent, gel-like flesh towards ripening (Fig. 1B).

Block B was harvested one month after Block A. Block B normally ripens somewhat later than block A, but this long delay was mainly due to untimely rain, twice before harvest. The firmness of the berries in block B, which usually bears the soft berries, seemed to increase as they were left on the vines to dry off. Thus, firm and soft berries were harvested from block B (Fig. 2). The plantwaterstatus of block A and B was measured using a pressurebomb but no differences was found (data not shown). Distinction between firm and soft berries was made arbitrarily on account of their firmness when pressed between the thumb and index finger. The flesh of the firm berries had an opaque colour and a high consistency while the soft berries had a gel-like appearance. The very firm

berries' inner and outer mesocarp consisted of opaque flesh (Fig. 2A) while berries of normal firmness (Fig. 2B) only had the opaque flesh in the outer mesocarp. The soft berries (Fig. 2D) had a gel-like flesh stretching from the seeds to the skin with exception of a few which had a very thin layer of the opaque flesh similar to that found in the firm berries but just under the skin (Fig. 2C).

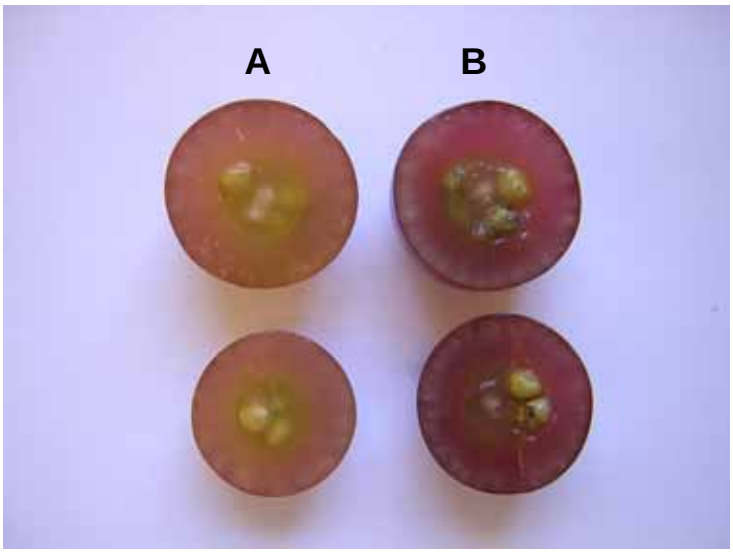

Figure 1 Firm (A) Redglobe berries harvested form block A (firm block) and soft (B) Redglobe berries harvested from block B (soft block) on the De Hoop farm, Paarl, 2006/2007.

Figure 2 Redglobe berries harvested from block B, showing that very firm berries (A), firm berries (B) similar to those of block A, as well as soft berries (C) and very soft berries (D) originated from the same block.

3.3.1.2 Cell size and shape

As described by Nii & Coombe (1983) for cv. Grenache, the general cell size of the exocarp (skin) cells of Redglobe was found to be much smaller, with thicker cell walls and contained more cytoplasm than the mesocarp (flesh) cells (Fig. 3).

Rapid expansion of cells occurred from pea size to 15 mm berries (Table 1). This expansion of cells is due to the accumulation of solutes (Possner & Kliewer, 1985) and the import of water (Keller, undated). In general it is accepted that berry growth will continue after vèraison during phase III of berry development also due to the enlargement of cells (Pratt, 1971). As seen in Table 1, little cell growth occurred during the period between vèraison and harvest. This can be due to too little sampling dates or because not enough were sections made. A lack of time, high expenses and problems with the availability of the microtome and electron microscope leads prevented that more cutting and viewing of the samples could be done. From vèraison up to harvest the cell sizes did not differ between firm (Block A) and soft (Block B) berries (Table 1). Unfortunately, no conclusion could be made because of a lack of proper data.

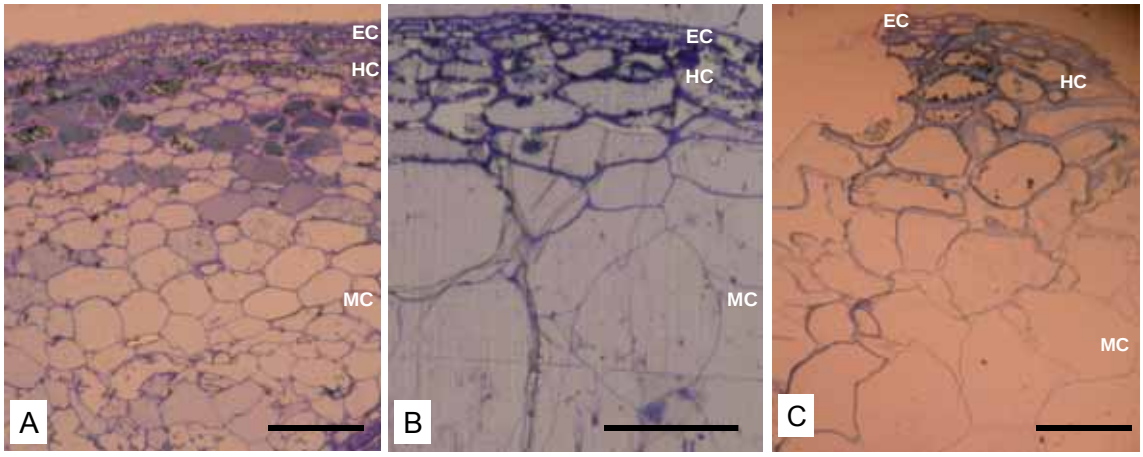

Figure 3 Light microscope sections of skin (exocarp) and flesh (mesocarp) of Redglobe at pea berry size (A), véraison (B) and harvest (C). EC: two epidermal cell layers, HC: 6-8 hypodermal cell layers, MC: Mesocarp cells. Scale bar: ± 0,5 mm.

Differences in the shape of cells were observed between the outer (white opaque coloured flesh) and inner mesocarp (translucent gel type flesh). As seen in Fig. 4, the cells in the outer mesocarp were more turgid and oval than those in the inner mesocarp. Similar differences occurred for berries from the firmer block A and softer block B (photos for last mentioned not shown). In general, berry firmness seems to be determined by the thickness of the mesocarp with more oval, turgid cells. In accordance with this, Liang *et al*. (undated) stated that when

cells do not develop enough turgor pressure, berries will be not very firm. As discussed above, the thicker this zone, the more firm the berry will be.

Table 1 Mean[1] cell size of different berry mesocarp tissues of firm (Block A) and soft (Block B) Redglobe berries at different phenological stages.

Tissue	Berry type	Mean number of cells per mm^2 at each phenological stage				
		Pea size berry	15 mm berry size	Véraison	14 days past véraison	Harvest
Outer mesocarp	Firm	240	18	10	6	10
	Soft	98	28	14	6	12
Inner mesocarp	Firm	128	10	12	6	10
	Soft	110	14	12	8	6

[1] The means of cell counts from photos are given. The data could not be analysed statistically because, in a few instances, only one or two photos of that particular tissue could be taken.

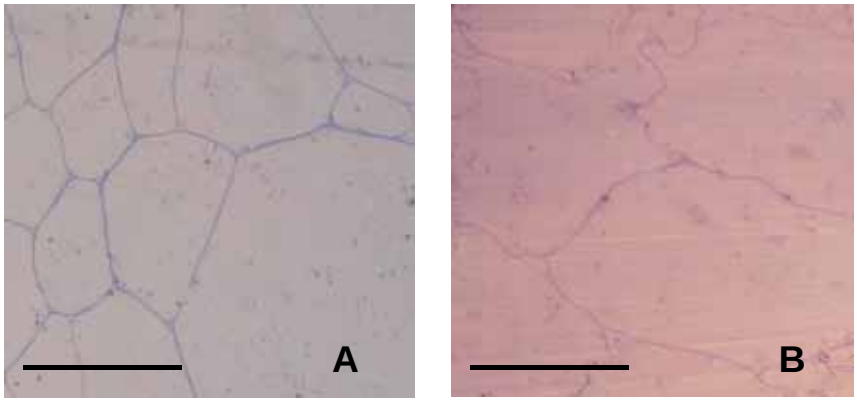

Figure 4 Light microscope sections of outer mesocarp (A) and inner mesocarp (B) of Redglobe berries from block B at harvest. Scale bar: ± 0,1 mm.

3.3.1.3 Cell structure

At harvest, the appearance of the cell walls of the mesocarp of berries from block A and block B did not differ. Cell walls of the inner and outer mesocarp tissues of both blocks were also similar (not shown). No significant differences in the thicknesses of cell walls were observed between the firm (block A) and soft (block B) berries (Table 2). This is in agreement with work done by Nunan *et al*. (1998) on cv. Muscat Gordo Blanco, where cell wall thickness of firm and soft berries did not differ.

Differences were however observed between the cell contents and the plasmalemmas of inner and outer mesocarp tissues. Differences in cell shape between the translucent (gel like) and opaque (firm) mesocarp tissues were ascribed to the differences in intactness of the plasmalemma and tonoplast (Fig. 5). Both membranes seem to be intact in the outer mesocarp cells while none of them could be observed for the inner mesocarp cells. In some of the inner mesocarp cells, the membranes were visible but were further subtracted from the cell wall than for the outer mesocarp (Fig. 6). It seemed that the turgidity of the cells in the mesocarp tissues differed according to the level to which the vacuole was maintained and the tonoplast stayed intact. The better it was maintained, the firmer the tissue.

Table 2 Mean cell wall thickness of different berry tissues of firm (block A) and soft (block B) Redglobe berries at different development stages.

Tissue	Berry type	Thickness of cell walls (nm)[1]				
		Pea size berry	15 mm berry size	Véraison	14 days past véraison	Harvest
Outer mesocarp	Firm	130 ± 35	226 ± 45	136 ± 102	385 ± 93	454 ± 184
	Soft	178 ± 21	246 ±78	266 ± 104	310 ± 134	310 ± 87
Inner mesocarp	Firm	180 ± 43	308 ± 73	410 ± 85	572 ± 303	333 ± 197
	Soft	120 ± 35	267 ± 125	370 ± 81	488 ± 313	401 ± 127

[1.] Mean cell wall thickness was calculated with the standard deviations at p≤0.05.

Regarding the fixation process used in these trials, Diakou & Carde (2001) found that when a glutaraldehyde sodium phosphate buffer solution is used as fixative, the fine exocarp and mesocarp structure of grape berry tissue after vèraison is not well preserved. The plasmalemma and tonoplast of the mesocarp cells are disrupted due to the high osmotic pressure in the cells and cell walls. Whether this is the reason for the observed disintegration of the membranes of the inner mesocarp tissues or not, it can be concluded that the membranes of the outer (firmer) mesocarp tissue was better preserved than that of the inner (softer) mesocarp. There are therefore definite differences in susceptibility for disruption of the plasmalemma and tonoplast between the outer and inner mesocarp cells. The thickness of the opaque outer (firmer) mesocarp will determine berry firmness and its development might be linked to the transport of solutes and

water into the berry as well as between the different mesocarp layers inside the berry.

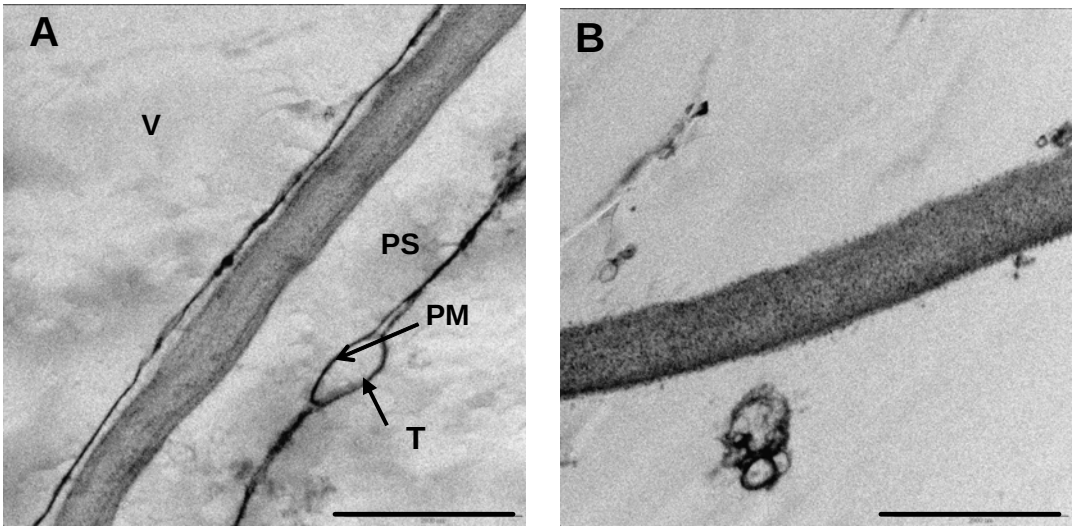

Figure 5 Transmission electron microscope sections of Redglobe berries (block B) at harvest. A = outer mesocarp, B = inner mesocarp, V = vacuole, PS = periplasmic space, PM = plasmalemma, T = tonoplast, Bar = 2 000 nm.

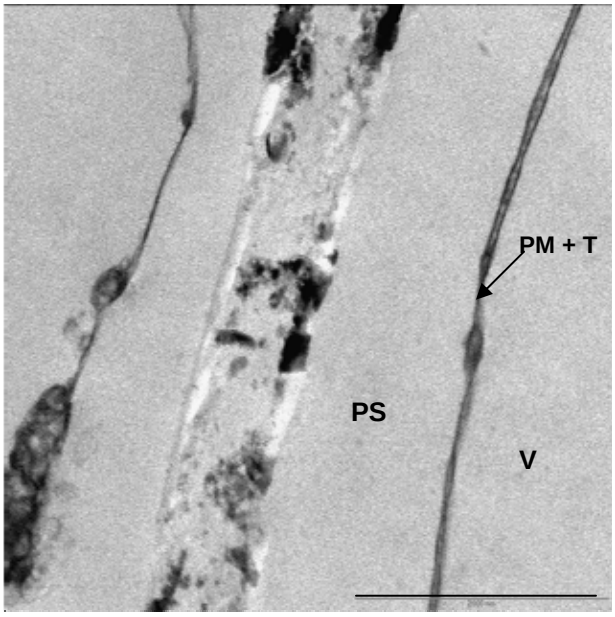

Figure 6 Transmission electron microscope section of the inner mesocarp of Redglobe berries (block B) at harvest. V = vacuole, PS = periplasmic space, PM = plasmalemma, T = tonoplast, Bar = 2 000 nm.

3.3.2 WALTHAM CROSS

3.3.2.1 General appearance

The flesh of the firm and soft berries differed as in the case of Red Globe. The firm clone (clone 8) produces berries with an opaque mesocarp which stretches nearly to the seed cavity (Fig. 7A). The inner part of the mesocarp of the soft berries (clone 13) however had a gel-like appearance (Fig. 7B). Almost the whole mesocarp of the soft berries consisted of this gel-like tissue.

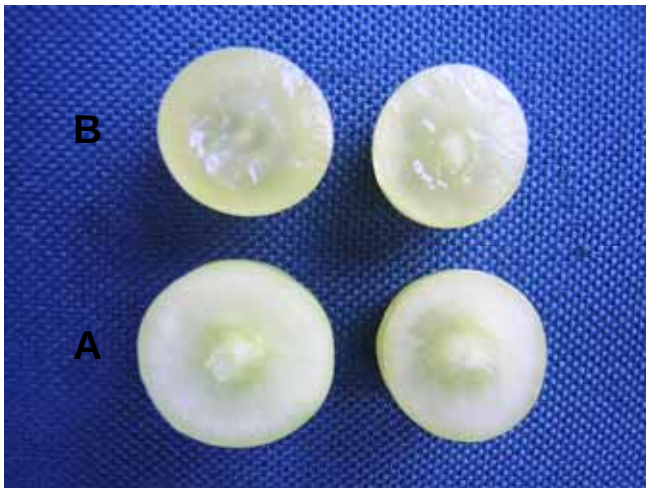

Figure 7 Waltham Cross berries of the firm clone 8 (A) and soft clone 13 (B) that are produced in the same block, De Doorns, sampled at harvest.

3.3.2.2 Cell size and shape

The shape of exocarp cells (epidermal and hypodermal cells) changed with the development of the grape berry (Fig. 8). The cells elongate tangentially from pea size to 15 mm berry size and retain its shape until harvest. This is in accordance with Nii & Coombe (1983) founding's that the cells expand tangentially during phase I of berry development. Similar differences in cell shape were observed as for Redglobe berries, i.e. the cells of the outer mesocarp were more turgid and oval than those of the inner mesocarp (Fig. 8). As said above, the thickness of the outer mesocarp seemed to determine the firmness of the grape berry. In the case of Waltham Cross, where these differences occur naturally between two clones, the cell shape may be determined genetically.

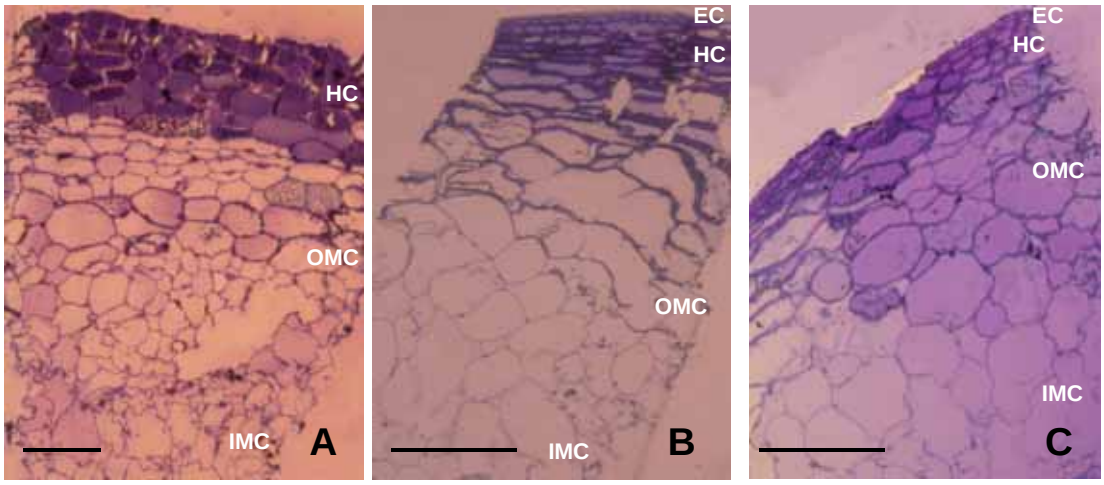

Figure 8 Light microscope sections of skin (exocarp) and flesh (mesocarp) Waltham Cross at pea berry size (A), 15 mm berry size (B) and harvest (C). EC: two epidermal cell layers of the exocarp, HC: 6-8 inner hypodermal cell layers, OMC: Outer mesocarp cells, IMC: Inner mesocarp. Scale bar: ± 0, 5 mm.

The dramatic decrease in cell numbers per mm^2 throughout early berry development indicates that rapid cell expansion occurred from pea berry size to 15 mm berry size, but not from vèraison to harvest (Table 3). These results were obtained for both Waltham Cross clones and were similar to those found for Red Globe. After the termination of cell division in Phase I of berry development, cell volume increase because of sugar accumulation (Possner & Kliewer, 1985; Nakagawa & Nanjo, 1965). The results obtained can therefore not be explained, except that too few replications of photos were observed.

Table 3 Mean[1] cell size of different berry tissues of firm (clone 8) and soft (clone 13) Waltham Cross berries at different development stages.

Tissue	Berry type	Mean number of cells per mm^2 at each phenological stage			
		Pea size berry	15 mm berry size	Véraison	Harvest
Outer mesocarp	Firm	62	25	34	20
	Soft	74	28	24	10
Inner mesocarp	Firm	108	No photos	14	16
	Soft	162	12	12	12

1. Only the means of cell counts from photos are given. The data could not be analysed statistically because, in some instances, only one or two photos of that particular tissue could be taken.

3.3.2.3 Cell structure

It seems that the thickness of cell walls in the inner mesocarp tissue increased from pea berry size to harvest, while it slightly decreased in the outer mesocarp tissue (Table 4). This occurs in both the firm and soft clone. The increase of cell wall thickness in the inner mesocarp tissue may be incorrectly interpreted due to the separation of cell, with enlarged middle lamellae (Bartley & Knee, 1982) that occurs towards ripening (Fig. 9). Bartley & Knee (1982) also found that extensive cell separation occurs during fruit ripening, due to changes in the middle lamella.

Table 4 Mean cell wall thickness of different berry tissues of firm (clone 8) and soft (clone 13) Waltham Cross berries at different development stages.

Tissue	Berry type	Thickness of cell walls (nm)[1]				
		Pea size berry	15 mm berry size	Véraison	14 days past véraison	Harvest
Outer mesocarp	Firm	317 ± 44	378 ± 32	421 ± 85	374 ± 36	236 ± 60
	Soft	798 ± 128	567 ± 45	357 ± 52	726 ± 109	203 ± 54
Inner mesocarp	Firm	309 ± 75	580 ± 54	773 ± 427	817 ± 115	1034 ± 278
	Soft	375 ± 115	578 ± 80	512 ± 148	986 ± 307	894 ± 254

[1.] Mean cell wall thickness was calculated and the standard deviations (P≤ 0.05).

The two Waltham Cross clones showed similar variations in the intactness of the plasmalemma and tonoplast as found for firm and soft Redglobe berries. The membranes were still intact for both clones at pea berry size (Fig. 10 1A & 1B). At harvest the plasmalemma and tonoplast of the cells in the outer mesocarp of the softer clone 13 (Fig. 10 2B) is more subtracted from the cell wall than those of the firmer clone 8 (Fig. 10 2A). These differences are even more pronounced in the inner mesocarp of the berry (Fig. 11), indicating that the turgidity of the vacuoles (Liang *et al*., undated) (or the intactness of the membranes) affects the firmness and appearance of grape berry tissues. It therefore seems that berry firmness is determined by vacuole turgidity. This turgidity can however be affected by possible physiological, water relational or nutritional factors as well as genetic factors (as demonstrated by the differences between the two Waltham Cross clones).

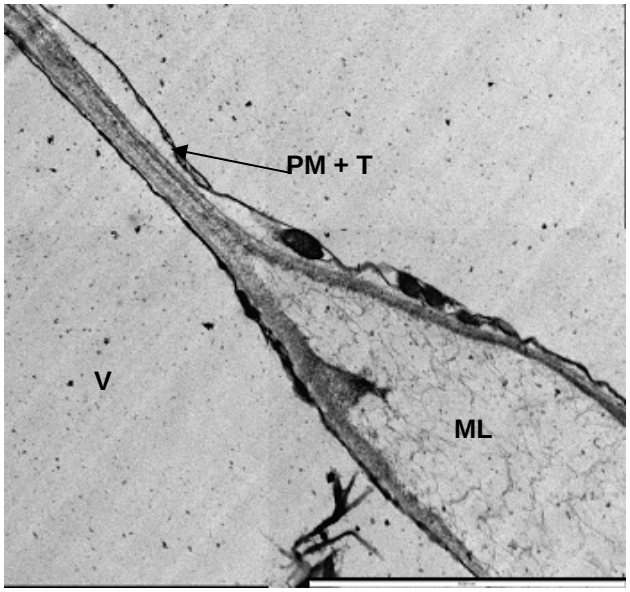

Figure 9 Transmission electron microscope section of inner mesocarp of Waltham Cross (firm clone 8) berries at harvest. ML = middle lamellae, V = vacuole, PM = plasmalemma, T = tonoplast. Bar = 5 000 nm.

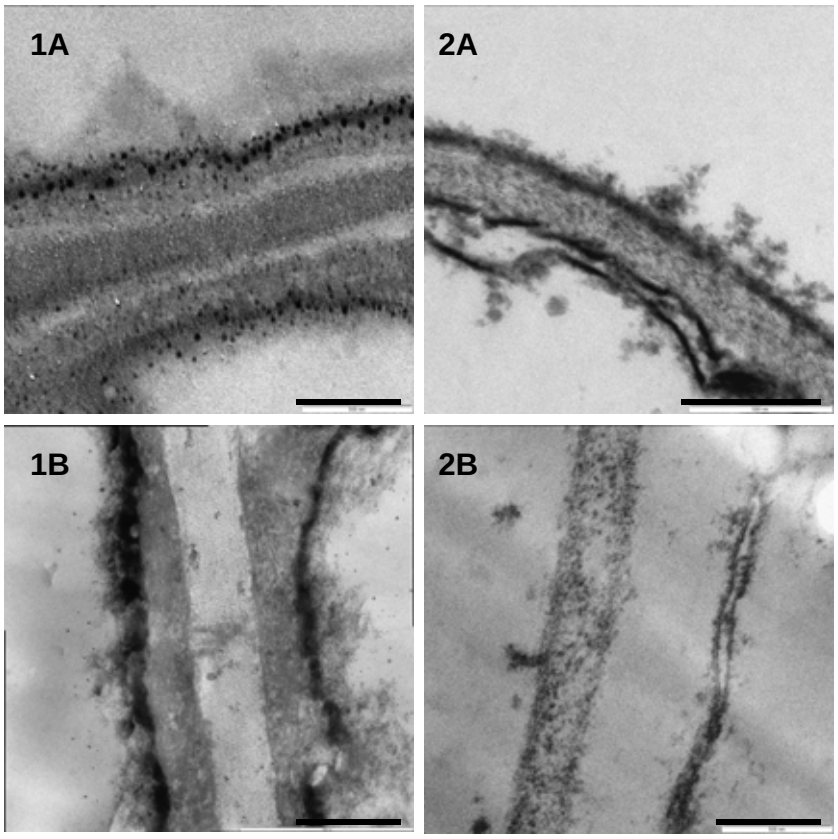

Figure 10 Transmission electron microscope micrographs of sections of the outer mesocarp of Waltham Cross berries of clone 8 (top two photos; i.e. A) and clone 13 (bottom two photos; i.e. B) respectively at pea size berries (number 1) and harvest (number 2). Bar = 500nm.

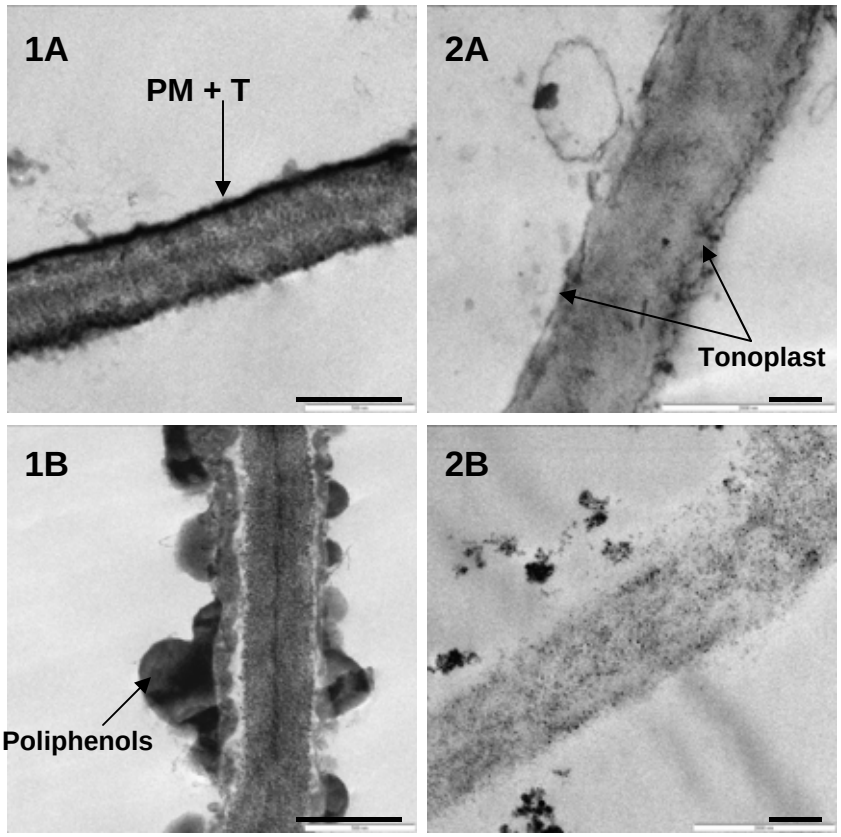

Figure 11 Transmission electron microscope micrographs of sections of the inner mesocarp of Waltham Cross berries of clone 8 with firmer berries (top two photos; i.e. A) and clone 13 with softer berries (bottom two photos; i.e. B) respectively at pea size berries (number 1) and harvest (number 2). Bar = 500nm.

3.4 CONCLUSIONS

Firm berries had opaque coloured flesh while soft berries had gel-like translucent flesh. In extremely firm berries the opaque flesh stretched from the skin to the seeds, including the outer and inner mesocarp. For berries of normal firmness, the opaque flesh was limited to only the outer mesocarp. The mesocarp of soft berries consisted of mostly gel-like flesh while in some cases a very thin layer of opaque flesh could be observed just under the skin.

Cell shape seemed to play an important role in the firmness of berries. The cells in the outer mesocarp (white opaque coloured flesh) are much more turgid and oval than those in the inner mesocarp (translucent gel-like flesh). The thickness of the outer mesocarp, consisting of the turgid oval cells, therefore determines the firmness of berries, i.e. the thicker the outer mesocarp, the firmer the berry. More research is needed on how to increase the thickness of the firmer outer mesocarp by ways of managerial practices or sprays.

The cell wall thickness of soft and firm berries did not differ. The appearance of cell walls in soft and firm berries, as well as the outer and inner mesocarp was similar. There was however a difference in the plasmalemma between the cells of the outer and inner mesocarp. Difference in cell shape between the gel-like (inner) and opaque (outer) mesocarp tissues can probably be linked to the intactness of the plasmalemma and the tonoplast. Both the membranes were intact in the outer mesocarp cells while it was subtracted from the cell wall, or not even visible, in the inner mesocarp cells. The fixation method used might have caused disintegration of membranes after vèraison. More research on the fixation method used in this trial to determine its suitability to use on berries after vèraison, is required. It can however be remarked that if potential disintegration of membranes after vèraison might be a shortcoming in this method, the inner, more gel-like mesocarp tissues was more susceptible to this kind of disintegration.

From this study it can therefore be concluded that (i) improved firmness of table grape berries will be obtained if the thickness of the outer, opaque coloured mesocarp could be increased; (ii) improved berry firmness will be obtained if vacuole turgidity could be increased. Gibberrelic acid, synthetic cytokinin and calcium applications are some of the most common practices applied by South African table grape producers to try to improve berry firmness. The effect of these practises on berry firmness is discussed in the next chapter.

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Chapter 4

RESEARCH RESULTS

Utilisation of gibberellic acid (GA_3), CPPU and bunch applied calcium sprays to increase berry firmness of table grapes: (I) Redglobe.

UTILISATION OF GIBBERELLIC ACID (GA_3), CPPU AND BUNCH APPLIED CALCIUM SPRAYS TO INCREASE BERRY FIRMNESS OF TABLE GRAPES: (I) REDGLOBE

4.1 INTRODUCTION

Table grapes are an aesthetic product, therefore berry appearance, taste and texture should meet the demands of consumers. Firm grapes are preferred. To meet the high standards of the export market producers must be able to exert some control over the development of berry firmness in order to obtain the highest price. Little research has been done to develop managerial practices that producers can apply to improve or ensure berry firmness. Calcium applications to the bunches and calcium fertilisation, as well as gibberellic acid (GA_3) and synthetic cytokinin (CPPU) applications, are performed by South African table grape producers to enhance berry firmness. However, these practices are not supported by scientific research. It can therefore not be prescribed for this purpose before its efficacy to increase berry firmness has been tested and validated for commercial production purposes.

Gibberellic acid enhances the division and expansion of pericarp cells (Sachs & Weaver, 1968) and has been reported to be most effective during the first growth phase of the grape berry (Iwahori *et al.*, 1968). The enlargement of cells results in a decrease of cell density (Ben-Arie *et al.*, 1997). Synthetic cytokinins (CPPU), on the other hand, increased cell density because it promotes cell division (Ben-Arie *et al.*, 1997). Grapes treated with GA_3 at vèraison were found to had increased firmness (Singh *et al.,* 1978; Ben-Arie *et al*., 1997). The firmness of berries was also increased by application of CPPU (Ebisuda & Dokoozlian, 2003). Ben-Arie *et al.* (1997) proposed that greater firmness obtained for berries treated with CPPU was probably due to its delaying effect on fruit maturation. Coombe & Hale (1973) ascribed the increased firmness to modifications in the anatomy of the berry such as increased skin thickness (Ben-Arie *et al*., 1997).

Calcium plays a major role in the structure of the cell wall (Grant *et al*., 1973). It acts as a binding agent in the middle lamellae (Dey & Brinson, 1984) and the ions increase the cohesion of cell walls (Demarty *et al.,* 1984). Low Ca^{2+} concentration in the cell wall increases its elasticity and the risk to rupture while

high concentration makes it more rigid and less flexible (Hepler, 2005). Most research relating calcium and fruit firmness was done on apples. Sams & Conway (1984) found that fruit softening of apples treated with calcium was delayed because Ca^{2+} slowed down the degradation of cell wall polymers. These apples also retained their firmness and cell-to-cell contact, which plays an important role in the firmness of fruit. The untreated apples softened while their cell walls swelled and separated. The cell walls of Ca^{2+} treated apples were reported to be well preserved with very little degradation (Poovaiah *et al.*, 1988). Indications whether calcium applications have the same potential effect on table grapes, could not be found in literature. The potential commercial use of GA_3, CPPU and bunch applied calcium sprays to increase or ensure berry firmness of Redglobe was therefore investigated in this trail.

4.2 MATERIALS AND METHODS

4.2.1 EXPERIMENTAL VINEYARD

The experiment was conducted on a twelve year old *Vitis vinefera* L. cv. Redglobe vineyard. These vines were grafted on the rootstock Ramsey and trained on a tirole trellis system. The vineyard is situated on the farm De Hoop in the Hex River Valley, De Doorns. It has a history of consistently producing soft berries. The spacing between the vines is 3 m x 2 m. Micro-irrigation was applied weekly.

4.2.2 EXPERIMENTAL DESIGN AND TREATMENTS

The trial was laid out in a randomized block design with four treatments (Table 1) that were replicated five times. The abbreviations used in Table 1 for the treatments will be used in the rest of this chapter. It is standard practice to apply a combination of GA_3 and CPPU to improve berry size. The treatments (including wetting agent) were applied using a 20L knapsack sprayer just after sunrise. All the treatments were bunch directed. Each experimental unit consisted of four vines, of which only the central two was used for experimental measurements and for sampling.

Table 1 Description of the treatments applied.

Treatment abbreviation	Experimental treatments Redglobe
Control	No plant bioregulators applied.
GA_3	20 mg/L GA_3 (ProGibb[1]) applied at 10 mm mean berry size.
CPPU	20 mg/L GA_3 (ProGibb[1]) plus 3 mg/L CPPU (Sitofex[2]) applied at 10 mm mean berry size.
Ca	Mixture of 8L/ha Stopit[3] plus 5 L/ha Caltrac[4] applied directly to bunches every two weeks from berry set to vèraison (Total of three applications).

1. ProGibb = 400g/kg gibberellic acid
2. Sitofex 10EC = 10g/L forchlorfenuron
3. Stopit = $CaCl_2$ at 160 g Ca^{2+}/L.
4. Caltrac = $CaNO_3$ at 400 g Ca^{2+}/L

4.2.3 BERRY SAMPLING FOR MICROSCOPE STUDIES

Berry samples (four berries each) were randomly taken at five developmental stages (pea size, 15 mm diameter, vèraison, 14 days past vèraison and at harvest) from each experimental unit for transmission electron microscope (TEM) and light microscope (LM) studies. The berries were always sampled from the middle part of the bunch. From each berry one radial section was made for electron and light microscope studies.

4.2.4 BERRY MEASUREMENTS

Berry samples (50 berries), randomly taken at the abovementioned stages, were used to determine berry mass (g), berry volume (cm^3), total soluble solids (˚Brix) and berry firmness (g/cm^2). Berry volume of ten berries was determined by filling a 1 000 ml measuring cylinder with enough water to cover all the berries and take a reading. The ten berries were then added to the cylinder and a second reading taken. The first reading was subtracted from the second one to determine the volume of ten berries. Total soluble solids (°Brix) were determined by using a digital refractometer (Atago, Tokyo). Berry firmness of ten randomly selected berries was measured with an ISICUDISI grape and soft fruit compression tester (Stellenbosch University, Stellenbosch) (Avenant & Avenant, 2006).

One carton per experimental unit were packed at optimal ripeness (15 ˚B) and stored for three weeks at 0˚C followed by one week at 10˚C as a shelf life period. After cold storage (three weeks at 0°C and one week at 0°C) the grapes were evaluated for loose berries, rot, berry split and rachis browning. The weight of the affected berries was determined and expressed as a percentage of the

total weight of the carton. The eating quality of the grapes was evaluated by a panel 20 of tasters that represented regular consumers. Training was done prior to the tasting. The criteria, rated on a five point scale, for the tasting were: General impression (attractive to repulsive), colour (typical colour to unacceptable blush or colour), taste (delicious to off flavours or tasteless), firmness (crispy to unacceptably soft) and skin (soft and not observable to tough). All these criteria were evaluated to the different frequencies of classes occurred with the highest class scoring 5 and the lowest class scoring 1.

From each carton, 50 berries were also sampled after four weeks' storage to determine berry firmness after cold storage (four weeks after harvest).

4.2.5 LIGHT AND ELECTRON MICROSCOPY

The procedure followed for the preparation of TEM and LM samples were adapted from that described by Diakou & Carde (2001). Radial sections (1 mm^2) of the fresh berries were made in the vineyard with a razor blade and immediately fixed and kept overnight (16 hours) in 2.5 % glutaraldehyde (in 0.1 M phosphate buffer, pH 7.4). Sections included the epidermis and the mesocarp. After fixation they were then washed two times (5 minutes each wash) in 0.1 M phosphate buffer, pH 7.4 and stained with a 1 % tannic acid (in 0.1 M phosphate buffer, pH 7.4) solution for 30 minutes. The samples were then washed twice (5 minutes each wash) in 0.1 M phosphate buffer, pH 7.4, after which it was placed in 1 % osmium tetroxide (in 0.1 M phosphate buffer, pH 7.4) for an hour. Again it was washed two times (5 minutes each wash) in 0.1 M phosphate buffer, pH 7.4, after which it was rinsed twice (5 minutes each wash) in distilled water. The samples then underwent a dehydration process using ethanol (1 x 10 minutes in 50 % ethanol; 1 x 10 minutes in 70 % ethanol; 1 x 10 minutes in 90 % ethanol; 1 x 10 minutes in 95 % ethanol; 2 x 10 minutes in 100 % ethanol (EM grade) and 2 x 10 minutes in acetone) and subjected to a process of infiltration and embedding: The samples were placed overnight in a 50/50 solution of acetone/Spurr's resin, followed by 75:25 resin:acetone for 8 hours and then 100 % resin overnight. The samples were then put in fresh 100 % resin in the morning for two hours. They were then orientated into a mould and resin added. The mould with the samples was then placed in a 60˚C oven for 24 hours.

The mould contained a transverse section which included the exocarp and mesocarp. From each mould two sections were made to represent the outer mesocarp (directly below the hypodermal cell layers) and the inner mesocarp (directly above the endocarp) respectively. For the light microscope studies, 1 µm

sections were cut with a Reichert ultracut S ultramicrotome. These sections were stained with 1 % (w/v) toluidine blue and then washed under water. For the TEM studies, 120 nm ultrathin sections were obtained using the same ultamicrotome. These sections were collected on 200 mesh copper grids and stained with 2 % uranyl acetate and Reynolds lead citrate. Samples were studied with a Leo Omega 912 transmission electron microscope. The electronmicroscope photos were taken with a Proscan CCD camera mounted on the LEO 912 TEM. The images were viewed and analysed using the EasiVision Pro software which was developed by Soft Imaging System GmbH. Mean cell wall thickness could be measured using this software and was determined by measuring two nearby cells' cell walls including the middle lamellae. This measurement was divided in two to determine the mean thickness of one cell wall. Cell size was determined by counting the amount of cells in a 1 mm^2 area under a light microscope.

4.2.6 STATISTICAL ANALYSIS

The analysis of variance was performed using SAS version 8.2 (SAS Institute Inc., 1999). Non-normality was tested using the Shapiro-Wilk test (Shapiro & Wilk, 1965). Student's t-test for least Significant Differences (LSD) were calculated at $p \leq 0.05$ and the standard deviations were used to calculate the standard errors at a 95% confidence level.

The post harvest life of the grapes were calculated by expressing the weight of berries affected by rot, loose berries, berry split or rachis browning as a percentage of the total weight of a carton.

The frequencies of observations made by the tasting panel were subjected to a general linear model (GLM) technique with a logistic link function. The maximum likelihood estimators (X-beta's) were calculated on an underlying scale (McCullagh & Nelder, 1989). These estimators, placed on an interval scale, were subjected to analysis of variance as mentioned above. The cut-off points for the respective classes were given as intercepts.

4.3 RESULTS AND DISCUSSIONS

4.3.1 THE EFFECT OF PLANT BIOREGULATORS (PBR'S) AND CALCIUM SPRAYS ON BERRY RIPENING

All treatments showed a delay in sugar accumulation. CPPU had a significant effect on the accumulation of total soluble solids (TSS) (Fig. 1). This is in accordance with research done by Reynolds *et al*. (1992) where CPPU treated

fruit had a lower degree Brix (°Brix) and pH as well as lighter skin colour. Grapes from the CPPU treatment were not harvested (10 days later) until it reached 14.5 ˚Brix TSS. This was done for experimental purposes so that berries of similar ripeness could be compared regarding post-harvest keeping and eating quality.

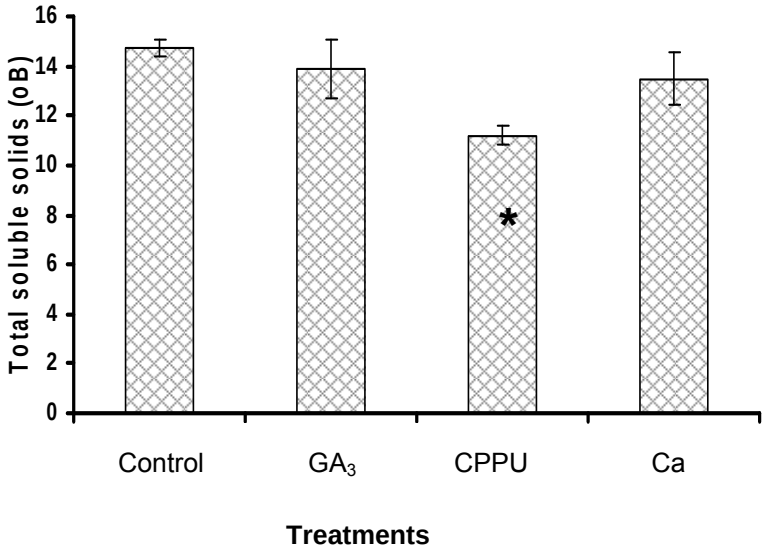

Figure 1 Effect of plant bioregulators and bunch applied Ca on the total soluble solids accumulated at the time of harvest of the control treatment of cultivar Redglobe (14.5^{o}B). Error bars indicate 95% confidence intervals. *Significant compared to control.

The calcium and CPPU treatments delayed colour development (Fig. 2). The CPPU treatment eventually developed an acceptable colour level as the TSS increased to 14.5 ˚Brix while colour of the calcium treatment remained poor. Reynolds *et al*. (1992) found a decrease in colour development of red and black table grape cultivars when it was treated with CPPU, this can be linked to the delay in fruit ripening brought on by CPPU. The calcium treatment may have an affect on the pH of the skin which may result in a different tone of colour (P. Raath: Personal communication, 2007). It therefore seems that Ca^{2+} applications to red and black grapes should be done with caution. The extent of this colour effect requires further investigation.

4.3.2 THE EFFECT OF PBR'S AND CALCIUM SPRAYS ON BERRY GROWTH

The berry expansion rate was the fastest at vèraison (70 days after anthesis (DAA)) and berries continued to expand until harvest (Fig. 3). The growth pattern is different from the generally accepted double sigmoidal growth pattern where

the berry growth rate reduces shortly before vèraison. The growth pattern was not double sigmoidal, probably because the sampling dates were too far apart as the lag phase (phase II) of berry development can be as short as five days.

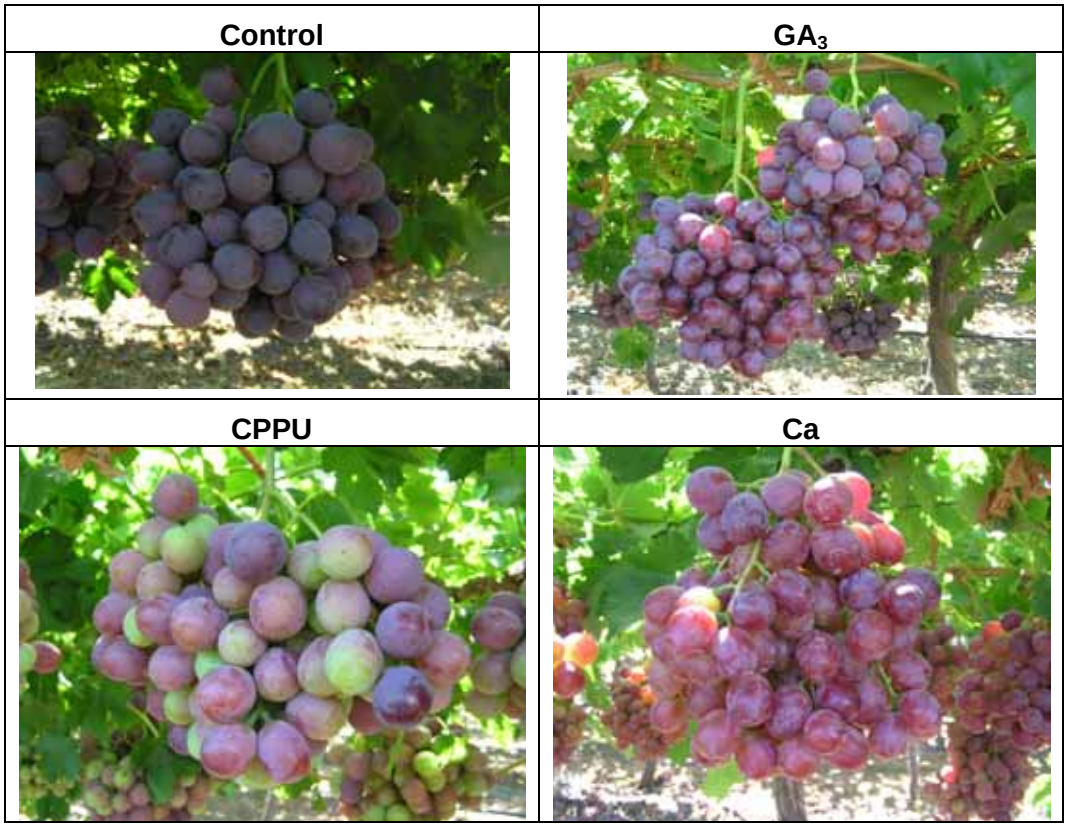

Figure 2 The effect of plant bioregulators and bunch applied Ca on berry colour development. The photos of bunches in the different experimental plots were taken on the same day – when the control treatment was ready to be harvested at *circa* 14.5°Brix.

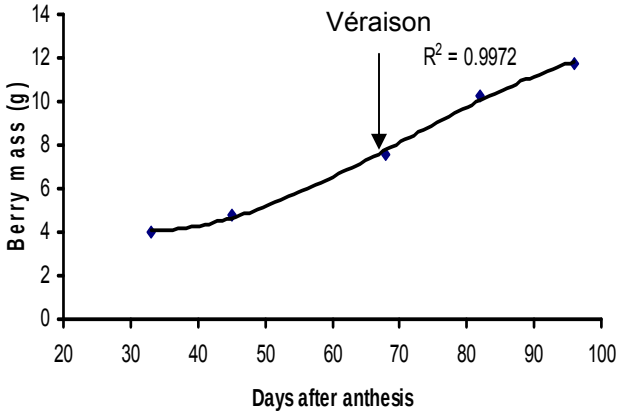

Figure 3 Pattern of berry growth from 15 mm berry size up to harvest for Redglobe (N=20 plots x 10 berries) in the Hexriver valley.

A very strong correlation between berry mass and berry volume was obtained (R^2 = 0.9911). Berry size is, however, significantly affected during the whole period from 45 DAA up to harvest. This is illustrated by the fact that both PBR's, with CPPU significantly the biggest, had a significant effect on final berry size. This was expected as Retamales *et al*. (1995) also found the same results in their research. Calcium treatments also have a significant positive effect on berry size (Fig. 4). In the case of PBR's, the berry enlargement effect was probably early in development, because this is the time when they affect berry size the most (Iwahori *et al*., 1968). Alcaraz-López *et al*. (2005) found an increase in berry size when berries were treated with calcium in combination with titanium. Calcium displacement or release may be required to make cellular growth possible (Cleland & Rayle, 1977). When new cell walls form, it needs new wall material to be incorporated into the wall, this includes calcium (Hall *et al*., 1974). It could be possible that if more calcium is available, more new cell walls could be formed which may lead to an increase in berry size.

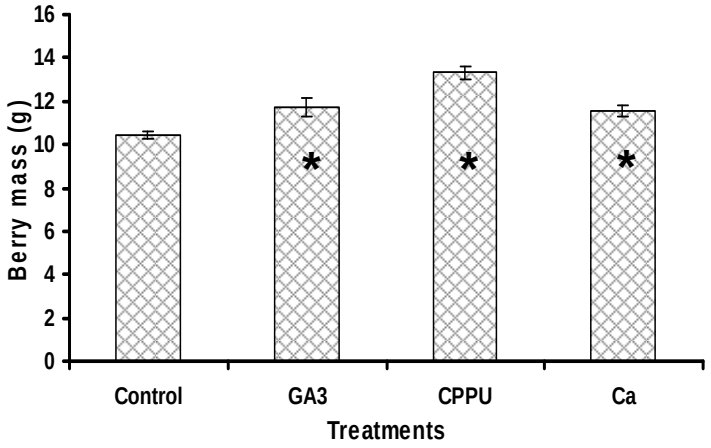

Figure 4 Effect of plant bioregulators and bunch applied Ca on berry size of cultivar Redglobe at harvest. Error bars indicate 95% confidence intervals. ***** Significant compared to control.

Berry growth, due to cell enlargement, is expressed as the reduction of cells per mm^2 over time. Except for the CPPU treatment that showed the largest cell size at 15 mm berry size in the inner mesocarp of the berry (Fig. 5), cell sizes did not show a consistent pattern between the various treatments during the rest of the berry developmental stages. This could be due to a lack of sufficient numbers of samples. The large cell size observed for the Ca-treatments at 15 mm berry size in the outer mesocarp, is probably due to experimental error since Ca had no further effect on cell size during the rest of berry development. Growth between 15 mm berry size and véraison can be ascribed to significant cell

growth/enlargement. Cell enlargement during this stage (Phase I) is mainly due to the accumulation of solutes (Possner & Kliewer, 1985) and the import of water (Keller, undated). The amount of cells per mm^2, however, did not decrease significantly from vèraison till harvest; therefore berry growth was not reflected in dramatic cell expansion during this period. Berries grow fast at the beginning of ripening (véraison) and slow down towards maturity (Ollat *et al*., 2002). The lack of cell expansion observed could have been a sampling error – the samples could have been taken a few days after véraison which could have excluded the fast growing period that occurs shortly after véraison.

The increased berry size obtained by CPPU was therefore not reflected in a significant difference in the amount of cells per mm^2 at harvest in either the inner nor outer mesocarp (Fig. 6). Although not significant, the GA_3 and CPPU treatments however showed the largest cells at harvest in the outer mesocarp, pointing to the effect (significant for CPPU) these plant bioregulators had on cell expansion of the outer mesocarp tissues. These treatments also had the firmest berries (discussed below).

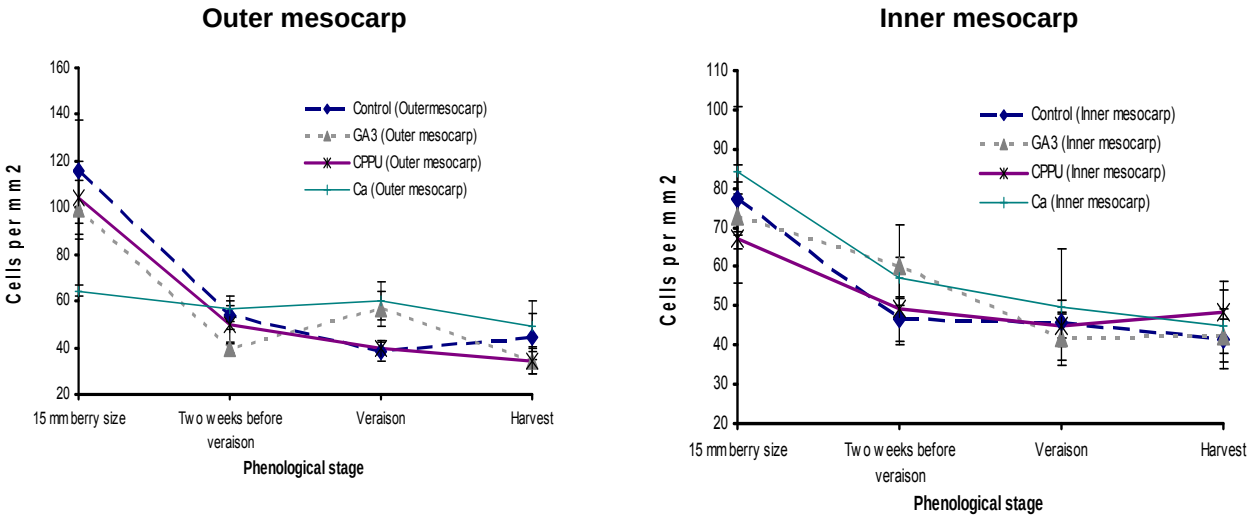

Figure 5 Cell growths, expressed as reduction in cells per mm^2, of Redglobe berries from the Hexriver valley, 2006/07 season, as affected by plant bioregulators and bunch applied Ca. Error bars indicate 95% confidence intervals.

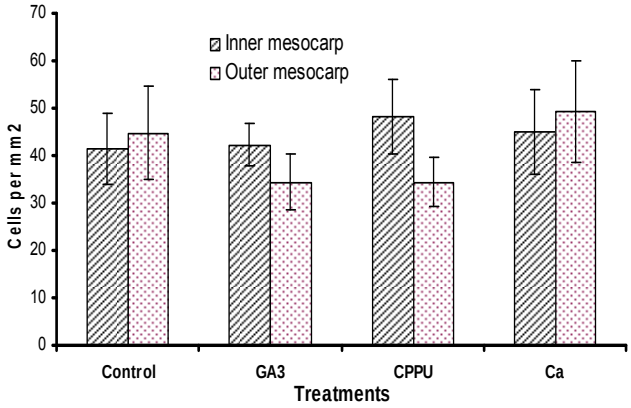

Figure 6 Effect of plant bioregulators and bunch applied Ca on the amount of cells per mm^2 of different tissues of Redglobe berries. Error bars indicate 95% confidence intervals.

4.3.3 EFFECT OF PBR'S AND CALCIUM SPRAYS ON BERRY FIRMNESS

Significant increases in berry firmness were obtained with GA_3 applications at harvest, but not with bunch applied Ca (Fig. 7) or a combination of CPPU and GA_3. In accordance with this, Singh *et al.* (1978) and Ben-Arie *et al*. (1997) also found increased firmness with berries treated with GA_3 while contrary to this; Ebisuda & Dokoozlian (2003) found increased berry firmness with the use of CPPU. One would expect the CPPU treatment also to be firmer than the control, since the amount of GA_3 applied is the same. The large variance obtained for the CPPU treatment explains the lack of significant difference in firmness. The CPPU compared to the other treatments did not show a significant decrease of berry firmness during cold storage (Fig. 7), but remained significantly firmer than the control after cold storage. CPPU increase the thickness of the skin (Ben-Arie et al., 1997), this may result in less water loss during cold storage which may have an effect on berry firmness. The calcium treatment showed the most decrease in berry firmness during cold storage which indicated that calcium may not play a role in maintaining berry firmness during cold storage.

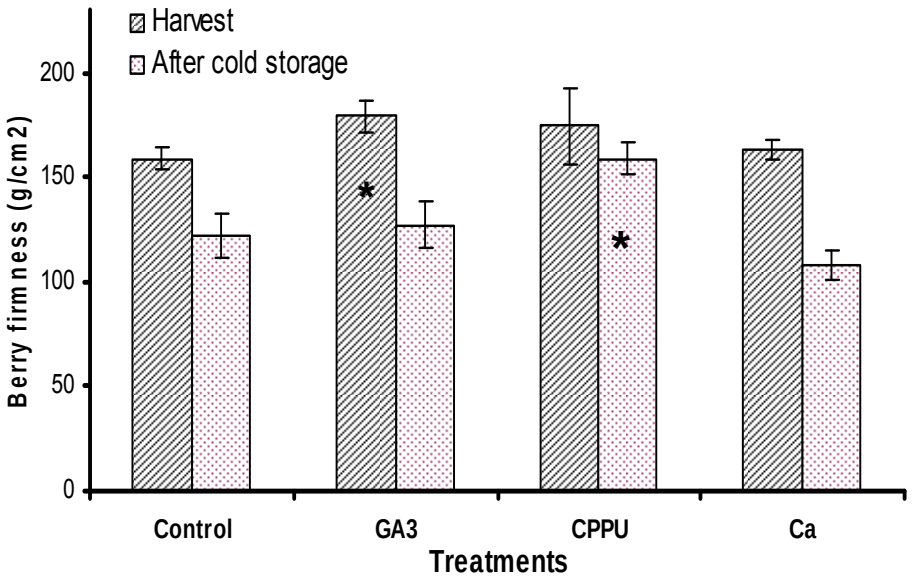

Figure 7 Effect of plant bioregulators and bunch applied Ca on the firmness of Redglobe. Error bars indicate 95% confidence intervals. ***** Significant compared to control.

Fig. 8 indicates berry firmness during berry development of Redglobe of all treatments. Berry firmness increased significantly from vèraison to harvest while it decreased significantly during cold storage. The increase in firmness from véraison to harvest is ascribed to increased cell turgidity as sugar and potassium (K^+) are downloaded in the berries, stimulating water uptake (Läuchli & Pflüger, 1978), driven by osmotic pressure, of the berries (Saxton, 2002). Since water loss occurs during cold storage (M. Huysamer: Personal communication, 2005) the decrease in berry firmness during cold storage is ascribed to water loss, resulting in less turgid vacuoles/cells.

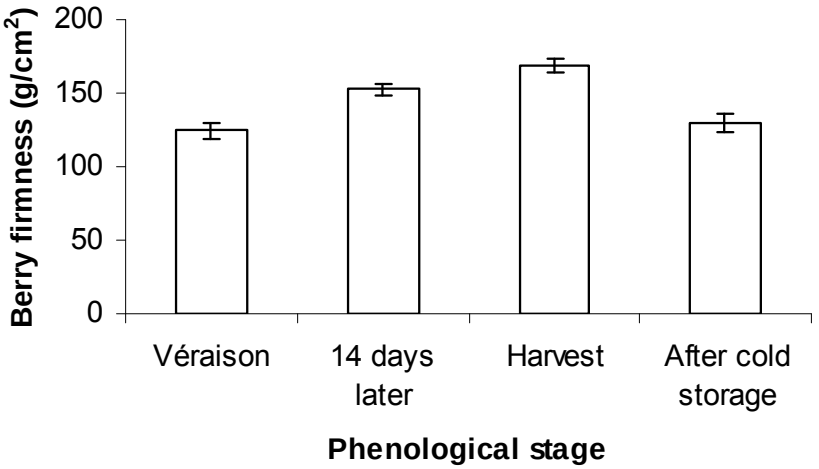

Figure 8 Changes in firmness of Redglobe berries throughout development and cold storage. (N = 10 berries x 20 experimental plots). Error bars indicate 95% confidence intervals.

Cell wall thickness of mesocarp cells were investigated to find an additional explanation for the differences in berry firmness related to PBR's. Cell walls of the outer mesocarp were found to be significantly thicker than the inner mesocarp cells (Fig. 9). The thicker cell walls in the outer mesocarp may contribute to the formation of the firm opaque coloured flesh (as discussed in chapter 3) present in the firmer berries. The PBR treatments showed no differences in the cell wall thicknesses of the outer mesocarp while the calcium treatments' cell walls were significantly thinner than those of the control. The cell wall thickness of the inner mesocarp of the berries treated with Ca was significantly thicker than the control (Fig. 9), while the outer mescarp was thinner. Calcium contributes to the rigidity of the cell wall (Dey & Brinson, 1984), this may contribute to the formation of thicker cell walls. Why cell wall thickness of the outer mesocarp of the Ca treatment is less than the control (contrary to expectations) is unclear. It seems that a correlation may exist between cell wall thickness in the outer mesocarp and berry firmness after cold storage.

At harvest, the cell walls of all treatments in the inner (Fig. 10) and outer (Fig. 11) mesocarp seemed to be well preserved – the cell walls was not separated and the tonoplast and plasmalemma was intact and close to the cell wall. The plasmalemma and tonoplast of the CPPU and Ca treatments seemed to be the best preserved (most intact) for the inner mesocarp (Fig. 10) The plasmalemma of the control in the outer mesocarp tissue (Fig. 11A) was least preserved and most subtracted from the cell wall. As discussed in chapter 3, the intactness of

the plasmalemma can be an indication of the firmness of the tissue. Firmer tissue seems to have cells where the plasmalemma, and especially the tonoplast, is well intact and the vacuoles turgid. This is in accordance with previous work where it was found that higher turgor pressure in the vacuoles of cells result in firmer berries (Liang *et al*., undated).

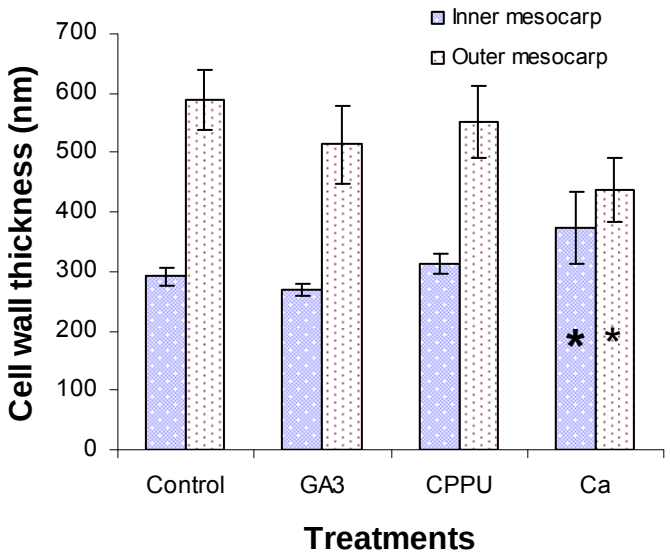

Figure 9 Effect of plant bioregulators and bunch applied Ca on the cell wall thickness of different tissues of Redglobe berries at harvest. Error bars indicate 95% confidence intervals. * Significant compared to control.

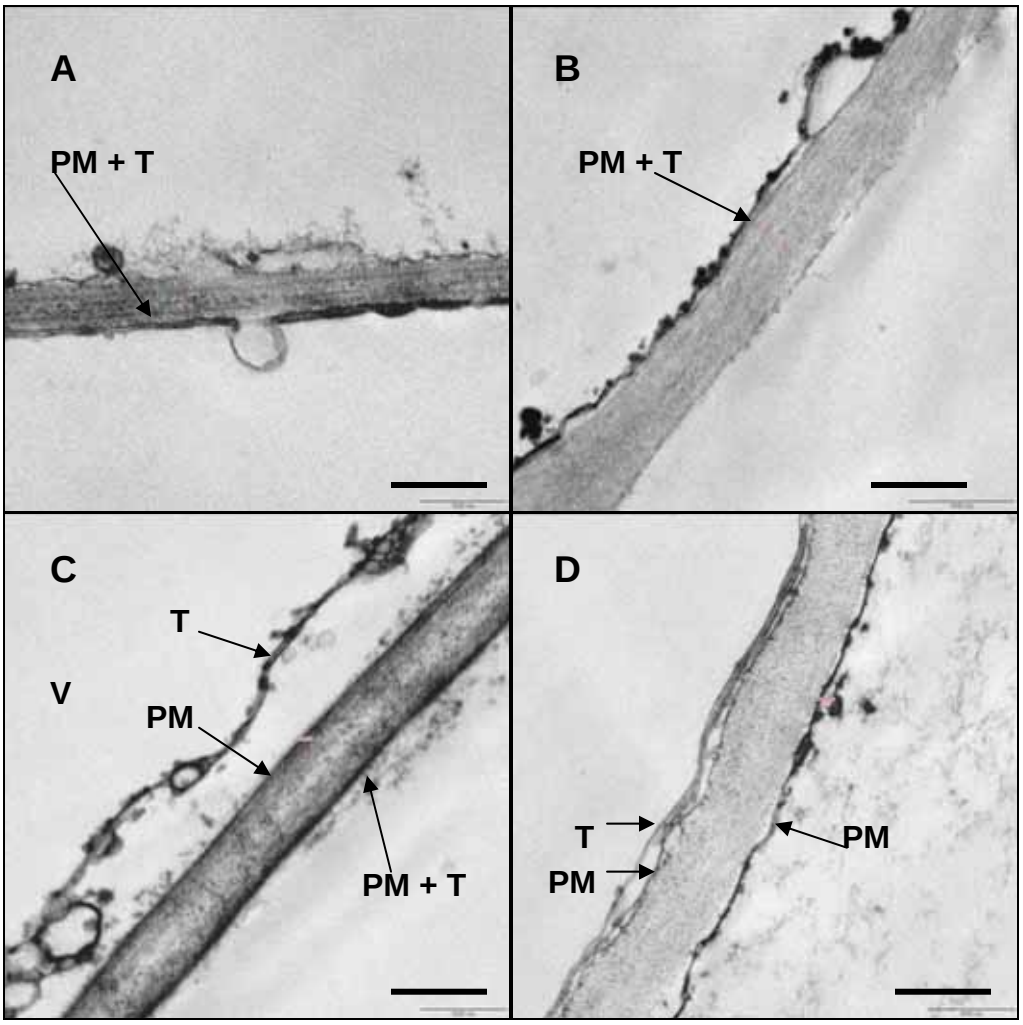

Figure 10 Transmission electron microscope section of the inner mesocarp of Redglobe berries at harvest. A) Control, B) GA_3, C) CPPU and D) Ca. V = Vacuole, PM = Plasmalemma, T = Tonoplast. Scale bar = 1000 nm.

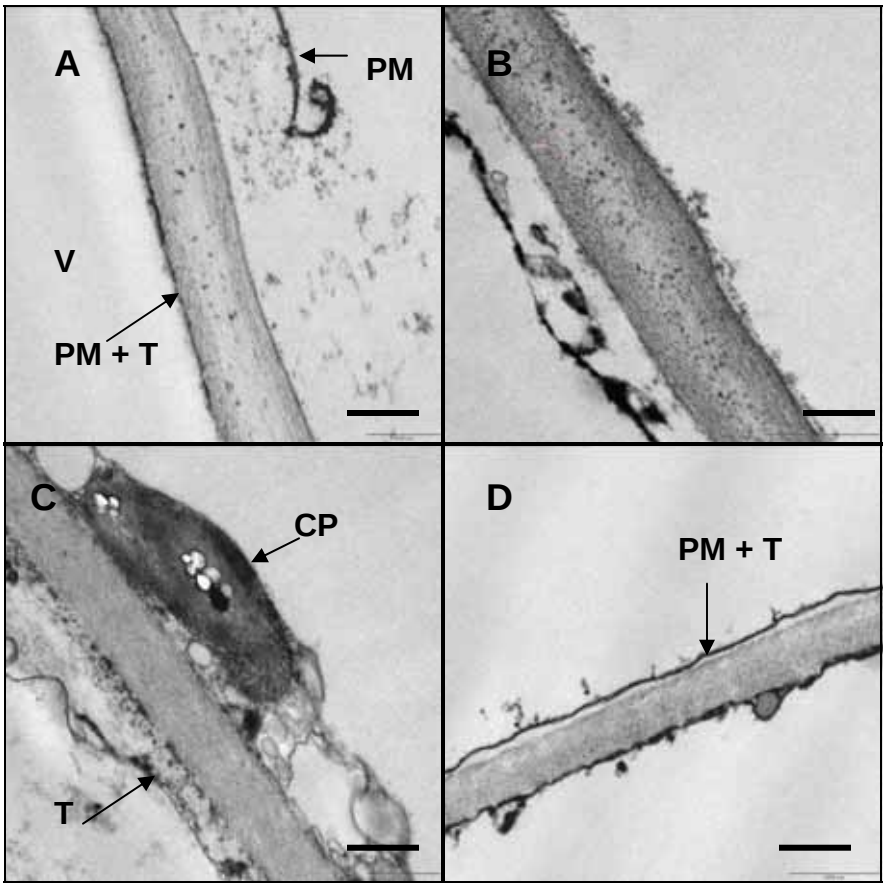

Figure 11 Transmission electron microscope section of the outer mesocarp of Redglobe berries at harvest. A) Control, B) GA_3, C) CPPU and D) Ca-sprays. V = Vacuole, PM = Plasmalemma, CP = Chloroplast, T= Tonoplast. Scale bar = 1000 nm.

4.3.4 EFFECT OF PBR'S AND CALCIUM SPRAYS ON BERRY QUALITY

The Ca and GA_3 treatments induced significantly more *Botrytis* rot than the control and CPPU treatments after cold storage (Table 2). The CPPU treatment did not reduce loose berries or *Botrytis* rot compared to the control, although it must be recognised that its occurrence was almost negligible. The GA_3 application seemed to have increased loose berries compared to CPPU, but not significantly more than for the control. None of the treatments induced cracked berries. These results is contrary to results found by Pool *et al*. (1972) that grapes treated with an optimal concentration of GA_3 results in berries with better storage life. A higher concentration of ProGibb, than prescribed by the manufacturer, in order to try to enhance results, was used in this trial. This may be the reason why this GA_3 treatment results in grapes of poorer quality.

Table 2 Effect of plant bioregulators and bunch applied Ca on berry quality after cold storage of Redglobe.

Treatment	Quality parameters		
	Botrytis rot (%)	Loose berries (%)	Cracked berries (%)
Control	1.9 b	0.16 ab	0.97
GA_3	7.9 a	0.45 a	1.75
CPPU	1.0 b	0.02 b	3.03
Ca	7.0 a	0.17 ab	2.45
P≤ 0.05	LSD* = 4.6	LSD = 0.42	NS**

* LSD = Least significant difference, means within columns followed by the same letter do not differ significantly.
** NS = Not significant at a confidence level of $p \leq 0.05$.

Table 3 shows that the general impression of the grapes after cold storage, as observed by the tasting panel, was not independent of the treatments (Chi-Square ≤ 0.0001). The general impression of the grapes was thus dependent on the treatment. It was classified in five different classes. Most of the tasting panel (57.3 %) classified the general impression of the control fruit as class 5, meaning that the control treatment made the best general impression after cold storage. There was little difference between the classified classes in the case of the GA_3 treatment, while 39.1 % and 40.0 % of the tasters classified the CPPU and Ca treatment respectively as class 3. All the treatments therefore had a negative effect on the general impression of the grapes.

Table 3 Effect of plant bioregulators and bunch applied Ca on general impression after cold storage of Redglobe. (Class 1 indicates the lowest awarded score and class 5 the highest), as observed by an independent tasting panel of 20 people. Observations are not independent of the treatments (Chi-square ≤ 0.0001).

Treatment	Number of tasters that preferred a specific class (%)				
	Class 1	Class 2	Class 3	Class 4	Class 5
Control	0	0	15.5	27.3	57.3
GA_3	6.4	11.8	24.6	35.5	21.8
CPPU	1.8	4.6	39.1	31.8	22.7
Ca	10.0	26.4	40.0	17.3	6.4

The highest percentage of tasters classified the colour of the control grapes as class 5 (the most ideal colour) while there was little difference between the

percentages of tasters that classified the grapes of the GA_3 treatment (Table 4). For both the CPPU and Ca-treatments, 42.7 % and 54.6 % of the tasters classified the grapes in class 3 respectively. All the treatments had a negative effect on the colour of the grapes, indicated by the highest percentage of tasters placing the control in Class 5.

Table 4 Effect of plant bioregulators and bunch applied Ca on the colour after cold storage of Redglobe. (Class 1 indicates the lowest awarded score and class 5 the highest), as observed by an independent tasting panel of 20 people. Observations is not independent of the treatments (Chi-square ≤ 0.0001).

Treatment	Number of tasters that preferred a specific class (%)				
	Class 1	Class 2	Class 3	Class 4	Class 5
Control	0.0	0.9	6.4	24.6	68.2
GA_3	9.1	11.8	24.6	28.2	26.4
CPPU	0.9	4.6	42.7	33.6	18.2
Ca	10.0	23.6	54.6	7.3	4.6

A strong correlation (R^2 = 0.9587) was found between the general impression and the colour of the grapes (Fig. 12). This is an indication that the colour of table grapes is a very important factor determining the consumers' impression of it. In Fig. 12 the negative effect of the treatments on the observed berry colour is also clearly illustrated, with the control treatment considered to have the best colour (all replications > 1,5) and the Ca treatment the worst (all replications < 0).

The highest percentage of tasters classified the GA_3, CPPU and Ca-treatments as class 3 (Table 5). A smaller percentage of tasters classified the control in class 3 than the other treatments. It therefore seems that all the treatments had a negative effect on the observed taste of the grapes.

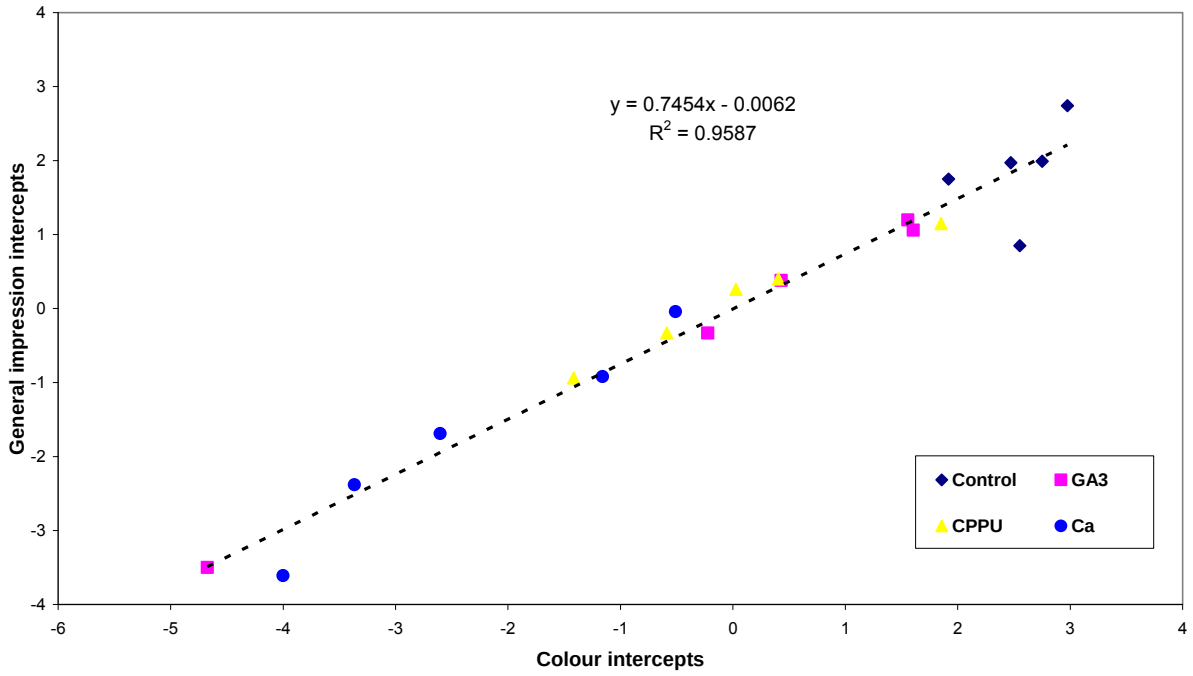

Figure 12 The correlation between general impression and colour classes classified by the tasting panel of Redglobe grapes after cold storage.

Table 5 Effect of plant bioregulators and bunch applied Ca on the taste after cold storage of Redglobe. (Class 1 indicates the lowest awarded score and class 5 the highest), as observed by an independent tasting panel of 20 people. Observations is not independent of the treatments (Chi-square ≤ 0.0001).

Treatment	Number of tasters that preferred a specific class (%)				
	Class 1	Class 2	Class 3	Class 4	Class 5
Control	2.8	10.9	35.5	26.4	24.6
GA_3	12.7	23.6	39	19.1	5.5
CPPU	7.3	23.6	46.4	15.5	7.3
Ca	10.9	31.8	45.5	9.1	2.7

4.4 CONCLUSIONS

The PBR and Ca treatments resulted in delayed sugar accumulation. Both CPPU and calcium treatments results in poor colour development. The poor colour development in the case of CPPU was the result of the delaying effect it had on

sugar accumulation, colour accumulation and organic acid respiration. The colour developed as the grapes accumulate sugar. Producers should be careful to apply CPPU to sensitive cultivars as it may affect the colour development negatively and it may prolong grape ripening. No improvement on the colour of the calcium treated grapes was observed as it accumulates sugar. Further research is needed to investigate the effect of bunch directed calcium sprays on grape colour. Producers should evaluate the use of calcium sprays very carefully. It may affect the colour and general appearance of red grapes negatively.

Berry firmness brought on by the use of CPPU may be related to the density of cells in the mesocap tissue as CPPU increase cell density or to skin thickness as CPPU increase skin thickness. Cell size and berry firmness seems to be closely related as the firmer treatments also had the biggest cells in the outer mesocarp. Plant bioregulators should therefore be applied on the correct time (during Phase I) during berry development to have the best effect on berry firmness and size.

Berry firmness can be maintained during cold storage through the application of CPPU. This may be ascribed to lower water loss that occurs in CPPU-treated berries as CPPU increase the thickness of the grape berries' skin.

The walls of cells in the outer mesocarp were significantly thicker than those in the inner mesocarp. The thicker cell walls in the outer mesocarp may result in the formation of the firm, opaque flesh (as discussed in chapter 3). The cell walls in both the inner and outer mesocarp tissues for all the treatments seem to be well preserved. More research is needed to investigate methods on how to increase cell wall thickness.

The control treatment was not sprayed with water when the other treatments were applied, this could be the reason why more *Botrytis* rot occurred for especially the Ca treatment as the Ca treatment was applied three times while the other treatments was applied only once. The thicker skin formed with the use of CPPU may have prevented *Botrytis* infections. None of the treatments decreased the occurrence of quality defects which may develop during cold storage.

The tasting panel was generally more impressed by the control treatment, and preferred its colour and taste. It seems as if the PBR's and Ca treatments had a generally negative effect on the eating quality of the grapes.

The CPPU treatments seems to have the best over all effect on the grapes regarding berry size, berry firmness, keeping and eating quality (although last mentioned was reduced when compared to the control). The use of CPPU can cause the grapes to reach the required sugar level at a later stage than untreated

grapes which can result in a loss of income when the specific cultivar arrive too late at an export market. The increased berry firmness measured for the CPPU treatment do not affect the storing capacity, but it has a negative effect on the ripening and eating quality of the grapes. CPPU applications can make a positive contribution to grape quality when general problems regarding berry size and firmness may occur.

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Chapter 5

RESEARCH RESULTS

Utilisation of gibberellic acid (GA_3), CPPU and bunch applied calcium sprays to increase berry firmness of table grapes: (II) Waltham Cross

UTILISATION OF GIBBERELLIC ACID (GA_3), CPPU AND BUNCH APPLIED CALCIUM SPRAYS TO INCREASE BERRY FIRMNESS OF TABLE GRAPES: (II) WALTHAM CROSS

5.1 INTRODUCTION

In this chapter, the experimental results investigating the utilisation of gibberellic acid (GA_3), CPPU and bunch applied calcium sprays to increase berry firmness of Waltham Cross table grapes is presented. Since exactly the same treatments than that of the previous chapter (applied to Redglobe) was applied in this case to Waltham Cross the relevant literature and research dealt with in chapter 4 also applies to this chapter.

5.2 MATERIALS AND METHODS

5.2.1 EXPERIMENTAL VINEYARDS

The experiment was conducted on a ten year old *Vitis vinefera* L. Waltham Cross vineyard. The vines were grafted on Ramsey and trained on a factory trellis system. This vineyard is situated on the farm Non Pareil in the Hex River Valey, De Doorns. It contains two clones, Waltham Cross clone 8 and Waltham Cross clone 13, separated in two sections. Clone 8 consistently produces firmer berries than the most commonly produced clone 13. The vines are also spaced 3 m x 2 m. Irrigation scheduling of this block was done by using tensiometers.

5.2.2 EXPERIMENTAL DESIGN AND TREATMENTS

The trial was laid out in a randomized block design with four treatments (Table 1) that were replicated three times in each clone. The abbreviations used in Table 1 for the treatments will be used in the rest of this chapter. It is standard practice to apply a combination of GA_3 and CPPU to improve berry size and firmness. The treatments were applied using a 20L knapsack spray just before sunrise. Each experimental unit consisted of four vines and were separated on either side from one another by four non-experimental vines.

Table 1 Description of the treatments applied.

Treatment abbreviation	Experimental treatments Waltham Cross
Control	No plant bioregulators applied
GA_3	10 mg/L GA_3 (ProGibb[1]) applied at 10 mm mean berry size.
CPPU	10 mg/L GA_3 (ProGibb[1]) applied at 10 mm mean berry size plus 3 mg/L CPPU (Sitofex[2]).
Ca	Mixture of 8L/ha Stopit[3] and 5 L/ha Caltrac[4] applied directly to bunches every two weeks from berry set to vèraison.

1. ProGibb = 400g/kg gibberellic acid.
2. Sitofex 10EC = 10g/L forchlorfenuron .
3. Stopit = $CaCl_2$ at 160 g Ca^{2+}/L.
4. Caltrac = $CaNO_3$ at 400 g Ca^{2+}/L.

5.2.3 DATA COLLECTION

For berry sampling for microscope studies, berry measurements, light and electron microscope studies and statistical analysis, refer to the previous chapter as the methods followed was exactly the same.

5.3 RESULTS AND DISCUSSION

5.3.1 EFFECT OF PLANT BIOREGULATORS (PBR'S) AND BUNCH DIRECTED CALCIUM (CA) SPRAYS ON RIPENING

All the treatments had a delaying effect on the accumulation of sugar in the berries, with that of the GA_3, CPPU and Ca treatment being significant (Fig. 1). Reynolds *et al*. (1992) also found the delaying effect of CPPU on fruit ripening. The CPPU treatment was not harvested until its total soluble solids (TSS) reached 15 ˚Brix, two weeks later than other treatments; it was done for experimental purposes so that berries of similar ripeness could be compared regarding post-harvest life and eating characteristics.

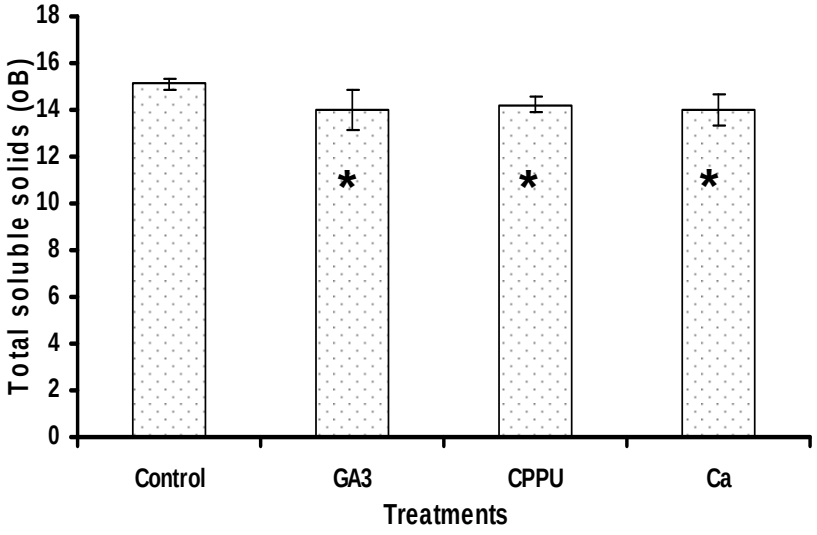

Figure 1 Effect of plant bioregulators and bunch applied Ca on the total soluble solids accumulated at time of harvest of the control treatment of Waltham Cross (15.0 ^{o}Brix). Error bars indicate 95% confidence intervals. * Significant compared to control.

5.3.2 THE EFFECT OF PBR'S AND BUNCH DIRECTED CALCIUM-SPRAYS ON BERRY GROWTH

As found for Redglobe, the pattern of berry growth for Waltham Cross also did not have the double sigmoidal pattern as generally accepted for grapes. The general double sigmoidal pattern could be expected when more data points existed – more sampling times are needed, as the Phase II of berry development can be as short as five days. Fig. 2 shows that berry growth was the most rapid during vèraison (70 days after anthesis (DAA)) and it continued until harvest.

As for Redglobe, a very strong correlation was found between berry mass and volume (R^2 = 0.9939). Berry size of the Waltham Cross berries gained 37 % of its final size from vèraison till harvest, but is significantly affected during the whole period from 45 DAA up to harvest. Any managerial practises applied in this period, that may affect berry development, will therefore potentially have an effect on berry size.

The CPPU treatment had a significantly positive effect on berry size (Fig. 3) compared to the control and the other two treatements. This is in accordance with research done by Retamales *et al*. (1995). The GA_3 treatments and Ca-sprays also seemed to increase berry size, but not significantly compared to the control. The PBR's are known to have the most significant effect on berry size when applied shortly after berry set (Iwahori et al., 1968).

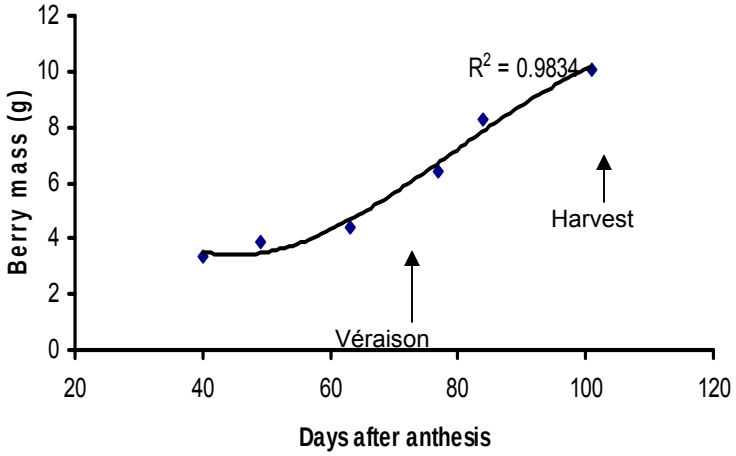

Figure 2 Pattern of berry growth from 15 mm berry size up to harvest for Waltham Cross (N = 24 experimental plots x 10 berries) in the Hexriver valley.

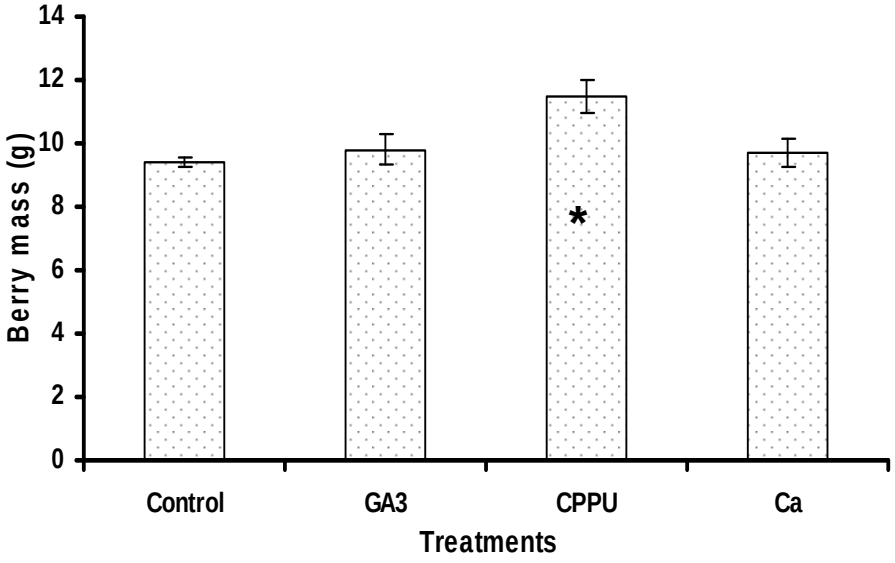

Figure 3 Effect of plant bioregulators and bunch applied Ca on mean berry size of both Waltham Cross clones (clone 8 and 13) at harvest. Error bars indicate 95% confidence intervals. *Sigificant compared to control.

Berry growth happens in the latter growth phases due to the enlargement of cells (Dokoozlian, 2002) and can therefore be expressed as the reduction of cells per mm^2. Fig. 4 shows that GA_3, CPPU or Ca sprays had no effect on the cell size of the inner and outer mesocarp during berry development compared to control. The increased berry size obtained for the CPPU treatment was therefore not due to increased cell size, but probably due to more cells formed during higher rates

of cell division as cytokinin promote cell division (Ogata et al., 1989). No differences in cell size were observed between the tissues of the two clones of Waltham Cross (data not shown). The difference in firmness between the two clone is therefore not related to cell size.

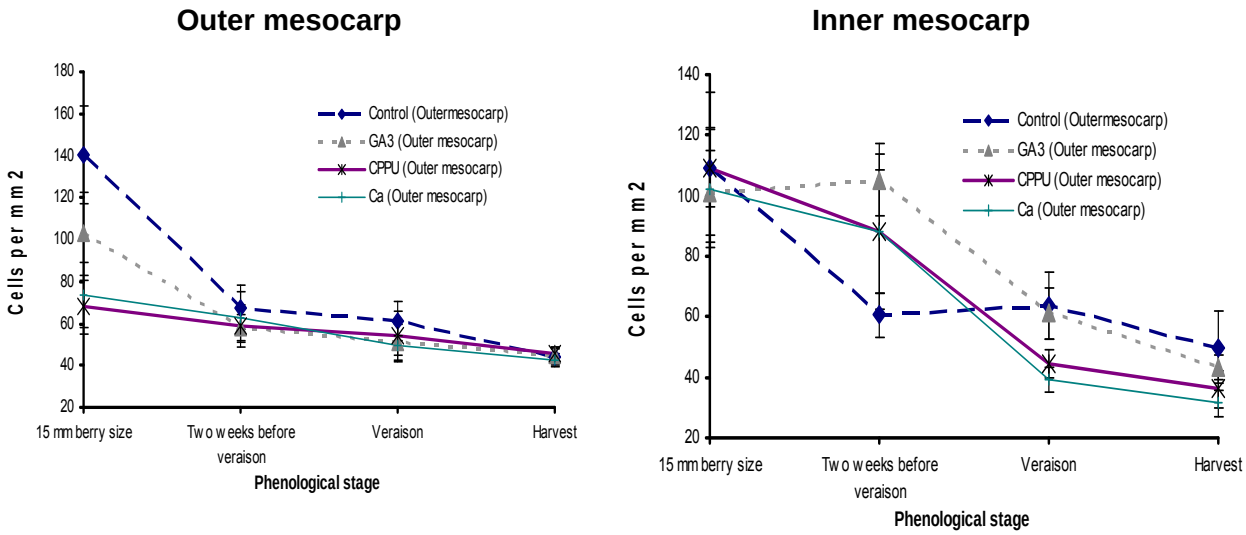

Figure 4 Cell growth, expressed as reduction in cells per mm^2, of Waltham Cross berries from the Hexriver valley, 2006/07 season, as affected by plant bioregulators and bunch applied Ca. Error bars indicate 95% confidence intervals.

5.3.3 THE EFFECT OF PBR'S AND BUNCH DIRECTED CALCIUM-SPRAYS ON BERRY FIRMNESS

Mean berry firmness of all the treatments decreased progressively through berry development and cold storage. In contrast to Redglobe, berry softening occurred rapidly between 60 and 80 DAA although vèraison was considered to be only at 80 DAA. This indicates to a possible earlier vèraison date as that determined by field observations as the deformability of the berry increase at véraison which results in the softening of the berry (Coombe, 1973). The firmness of berries did not change significantly between véraison and harvest (Fig. 5). The accumulation of solutes from véraison till harvest in the berry cause water uptake (Grange & Andrews, 1994) which will result in firm berries (Liang et al., undated). This phenomenon may be the reason why berry firmness does not change much from véraison till harvest. There was a significant decrease in berry firmness during cold storage. This may be due to water loss through respiration and the skin.

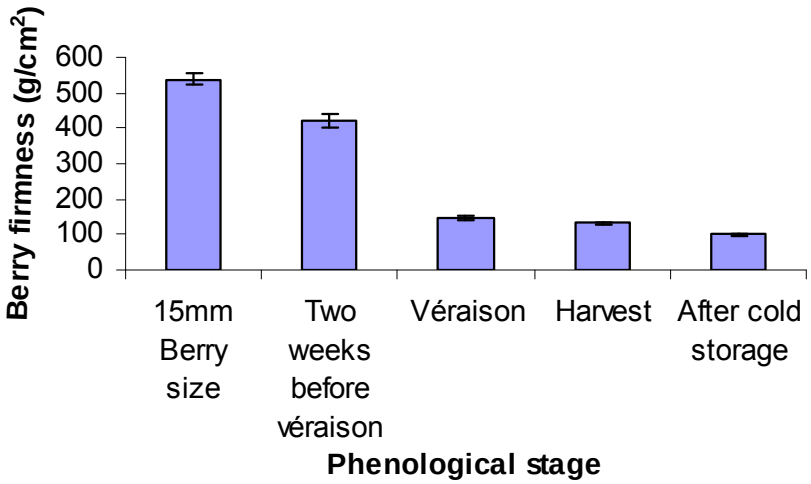

Figure 5 Changes in firmness of Waltham Cross berries throughout development and cold storage. Error bars indicate 95% confidence intervals.

The CPPU treatments were the only treatments that significantly improved the firmness of berries at harvest (Fig. 6). The greater firmness of berries could be ascribed to the effect of delayed maturation (Ben-Arie *et al*., 1997) or to modifications in the anatomy of the berry (Coombe & Hale, 1973). The increased skin thickness of CPPU treated berries could also be the cause of the increase firmness (Ben-Arie *et al*., 1997). A significant reduction of berry firmness was however obtained during cold storage for all the treatments. After cold storage the CPPU and GA_3 treatments however maintained their firmness significantly more than the control. The GA_3 and CPPU treatment start with firmer berries (significant for CPPU) before cold storage; this may be the reason why these treatments also were the firmest after cold storage.

A clear difference in firmness between the soft and firm clones were obtained when the mean berry firmness over the whole period of berry development was compared (Fig. 7A). This indicates that the clones do indeed differ in firmness. The firmness reactions of the two clones on the different treatments however did not differ significantly (data not shown), meaning that a clone x treatment interaction was not obtained. At harvest and after cold storage, there was no significant difference between the firmness of the clones (data not shown). The firmness during berry development between the clones differs but at harvest there was little difference in firmness. Both clones also react the same on the treatments, therefore not one nor the other are the better clone to plant if a producer wants firm Waltham Cross berries.

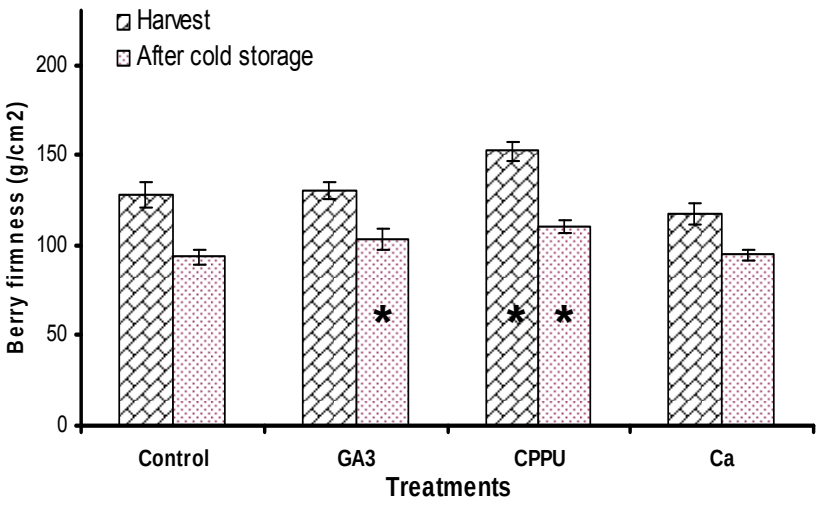

Figure 6 Effect of plant bioregulators and bunch applied Ca on the firmness of Waltham Cross berries. Error bars indicate 95% confidence intervals. * Significant compared to control.

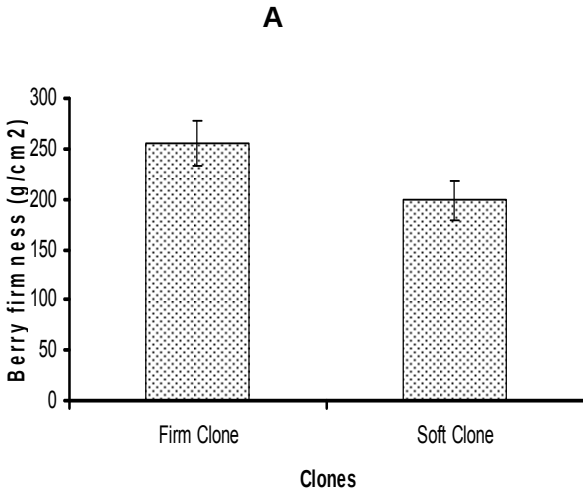

Figure 7 Berry firmness main effect clone means (Clone 8 (firm clone) and Clone 13 (soft clone)). Error bars indicate 95% confidence intervals.

In general, the thickness of cell walls in the inner and outer mesocarp cells differed significantly in all the treatments, i.e. the cell walls of the inner mesocarp cells were significantly thicker than those of the outer mesocarp (Fig. 8). As for Redglobe (chapter 4) it was hypothesised that differences in berry firmness, caused by PBR's, might be due to potential differences in cell wall thickness of the mesocarp cells. In Fig. 8 it can be observed that the CPPU treatment resulted in significantly thicker cell walls in the inner mesocarp. The reason why the outer

cell walls were not affected is unclear, and it is furthermore doubtful that thicker cell walls in the inner mesocarp will contribute to the firmness of the berries. This hypothesis is in accordance with the work done on Redglobe (chapter 4) where cell wall thickness could not be related to berry firmness.

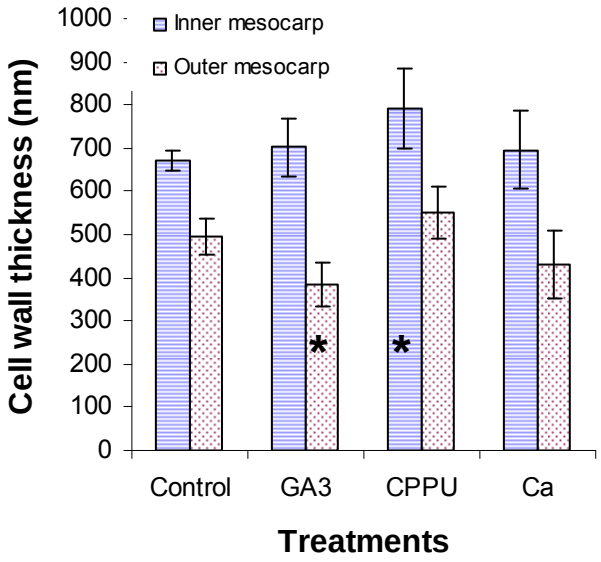

Figure 8 Effect of plant bioregulators and bunch applied Ca on the cell wall thickness of different tissues of Waltham Cross berries at harvest. Error bars indicate 95% confidence intervals. * Significant from control.

Furthermore, at harvest the cell walls of cells in the outer (Fig. 9) and inner (Fig. 10) mesocarp of the firm clone (Clone 8) seem to be well preserved. The plasmalemmas in the inner mesocarp, however, are more subtracted from the cell wall than in the outer mesocarp. As discussed in the previous chapter, the intactness of the plasma lemma can probably be linked to the firmness of the tissue and, as stated in chapter 3, the outer mesocarp of clone 8 is clearly distinguished from the inner mesocarp, giving rise to the firmer berries compared to clone 13, where the distinction is not as clear. The firmer the tissue, the more intact the plasma lemma will be because of the higher turgor that exists in the cells (Liang et al., undated).

There is not much difference between the appearances of the cell walls of the different treatments in either the inner or outer mesocarp for the firmer clone (clone 8). Similar trends exist between the inner (Fig. 11) and outer (Fig. 12) mesocarp of the softer clone (Clone 13).

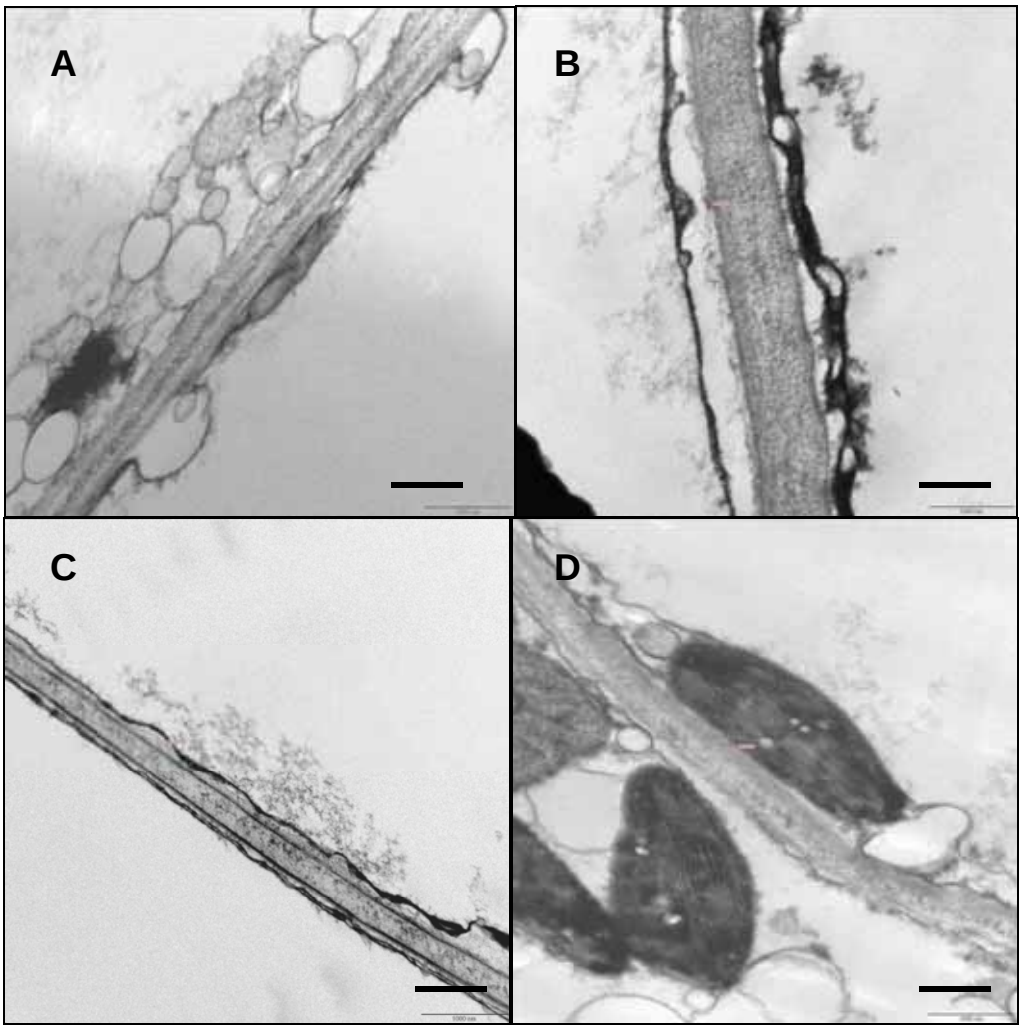

Figure 9 Transmission electron microscope sections of the outer mesocarp of firm (clone 8) Waltham Cross berries at harvest. A) Control, B) GA_3, C) CPPU, D) Ca. Scalebar ± 1000 nm.

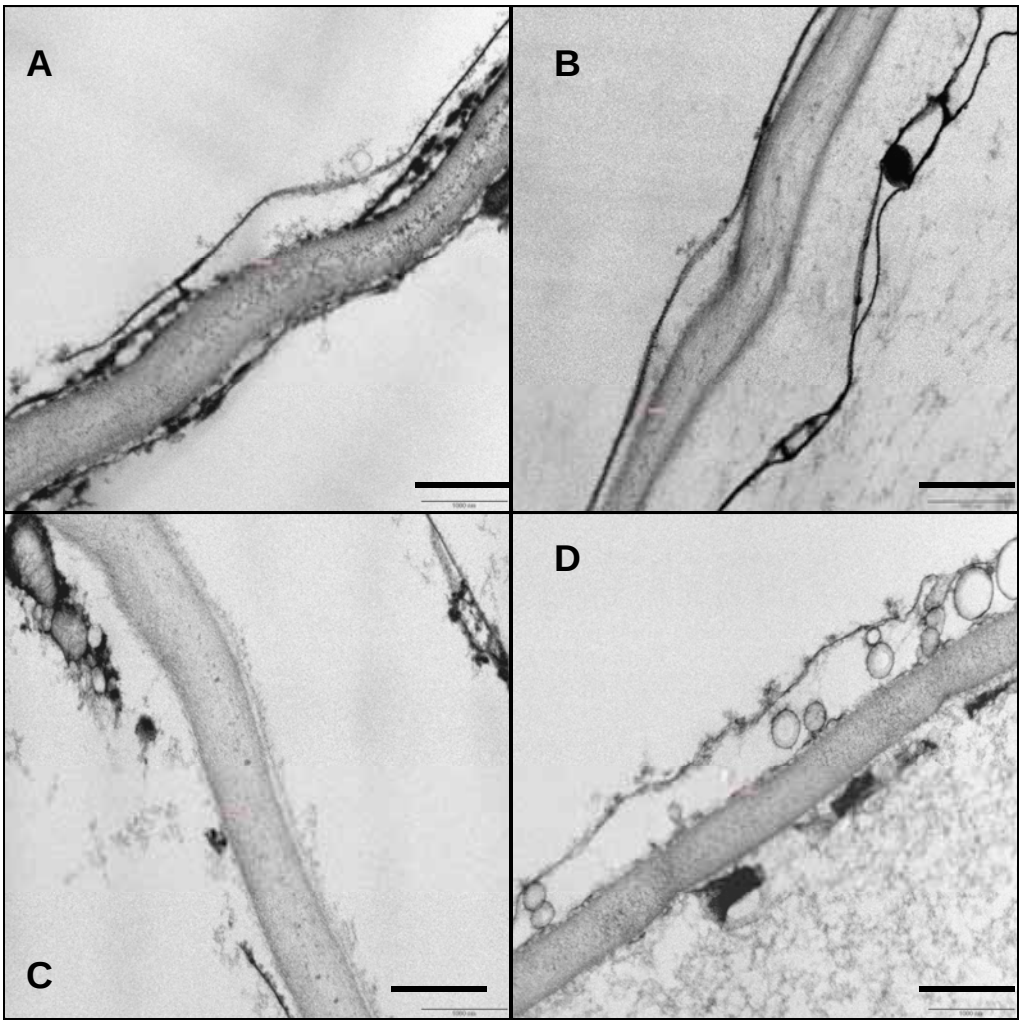

Figure 10 Transmission electron microscope sections of the inner mesocarp of firm (clone 8) Waltham Cross berries at harvest. A) Control, B) GA_3, C) CPPU, D) Ca. Scalebar ± 1000 nm.

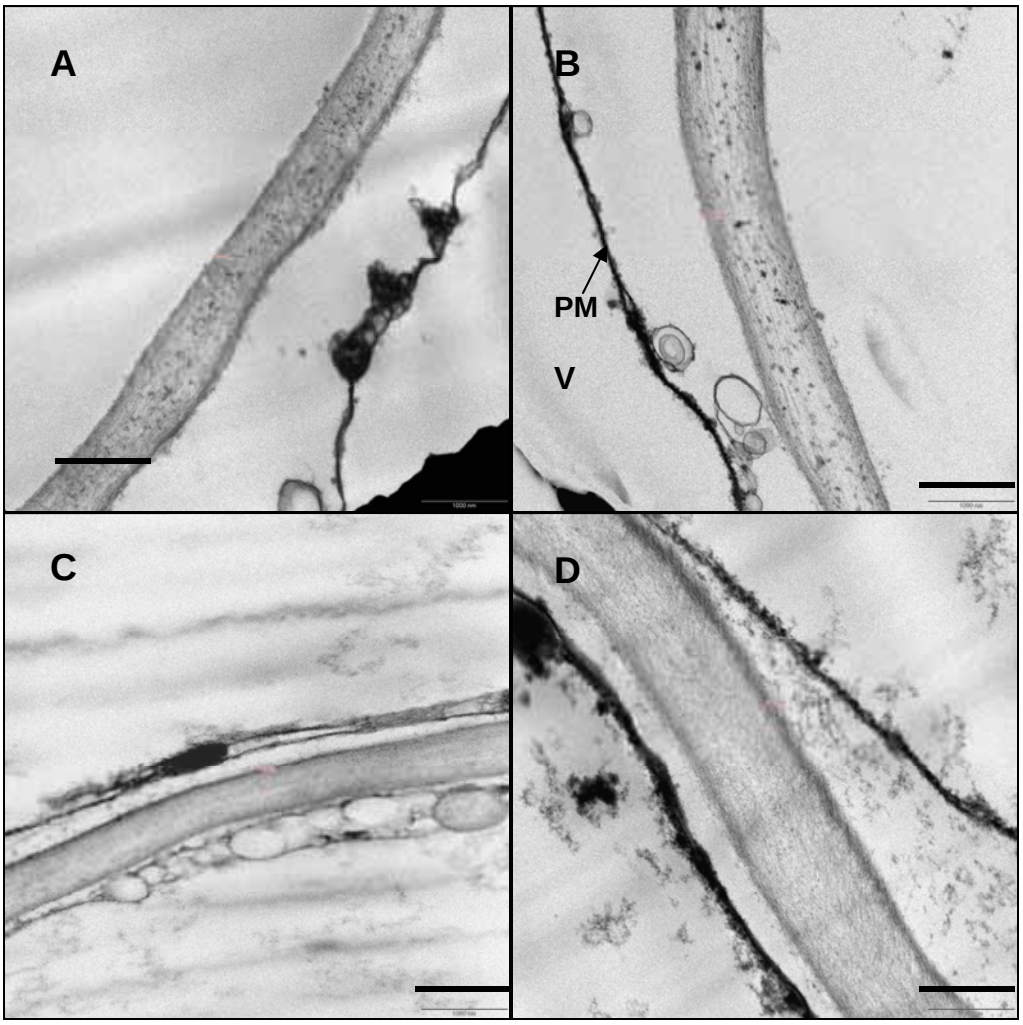

Figure 11 Transmission electron microscope sections of the inner mesocarp of soft (clone 13) Waltham Cross berries at harvest. A) Control, B) GA_3, C) CPPU, D) Ca. V = vacuole, PM = plasma lemma. Scalebar ± 1000 nm.

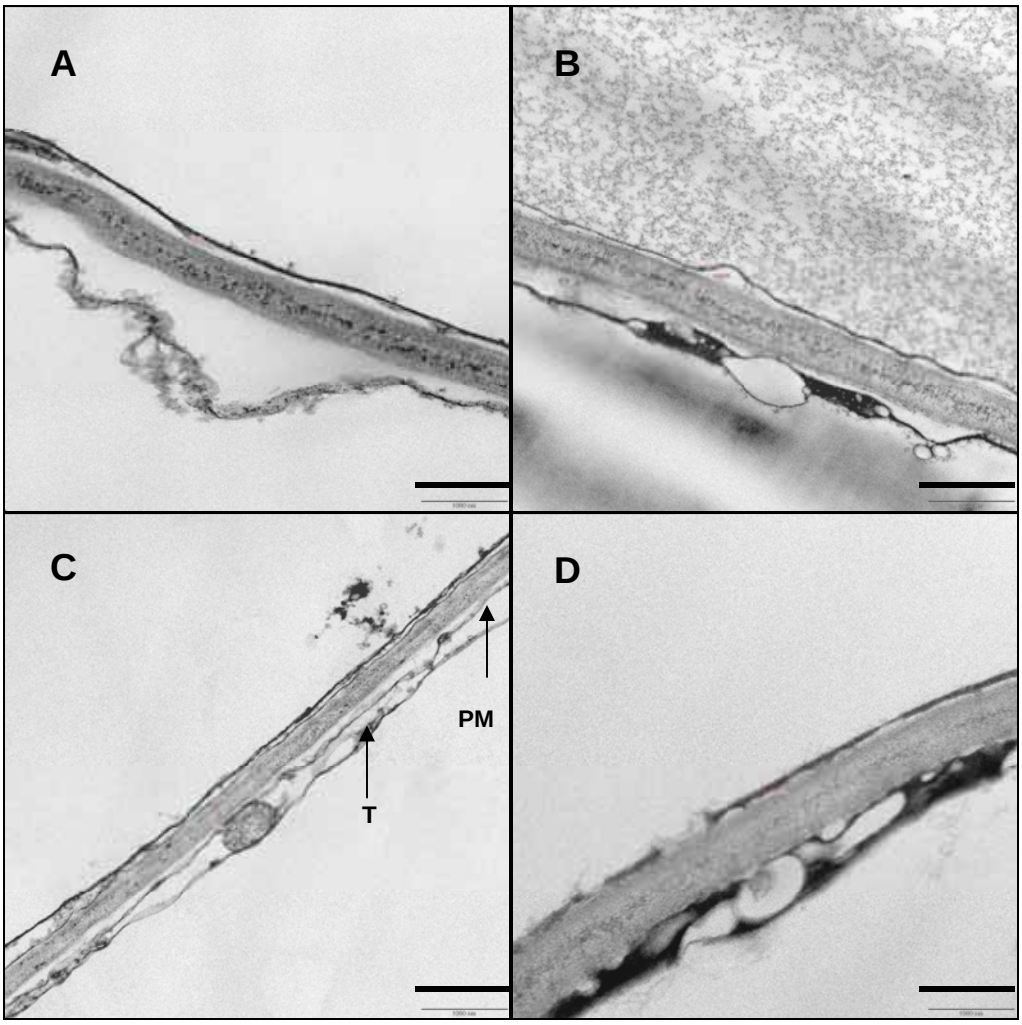

Figure 12 Transmission electron microscope sections of the outer mesocarp of soft (clone 13) Waltham Cross berries at harvest. A) Control, B) GA_3, C) CPPU, D) Ca. T = Tonoplast, PM = Plasma lemma. Scalebar ± 1000 nm.

5.3.4 THE EFFECT OF PBR'S AND BUNCH APPLIED CALCIUM-SPRAYS ON BERRY QUALITY

The post harvest life of Waltham Cross was not negatively affected by any treatments (Table 2). The Ca treatment also showed higher *Botrytis* infection levels compared to control, while CPPU showed a tendency to increase the occurrence of loose berries. The higher *Botrytis* infection in the case of the Ca treatment may be the result of the water in the sprays applied on this treatment – two more sprays than on the other treatments were applied. Although not significant at P≤ 0.05, there is a strong indication of the occurrence of these defects. In all cases, however, the occurrence of the quality defects was negligible.

Table 2 Effect of plant bioregulators and bunch applied Ca on berry quality after cold storage of Waltham Cross.

Treatment	Quality parameters		
	Botrytis rot (%)	**Loose berries (%)**	**Cracked berries (%)**
Control	0.18	0.93	0.13
GA_3	1.62	1.97	0.22
CPPU	0.15	1.73	0
Ca	0.47	0.95	0.15
P≤ 0.05	NS*	NS*	NS*

* NS = Not significant at a confidence level of P ≤ 0.05.

The eating quality was also not affected by neither PBR's nor Ca treatments. Table 3 shows that after cold storage the general impression of the grapes, as observed by the tasting panel is not independent of the treatments (Chi-square ≤ 0.0001). The highest percentage of tasters classified all the treatments' general impression mainly in classes 3. This indicates that the treatments had the minimum effect on the general impression of the grapes. However, from Table 3, it seems as if the GA_3 and CPPU treatments made the best general impression on the tasters as more tasters classified these treatments in class five than the other two treatments.

Table 3 Effect of plant bioregulators and bunch applied Ca on general impression after cold storage of Waltham Cross (Class 1 indicates to lowest awarded score and class 5 the highest), as observed by an independent tasting panel of 24 people. Observations are not independent of the treatments (Chi-square ≤ 0.0001).

Treatment	Number of tasters that preferred a specific class (%)				
	Class 1	**Class 2**	**Class 3**	**Class 4**	**Class 5**
Control	7.9	6.4	50.0	25.4	10.3
GA_3	4.0	7.9	42.1	22.2	23.8
CPPU	7.9	8.7	34.1	20.6	28.6
Ca	7.1	11.9	42.1	22.2	16.7

Most of the tasters classified all the treatments in taste class 3 (Table 4). This indicated that the taste of the grapes after cold storage was not affected by the treatments. However, the control and CPPU treatment tasted better than the other two treatments as they were classified in class 5 by a higher percentage of tasters than the other treatments.

Table 4 Effect of plant bioregulators and bunch applied Ca on taste preference after cold storage of Waltham Cross (Class 1 indicates to lowest awarded score and class 5 the highest), as observed by an independent tasting panel of 24 people. Observations are not independent of the treatments (Chi-square ≤ 0.0001).

	Number of tasters that preferred a specific class (%)				
Treatment	**Class 1**	**Class 2**	**Class 3**	**Class 4**	**Class 5**
Control	4.8	12.7	42.9	17.5	22.2
GA_3	11.9	23.8	41.3	13.5	9.5
CPPU	7.9	16.7	34.9	16.7	23.8
Ca	11.9	17.5	38.1	18.3	14.3

As seen in Table 5, most of the tasters classified the firmness of the grapes after cold storage in class 3 which indicates that none of the treatments affected berry firmness. The same trend exists in the case of the two clones, and little difference in firmness was observed between them (data not shown). Again, the CPPU treatment was classified by the highest percentage of tasters in classes four and five, which indicates that CPPU had the most positive effect on berry firmness.

Table 5 Effect of plant bioregulators and bunch applied Ca on firmness after cold storage of the cultivar Waltham Cross (Class 1 indicates to lowest awarded score and class 5 the highest), as observed by an independent tasting panel of 24 people. Observations are not independent of the treatments (Chi-square ≤ 0.0001).

	Number of tasters that preferred a specific class (%)				
Treatment	**Class 1**	**Class 2**	**Class 3**	**Class 4**	**Class 5**
Control	5.6	9.5	49.2	23.8	11.9
GA_3	6.4	5.6	57.9	19.8	10.3
CPPU	1.6	5.6	37.3	27.0	28.6
Ca	13.5	13.5	43.7	18.3	11.1

5.4 CONCLUSIONS

Commercial use of CPPU may have certain marketing implications for producers who have to sell their grapes at a specific time as it had a delaying effect on fruit ripening.

The bigger berry size of the CPPU treatment could not be ascribed to cell size but most probably to higher rates of cell division early in berry development. The best effect on berry size will therefore be obtained if CPPU is applied early in berry development.

The CPPU treatments were the only treatments with significantly firmer berries at harvest, while both the CPPU and GA_3 treatments maintained firmness during cold storage better than the other two treatments. Reduction of berry firmness during cold storage can be ascribed to water loss.

As discussed in the previous chapter, the intactness of the plasma lemma and tonoplast can be linked to the firmness of the tissue. The more intact the membranes are, the firmer the tissue will be. The outer mesocarp is indeed firmer (see chapter 3). More research is needed to investigate options on how to increase berry firmness by preserving the cell walls in tissues.

The CPPU treatments seem to have the best effect on berry size, firmness, storage capacity and eating quality of the grapes. It can therefore be concluded that CPPU applications can make a positive contribution to grape quality when general problems regarding berry size and firmness occur. Producers must keep in mind the delaying effect CPPU have on fruit ripening which can cause the grapes to arrive too late at a certain export market.

5.5 LITERATURE CITED

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Chapter 6

GENERAL DISCUSSION AND CONCLUSIONS

GENERAL DISCUSSION AND CONCLUSIONS

Table grape export markets demand only the best quality fruit. Quality defects such as poor colour, browning, soft tissue breakdown and inadequate berry firmness is a few defects that can cause great losses in export programs. Berry firmness is one of the determining factors of eating quality. Consumers prefer firmer berries above softer berries. Producers therefore have to try to enhance and maintain berry firmness during berry development. Firmer berries are also expected to have better keeping quality. Very little research has been done to develop managerial practices or sprays that can be applied during berry development to enhance and maintain berry firmness.

This study was done on Redglobe and two Waltham Cross clones. The aim of this study was two-fold. Firstly, to determine the cellular and ultracellular differences between firm and soft table grape berries as well as the effect of plant bioregulators (PBR's) such as gibberellic acid (GA_3), synthetic cytokinin (CPPU) and bunch directed calcium sprays on the cell structure. Secondly, the effect of GA_3, CPPU and bunch directed calcium sprays on eating quality and storage capacity was under investigation.

Firm berries had a white opaque coloured flesh with a firm consistency while softer berries had a gel-like translucent flesh. Soft berries' mesocarp consisted of mostly the gel-like translucent flesh with in some cases a thin layer of opaque coloured flesh just under the skin. In the case of berries of normal firmness the outer part of the mesocarp was opaque coloured. Extremely firm berries' whole mesocarp consisted of the opaque coloured flesh. The thicker this layer of opaque coloured flesh is, the firmer the berry. Producers therefore require practises to enhance the thickness of this opaque coloured flesh of the berries.

It was found that berry firmness is not related to cell size - the cell size of the firm and soft berry tissue were alike. Cell shape between the firm and soft tissues however differed. The firmer opaque coloured tissue's cells were more turgid and oval than those of the softer gel-like tissues. Again it can be concluded that berry firmness may be determined by the thickness of the opaque coloured flesh in the outer mesocarp. The thickness of the cell walls did not differ between the soft and firm tissues while there was a difference between the cell contents and the intactness of the plasmalemma and tonoplast. The plasmalemma and tonoplast in the softer tissue was further subtracted from the cell wall than in the firmer tissue. The firmness of the tissue seems to be determined by the shape of the cell: More turgid cells with well intact plasmalemmas and tonoplasts will have a firmer consistency. Berry development that enhances the accumulation of solutes that will increase osmotic influx of water, will lead to firmer berries. Further investigation is needed on the effect of plant water relations the development of firm berries.

The plant bioregulators and bunch applied calcium treatments causes delayed ripening of the grapes in both Redglobe and Waltham Cross. CPPU caused significantly bigger and firmer berries in both cultivars. In the case of Redglobe,

CPPU caused bigger cells in the outer mesocarp, suggesting a possible correlation between berry firmness and cell size. CPPU however did not increase cell size in Waltham Cross. The increased firmness of CPPU treated berries could also be caused by an increased rate of cell division earlier in berry development. The CPPU treatment was the only treatment that maintained berry firmness during cold storage for Redglobe while GA_3 and CPPU treatments did so in the case of Waltham Cross. The loss of berry firmness during cold storage is ascribed to water loss. CPPU reduced water loss due to thicker skins obtained. Further research is needed on why berries loose their firmness during cold storage.

GA_3 and calcium treatments had a negative effect on grape quality after cold storage on both cultivars. This can be due to the fact that the control treatment was less wet because it was not sprayed with water when the other treatments (especially Ca) were applied and due to the fact that CPPU can cause skin thickening which can make the berry more resistant to infections. The tasting panel prefers Waltham Cross treated with the CPPU treatment. For the Redglobe, tasters prefer the control.

The use of CPPU in combination with GA_3 had the best effect on berry size, berry firmness, eating quality and storage capacity. It can therefore be recommended for improvement of table grape berry firmness and keeping quality in cases where problems regularly occur. CPPU also maintained berry firmness better, making it useful for export of grapes (where storage of up to six weeks might be required). Ca applications are not recommendable on as it affects berry colour (especially red grapes) and the general appearance of the grapes negatively. CPPU and GA_3 must be applied during the right time of berry development as both these PBR’s are only active during the first phase of berry development. One of the negative effects found with the CPPU treatment is that it results in delayed fruit ripening which can affect the export of the fruit to specific markets.

More research is needed in this field of study. Other managerial practises such as different irrigation strategies and sprays must be evaluated for its effect on berry firmness. Furthermore, the role of fertilisation, irrigation and enzyme activitiy on berry firmness must be elucidated.