The Effect of Different Irrigation Frequencies in Combination with Boron and Calcium Bunch Applications on Berry Split of SoutherngrapeOne

by

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Thesis presented in partial fulfilment of the requirements for the degree of
Master of Agricultural Sciences

at
Stellenbosch University
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March 2010

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Date: 22 February 2010

Summary

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2010

The table grape industry employ a wide range of viticultural management practices in order to produce the high quality grapes demanded by the export market. A common contributor to degrading the quality of table grapes is the occurrence of berry split, which not only has an unattractive visual effect, but also increases the berries' susceptibility to infection by spoilage organisms.

A number of environmental conditions such as rainfall and humidity, and/or agricultural practices, such as irrigation, and high density canopies, can lead to higher plant cell water content. This in turn, can increase the potential of berry split to occur. To date, the main method of berry split prevention has been the management of plant water status by; *(i)* regulating irrigation withdrawal times, and *(ii)* covering of canopies if rainfall is predicted prior to harvest.

The aim of this study was to determine the effect that irrigation frequency, as induced by irrigation withdrawals; as well as boron (B) and calcium (Ca) treatments, applied as bunch directed sprays, have on pre- and post-harvest berry split. To this end, a newly released late ripening, white seedless cultivar, SoutherngrapeOne was chosen as a model cultivar as it has a high susceptibility to berry split. SoutherngrapeOne vines were subsequently subjected to a range of irrigation frequencies based on typical irrigation scheduling used in the table grape industry, which comprised of a low, medium and high frequency. The low frequency was duplicated in order to demonstrate the effect that a heavy irrigation, just before harvest may have on berry split. These treatments were further subdivided to investigate the effect that B and Ca may have on berry split. For the B treatment, four Solubor[1] bunch directed sprays were applied from 8mm berry size to véraison. The Ca treatment consisted of Stopit®[2] and Caltrac®[3] bunch directed sprays applied over the same period. In addition, a combination of the B and Ca treatment were applied to investigate any possible interaction. To account for the effect of water as solvent in the B and Ca treatments, and the spraying effect, pure water as treatment was also evaluated. Control vines received no sprays.

The applied irrigation treatments resulted in different plant water status conditions. Separate applications of B and Ca treatments resulted in a decrease of B and Ca content in the flesh respectively. The control and combination treatment, of B and Ca resulted in the same of B and Ca content in the flesh. Furthermore, none of the applied treatments resulted in an increase of either B or Ca content in the berry skin.

It was found that the medium frequency irrigation resulted in the best irrigation strategy to prevent pre-harvest berry split. Surprisingly, all the sub-treatments: B, Ca, and combination of B and Ca, resulted in an increased incidence of pre-harvest berry split when compared to the control group for the 2006/07 season. However, in the 2007/08 season only the B treatment resulted in an increase of pre-harvest berry split.

Concerning post-cold-storage physiological disorders, Ca treatments appear to have reduced berry drop, but increased decay. In the 2006/07 season, the B treatment resulted in reduced post-cold-storage berry split, whereas B

[1]Solubor (307.5g B/kg), applied at 1.5kg/ha.
[2]Stopit ($CaCl_2$ at 160g Ca^{2+}/L) applied at 8L/ha.
[3]Caltrac ($CaNO_3$ at 400g Ca^{2+}/L) applied at 5L/ha.

treatment in the 2007/08 season had no effect. Both B and Ca should be considered as having the potential to increase the appearance of hairline cracking. Calcium treatment also led to an increase in decay which may have been as a result of the splitting it contributed to. Low frequency irrigation recieving irrigation before harvest was found to result in browner stems.

Low irrigation frequencies decreased the cell size of the berry skin. The Ca treatment gave rise to thicker (weaker) cell walls, this morphological change may be responsible for the physiological disorders it caused.

From these findings, it can be deduced that poorly managed irrigation, together with unnecessary application of B and/or Ca may result in an increase of berry split and other physiological disorders, with subsequent financial losses for the producer.

Opsomming

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Die tafeldruifindustrie maak gebruik van 'n wye reeks wingerdkundige praktyke ten einde die hoë gehalte druiwe te produseer wat die uitvoermark vereis. Korrelbars is 'n algemene verskynsel wat afbreek maak tot die gehalte van tafeldruiwe. Behalwe dat voorkoms van die druiwe benaadeel word, verhoog dit ook in vatbaarheid vir infeksie deur verrottingsveroorsakende swamme. Hoë reënval en humiditeit, sowel as wingerdkundige praktyke soos besproeiing en hoë lowerdigtheid, wat kan lei tot verhoogde waterstatus in plante, kan lei tot 'n toename in korrelbars.

Daar word hoofsaaklik van twee metodes gebruik gemaak om korrelbars te beheer, naamlik die bestuur van plantwaterstatus deur; *(i)* beheer van besproeiingsontrekkingstye en *(ii)* bedekking van lowers indien reën voorspel word voor oestyd.

Die doel van hierdie studie was om vas te stel wat die invloed van besproeiings frekwensies sowel as trosgerigte boor (B) en kalsium (Ca), spuitbehandelings, op voor- en na-oes korrelbars het. Die onlangs vrygestelde laat rypwordende, wit, pitlose kultivar, SoutherngrapeOne is gebruik, aangesien dit hoogs gevoelig is vir korrelbars.

Stokke is aan verskillende besproeiings intervalle, soos tipies gebruiklik in die tafeldruifindustrie, blootgestel. Hierdie intervalle bestaan uit n' lae, medium en hoë besproeiings frekwensie. Die lae besproeiings frekwensie is herhaal ten einde die invloed van besproeiing net voor oestyd op korrelbars te ondersoek. Die invloed van B- en Ca-behandeling op korrelbars is ook ondersoek. Vir die B-behandeling is vier Solubor[1] trosgerigte spuite aangewend vanaf 8mm korrelgrootte tot deurslaan. Vir die Ca-behandeling is Stopit®[2] en Caltrac®[3] as trosgerigte spuite oor dieselfde tyd toegedien. Kombinasiebehandelings is ook aangewend om enige interaksie tussen B en Ca te ondersoek. Waterbehandelings is ook toegedien om die invloed van water as oplosmiddel van B- en Ca-behandelings sowel as die spuit-effek te ondersoek. Kontrole stokke is ook ingesluit en het geen spuitebehandeling ontvang nie.

Die besproeiingsbehandelings het verskillende plantwater toestande tot gevolg gehad, B- en Ca-behandelings het gelei tot 'n afname in B- en Ca-inhoud in die vleis onderskeidelik. Die kombinasie en kontrole behandelings het eenderse hoeveelhede B en Ca in die vleis tot gevolg gehad. Geen van die aangewende behandelings gelei tot 'n toename in B- en Ca-inhoud in die dop nie.

Die resultate toon dat medium besproeiings frekwensie die beste besproeiingsstrategie is om voor-oes korrelbars te voorkom. In vergelyking met die kontrole-behandeling in 2006/07, het B, Ca en die kombinasie van B en Ca, 'n toename in voor-oes korrelbars tot gevolg gehad. In die 2007/08 seisoen het slegs die B-toediening egter tot 'n toename in voor-oes korrelbars gelei.

Kalsium behandelings het 'n afname in los-korrels, maar 'n verhoging in korrelbars tot gevolg gehad. In 2006/07, het B-toediening tot 'n afname in korrelbars na koelopberging gelei, maar in die 2007/08 seisoen het dit geen effek gehad nie. Beide B- en Ca-toediening het die potensiaal om haarlyn barste te veroorsaak. Kalsium toediening het bederf verhoog wat moontlik

[1]Solubor (307.5g B/kg), toegedien teen 1.5kg/ha.
[2]Stopit ($CaCl_2$ at 160g Ca^{2+}/L) applied at 8L/ha.
[3]Caltrac ($CaNO_3$ at 400g Ca^{2+}/L) applied at 5L/ha.

aan die hoër bars wat dit induseer toegeskryf kan word.

Lae besproeiings frekwensie, het bruiner stingels veroorsaak, en ook gelei tot 'n afname van selgrootte in die dop. Die Ca-toediening het aanleiding gegee tot dikker selwande in die dop. Hierdie anatomiese veranderinge kan moontlik die rede wees vir die verhoging in fisiologise afwykings.

Van hierdie bevindinge kan ons aflei dat swak bestuur van besproeiing, sowel as die onnodige aanwending van B en/of Ca, kan aanleiding gee tot 'n toename in korrelbars en ander fisiologiese afwykings, en dus finansiële verliese vir die produsent inhou.

Acknowledgements

I would like to express my sincere gratitude to the following people and organisations who have contributed to making this work possible:

- Mr. P.J. Raath, my supervisor, who advised and guided me through example and with enthusiasm;
- Mr. J.H. Avenant, of the ARC Infrutec-Nietvoorbij, for providing this project, accommodating me and acting as my co-supervisor;
- The South African Society for Oenology and Viticulture for the bursary to complete my Masters degree;
- Anthony Hill, owner of the farm Clovally, where this study was conducted, for his interest in research and his willingness to donate resources and time for the study;
- Marieta Van Der Rijst, at the Biometrical department at the ARC for her assistance with the statistical analysis of the data;
- Trix Quixley, for her help during this study especially the electron microscopy work;
- The personnel of Infrutec-Nietvoorbij, for their willingness and assistance during this study;
- Dr. P.A. Myburgh for his assistance and advice in the completion of my thesis;
- Mr. P. Fouchë, from Giro-sprinklers, for his generosity and supplying necessary irrigation equipment;

- To all my friends for their support and help, especially Riaan Conradie, Kora Holm and in particular, Benita Pretorius ❀on whom I can always relay;

- My brothers, André, Johann and Wiehahnn, also Annelita for their support and enthusiasm during my studies.

Dedications

Hierdie tesis dra ek met liefde op aan my ouers wie ek admireer, Willie en Babs Koekemoer, wat my gedra het met liefde en ondersteuning deur my akademise loopbaan.

Scholasticus optio

ń Keuse tussen een, is geen keuse. Nog minder, is ń keuse tussen twee sonder ondersoek.
Choice between one, is no choice. Even less is a choice between two without investigation

(Leander Koekemoer 2009)

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Chapter 1

General introduction and project aims

The quality and value of table grapes is largely governed by the physical appearance of the grapes produced. Therefore, any loss of visual attractiveness results in decreased production value and considerable financial losses for the producer. Berry split is one of the leading causes of such production losses, in addition to the secondary effects that follow such as microbial infection, water loss, and SO_2 bleaching.

In order to prevent berry split, focus is placed on irrigation management to control berry turgor pressure, a critical factor close to harvest. The relationship between turgor pressure and cell wall strength determines cell growth. When the effective turgor pressure becomes too high for normal stress relaxation, cell walls may break (Matthews *et al.*, 1987). It is this breaking of individual cell walls that causes berries to split open (Considine & Kriedemann, 1972). The latter contributes to production losses in two ways; *(i)* loss of shipment weight (as these berries have to be removed before packing), and *(ii)* value, due to negative quality reports from importing countries if split develops during shipment.

The aim of this study was to investigate the effect that different irrigation frequencies may have on the occurrence of pre- and post- harvest berry split. The proposed advantages of boron (B) and calcium (Ca) treatments on cell wall strength were also evaluated under these different irrigation frequencies.

To this end, the following approaches were followed on a semi-commercial block in the Hex River Valley:

- SoutherngrapeOne was used as the model cultivar due to its high susceptibility to berry split.
- Irrigation frequency was managed according to scheduling most commonly used in the table grape industry.
- The effect of commercially available B and/or Ca treatments were evaluated by applying treatments during berry development.
- Subsequently, berry split and stem water potentials were measured throughout the seasons from 2006/07 to 2007/08.
- Following cold storage, grape quality was evaluated by quantifying physiological disorders in berries.
- Finally, berry skin thickness, cell size therein, and cell wall diameter were analyzed using light- and transmission electron microscopy.

In this thesis, Chapter 2 contains a literature review describing the mechanism of berry split, with specific focus on the contribution of B and Ca towards cell wall strength. It further describes the influence that turgor pressure, berry ripening, environmental conditions and viticultural practices have on berry split. In Chapter 3, the pre-harvest occurrence of berry split as influenced by these factors are discussed, Chapter 4 encapsulates the effect that these factors had on post cold storage fruit quality. The microscopy results are presented in Chapter 5, followed by a general conclusion in Chapter 6.

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Matthews, M., Cheng, G., Weinbaum, S., 1987. Changes in water potential and dermal extensibility during grape berry development. J. Amer. Soc. Hort. Sci. 112, 314–19.

Chapter 2

Literature review

2.1 Introduction to berry split

The excitement accompanying the release of the new, late ripening, white seedless cultivar SoutherngrapeOne, was accompanied by concern, as it seems that this cultivar is highly susceptible to grape berry skin rupture (Me. P Burger: Personal communication, 2006). Grape berry skin rupture, also known as berry split, is a common occurring phenomenon in the table grape industry. It can be the result of a variety of factors, including *(i)* fungal, and other infections that disrupt the integrity of the berry skin, *(ii)* physical damage, such as those caused by animals, or during harvesting, transport and processing of the fruit, as well as *(iii)* water stress followed by a abundance of free water, that is, the swelling and subsequent splitting of berries due to the sudden uptake of water (Sekse, 1998).

The latter cause of berry split, namely water stress, due to delayed irrigation followed by a sudden availability, forms the focus of this study. There are two major types of berry split that results from water stress; firstly, *cracking* which refers to the occurrence of fine openings just through the surface of the berry skin, mostly in the form of concentric rings. And secondly, *splitting* which refers to the rupture of the cuticle, epidermis, sub-epidermis, and many of the vacuolated cells of the outer pericarp (Swift *et al.*, 1974). It has been suggested that the main difference in the appearance of these two forms of berry skin rupture is due to the way in which the epidermis gives way to

turgor pressure. In this way, a slow increase in turgor pressure may result in cracking of the berries as Knoche *et al.* (2001) has shown working on cherries, whereas a rapid increase may result in the splitting of the berries (Sekse, 1998).

2.2 Mechanism of berry split

From the literature, it can be deduced that water stress induced berry split can be regarded as a factor of two opposing forces, namely *(i)* turgor pressure, that is, an increase in intracellular pressure due to the uptake of water after a water deficit, and *(ii)* cell wall strength, which refers to the capacity of the plant cell wall to withstand an increase in turgor pressure.

Turgor pressure is mainly due to the production of sugar-based photosynthate. These highly osmotically active substances regulate the flow of water by osmotic adjustment as part of the growth process (Considine & Kriedemann, 1972). However, the influx of water cannot continue *ad infinitum*, as it is restricted by the berry skin. In turn the strength of the berry skin is governed by the complex interactions of the structural building blocks namely hemicellulose, pectin, structural proteins, and cellulose micro-fibers in the cell walls (Brett & Waldron, 1990). In addition, B and Ca play inadmissible roles in composing the framework to withstand the increasing pressure on the plasma membrane as cell growth takes place (Cosgrove, 1987; Matoh & Kobayashi, 1998). Very importantly, it has been demonstrated that splitting of the berry skin is caused by the rupture of individual cell walls, and not by the separation of adjacent cells (Considine & Kriedemann, 1972). Thus in the event of a cells' turgor pressure exceeding the strength of its cell wall, the individual cell wall breaks open, this event is referred to as berry split (Considine & Kriedemann, 1972).

Furthermore, the thickness of the cell walls in the sub-epidermal region holds direct correlation to the occurrence of berry split (Yamamura & Naito, 1985). Considine & Knox (1979) reported that a thickening of the cell wall takes place during the second stage of berry development, followed by subsequent thinning of the cell wall at the onset of the third stage of berry development. In accordance, the third stage of berry development is regarded more susceptible to berry split. This finding is further supported by the observation

that berry skin cell walls lose their elasticity, along with their plasticity, after the third stage of berry development, contributing to berries increased susceptibility to splitting upon increased turgor pressure at this point (Meynhardt, 1964; Matthews *et al.*, 1987).

To date, only one *in planta* mechanism of berry split prevention has been suggested in grapes, namely water recycling. Water recycling via the xylem in the post véraison stage of berry development, lowers the turgor pressure in the berry. However, warm and rainy or humid nights, has been found to inhibit this back flow, causing the phloem influx to exceed the xylem efflux combined with the berry transpiration, ultimately resulting in berry split (Keller *et al.*, 2006).

2.3 Factors influencing berry split

The occurrence of berry split is influenced by a number of factors namely; *i* berry cell wall strength, *ii* turgor pressure, *iii* berry ripening, *iv* environmental conditions, as well as *v* common viticultural practices. The following section contains an overview of these factors and how they may potentially contribute to berry split.

2.3.1 The plant nutrients boron and calcium

Boron and Ca are well known for their contribution to cell wall strength (Matoh & Kobayashi, 1998; Kobayashi *et al.*, 1999). These nutrients are commonly employed as foliar or bunch directed treatments in the South African table grape industry. However, little is known about the mechanism and effect of such bunch directed treatments, and even less about how different cultivars react to such treatments.

2.3.1.1 The importance of boron in plants

Boron is an essential plant micro-nutrient that plays a key role in a wide range of plant physiological processes. It is generally considered phloem immobile,

which means that once it reaches the leaves, it is restricted from further translocation, and becomes fixed in the apoplast (Blevins & Lukaszewski, 1998).

Boron is involved in three main aspects of plant physiology, namely *(i)* membrane function and transport, *(ii)* metabolic activities, and *(iii)* cell wall structure (Blevins & Lukaszewski, 1998).

Boron has been found to support ion uptake by playing a key role in maintaining the reducing atmosphere in the apoplast, in addition to its possible involvement in the localization of ascorbate at the plasma membrane interface (Brown, 1979). Boron has further been found to be required for sufficient uptake of phosphorus (P), chloride (Cl), and rubidium (Ru) in maize (Pollard *et al.*, 1977), and also to act as a stabilizing factor in the plasma membrane (Cakmak *et al.*, 1995), preventing leakage of potassium (K), sucrose, phenolics, as well as amino acids (Goldbach, 1985; Cakmak *et al.*, 1995)

The importance of B in plants is especially evident from the many anatomical, physiological and biochemical changes that occur during states of B-deficiency. Scott (1943) recorded the first symptoms of B-deficiency in grapevines. He observed well developed patterns with chlorotic areas toward the leaf margins and between the leaf veins. He further noted that even in severe cases, the chlorotic areas always remained intact. The surface of affected leaves was found to be abnormally roughened with raised areas between the veins, resulting in an upward cupping of the leaf. He also found that older leaves remained chlorotic after boron-treatment, while young leaves produced after a B treatment, did not show any symptoms of B deficiency. Furthermore, shoots that grew from vines with B-deficiency were weak, with a tendency to produce several lateral shoots from a single node, especially from the nodes most distant from the trunk. The internodes were very short, leaves small, and often malformed. And although flower on bunches did develop, they were twisted, malformed, and failed to set fruit.

In later studies it was discovered that several enzymes such as ribonucleases, glucose-6-phosphatase dehydrogenase, phenylalanine ammonia lyase, beta-glucosidase, and polyphenoloxidase, all of which are usually attached to the plasma membrane or cell wall, are activated during B-deficiency, resulting in altered plant metabolism, and an increase in phenolic synthesis (Shkolnik, 1984; *teste* Dale & Krystyna (1998)). Furthermore lower ATPase activity in

plasmalemma-enriched vesicles was observed by Lawrence *et al.* (1995) in B-deficient chickpea roots than in control plants.

The requirement for B further increases dramatically during the reproductive stage of plant growth (Loomis & Durst, 1992). This statement is based on an observation made during an early study by Scott (1943) in which it was found that B application to previously B-deficient vines had a more pronounced effect on fruit production than on vegetative growth. Furthermore, B has been shown to not only be essential for pollen germination, but also critical for pollen tube elongation (Johri & Vasil, 1961; Jackson, 1989).

2.3.1.2 Borate complexes in the cell wall

The primary function of B as a micronutrient is undoubtedly its structural role in the cell wall (Ishii *et al.*, 2001). The majority of cellular B is localized in the cell wall fraction (about 80-90% of dry weight) (Loomis & Durst, 1992). The mechanical properties of growing cell walls can be modified by crosslinks between the major components; cellulose and matrix polymers such as hemicellulose and pectin polysaccharides. Recently, formation of borate esters with hydroxyl groups of cell wall carbohydrates and/or glycoproteins has been proposed as a mechanism of crosslinking cell wall polymers. Borate bridging is permitted by H-bonding of hydroxyl groups (Blevins & Lukaszewski, 1998).

Borate forms the most stable complexes or di-esters with cis-diols on furanoid rings, limiting these reactions to ribose and apiose (Loomis & Durst, 1992) on the cellulose micro-fibers (Fig. 2.3.1). It has been proposed that plant cell wall acceptor molecules effectively bind magnesium after loosely binding B, and that magnesium (Mg) competitively binds with Ca (Teasdale & Richards, 1990). Their work further support the notion that borate esters formed with hydroxyals of sugar such as apiose and fructose on the pectin or glycoprotein polymers, provide areas for the chelation of Ca or Mg in cell walls.

A beta-polysaccharide complex was isolated from radish root cell walls, where 80% of the cellular B was found to be localized in this complex (Matoh *et al.*, 1993). Subsequently a B-containing pectic polysaccharide complex, B-rhamnogalacturonan-II (RG-II-B), was isolated (Kobayashi *et al.*, 1996). This complex being essential to the function of the cell wall. Ishii *et al.* (2001)

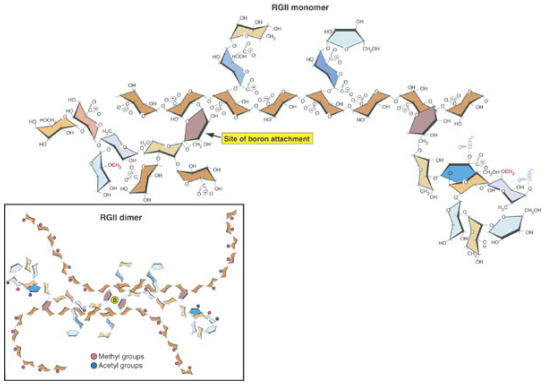

Figure 2.3.1: A proposed structure of the borate diester cross-linking two RG-II molecules via apiose residues of side chains. The backbone is that of a cellulose microfiber.

showed an inverse correlation concerning the cell wall thickness of the leaves and dimeric rhamnogalacturonan II B complex (dRG-II-B) formation. Also that an addition of B to such cells will result in the formation of dRG-II-B and in doing so firm-up the matrix to form a thin cell wall, the thickening or swelling is due to the lack of cross-linking of borate esters.

Borate bridging in cell walls could explain many of the characteristics of B-deficient and B-toxic plants. In such plants, the lack of B bridging could account for brittle leaves and rigidness of other plant parts, as opposed to the elasticity observed in plants with sufficient B supply (Loomis & Durst, 1992; Hu & Brown, 1994). It has further been postulated that insufficient B bridging may be the reason that meristematic tissue formed under conditions of B-deficiency are usually broken (Hu & Brown, 1994).

2.3.1.3 The importance of calcium in plants

Calcium is an essential, phloem immobile plant macronutrient (Demarty *et al.*, 1984) that is transported throughout the plant by apoplastic movement (Matoh & Kobayashi, 1998). Calcium is well-known for its ability to act as second messenger in external and cytosolic responses, as well as for its involvement in membrane function and cell wall growth (Powell *et al.*, 1982). It is at the cell wall that Ca is most readily bound, and therefore the highest concentrations of Ca is found in the cell walls (Demarty *et al.*, 1984).

Demarty and colleagues, hypothesized that the effect that Ca has on cell wall growth may be due to its ability to exchange protons with the cell wall. This proton exchange would then result in a change in the surrounding pH, which in turn leads to the activation of enzymes necessary for cell wall growth. They further suggested that this might be due to Ca's high affinity for uronic groups. In contrast to this Jones & Lunt (1967) separated cell growth in two stages. During the first stage the cell increased in plasticity and elasticity, which was decreased by Ca. The second stage of cell growth was identified by the biosynthesis and laying down of cell wall material, which was enhanced by Ca and prevented by auxin.

During plant growth, at the onset of lignification, Ca has been found to play an especially critical role. Lignin formation was almost completely suppressed in Ca-deprived plants. This is attributed to the fact that lignin precursors are transported by Ca-dependent transporters (Northcote, 1984). Furthermore, Eklund & Eliasson (1990) found evidence of Ca being responsible for the shift from primary cell wall enlargement to lignification, a statement which is a good indication of secondary wall formation.

Calcium deficiencies are rarely encountered in practice due to common soil corrections (Matoh & Kobayashi, 1998). However, disorders such as stem and bunch breakdown have been found in Canada Muscat and Himrod grapes when grown in Ca-deficient growth medium (Ontario Ministry of Agriculture, Food & Rural Affairs). Other deficiency disorders such as internal breakdown (Bangerth *et al.*, 1972) and bitter pit of apples (Sharples, 1976), black heart in celery, end rot of tomatoes, integral browning of brussels sprouts (Kirkby & Pilbeam, 1984), as well as corking disorders in pipfruit (Faust & Shear, 1968). Furthermore it was found that disturbances in Ca transport can lead to tipburn in lettuce (Collier & Tibbitts, 1982), and brownheart in brassicas (Millikan & Hanger, 1966).

2.3.1.4 Structural role of calcium in cell walls

Calcium also plays an essential role in cell wall structure (Gonzalez-Fontes *et al.*, 2008). Several mechanisms have been proposed to describe the role that Ca plays in the structural integrity of the plant cell wall. Cell wall constituents, other than pectic substances, have been suggested to form complexes with Ca

(Ito & Fujiwara, 1967). Complexes between Ca and cell wall proteins have been suggested to lend intercellular cohesion to the cell wall Ginzburg (1961). It has further been proposed that Ca ions can be fixed in the cell wall by coordinated linkages with hydroxylic groups of polysaccharide (Wuytack & Gillet (1978) *teste* Demarty *et al.* (1984)).

Work done on *Picea abies* cuttings showed that cellulose synthesis is not affected by states of Ca-deficiency (Eklund & Eliasson, 1990). They explained this finding with the assumption that cellulose synthesis can continue in the plasma membrane since the necessary precursors can be obtained from the cytosol, even when a Ca deficiency exist. An artificially high endogenous concentration of Ca can however inhibit the formation of cellulose (Delmer, 1987). In addition, Ito & Fujiwara (1967) found that the chemical fraction and sugar production of rice plant cell walls remaind the same when exposed to different levels of Ca-deficiency, a further indication that Ca does not influence the synthesis of cell wall components. However, this is in contrast with findings by Burstrom (1958) (*teste* Eklund & Eliasson (1990)) who reported a 20% decrease in cellulose content in Ca deficient roots, with little change in pectin and hemicellulose content. The latter findings may however be more plausible, as the *Picea abies* cuttings that were used in the Eklund & Eliasson (1990) study, may have had a sufficient Ca content to partially sustain growth.

Pectin molecules in cell walls contains a unique three dimensional structure that renders hydrogen bonding to other polysaccharides impossible (Preston, 1979). In addition, the pectin chain interactions with cations are relatively weak, attributed to the uncharged state of the chain (Grant *et al.*, 1973). However Ca has the ability to link pectin chains together, as well as pectins to polysaccharides by forming cross bridges (Wuytack & Gillet (1978) *teste* Demarty *et al.* (1984)).

In order for cross bridge linkage to take place, specific cell wall matrix conditions need to exist. Firstly, specific arrangements of pectin chains are required (Walkinshaw & Arnott, 1981a), along with the presence of galacturan chains with a minimum of seven consecutive carboxyl groups along the interior face of the participating chains (Ferguson, 1984; Powell *et al.*, 1982). Furthermore, participating carboxyl groups need to have a low ($<$30%) degree of methylation (Walkinshaw & Arnott, 1981b). Longer polygalacturan chains have been found to bind stronger to each other and polysaccharide chains

(Grant *et al.*, 1973). This is supported by findings by Morris *et al.* (1982) who presented evidence that the primary mechanism of Ca-induced linkage poly-D-galacturonate chain, is by dimerization. This is similar to linkage of poly-L-guluronate chains (Powell *et al.*, 1982), to give rise to the "egg-box" model (Fig. 2.3.2).

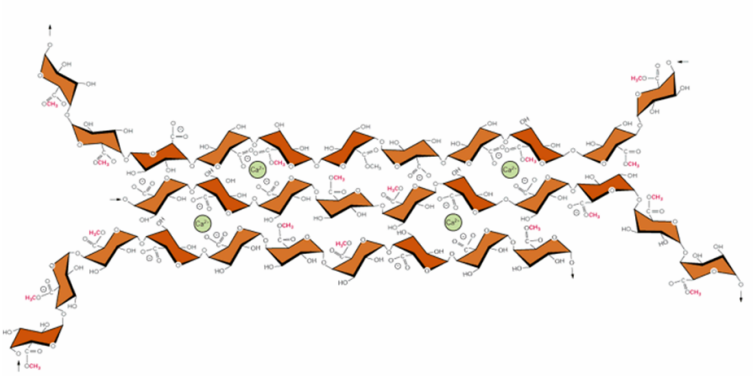

Figure 2.3.2: The proposed structure that Ca forms with pectin in the cell wall matrix.

When conditions are met, strong cohesive gel networks of cross-linked dimeric egg-box junctions (Walkinshaw & Arnott, 1981a,b) are formed. These networks are strong enough to withstand cell wall expansions when the concentration of unbound apoplastic Ca is in the order of 1000 μM Tagawa & Bonner (1957). However, lower, normal levels of Ca, is sufficient to prevent weakening of the cell wall (Demarty *et al.*, 1984). It is important to note that if the Ca concentrations are not carefully controlled, the cohesive gel networks can turn brittle, and break (Walkinshaw & Arnott, 1981a).

Furthermore, calcification increases the resistance of plant cell walls to infection, probably by increasing their resistance to polygalacturonidase (Bateman (1965) *teste* Jones & Lunt (1967)). The tight binding between Ca and pectin, also contributes to the stabilization of the cell walls (Demarty *et al.*, 1984). In fact, the formation of Ca-pectate plays an inadmissible role in the physical properties of the cell wall, and is essential to maintain rigidity, and strength of plant cell walls (Cormack, 1965; Ito & Fujiwara, 1967; Rasmussen, 1966; Tagawa & Bonner, 1957).

Literature supports the idea notion that Ca may play a regulatory role in the ripening of fruit during post-harvest storage. At high tissue concentrations, Ca has been shown to delay fruit ripening, as well as the breakdown of tissue (Bangerth *et al.*, 1972). This may be due to Ca-induced cohesive gel networks formed by pectin in the middle lamella and the cell wall (Demarty *et al.*, 1984; Ferguson, 1984; Platt-Aloia *et al.*, 1980). Since fruit ripening is associated with fruit softening, that is when the middle lamella becomes more loosely bound, high Ca concentrations are likely to impede these structural and physiological changes due to the formation of Ca cross-linked pectin polymers (Ferguson, 1984).

2.3.2 Turgor pressure

Turgor pressure refers to the pressure exerted on plant cell walls due to an influx of water into the cells, and is the driving force for cell growth to occur. Turgor pressure is governed by the cell's osmotic potential, and is regulated by osmoregulation and osmotic adjustment with the help of osmotically active substances. When the turgor pressure in the berry cells rise above its elasticity coefficient, berries may split open.

2.3.2.1 Water movement

Munch (1926, 1930) (*teste* Goeschl & Magnuson (1986)) proposed a pressure flow theory that involves two osmometers connected together by a thin tube. The first osmometer had a higher sugar concentration than the second, giving it a lower osmotic potential. When the system is submerged under water there is a net flow of water into the first osmometer where it builds up pressure and moves through the tube to the second osmometer. Because of the mass flow generated, the sugar moves to the second osmometer. The pressure created by the mass flow of water and sugar accumulation in the second osmometer expands its size and also increases its sugar concentration.

Dreier *et al.* (2000) postulated that smaller berries accumulated sugar faster because of the bigger skin surface area to berry volume, giving it a more positive allometric relationship. They added that there seems to be a relationship between sugar accumulation and the limiting process of phloem unloading.

Limiting, because it ultimately depends on the berry transpiration. This is supported by the close relationship they found between berry radius and sugar concentration which can mathematically be calculated if the evaporation of water through the berry skin is taken into account. This agrees with findings by Rebucci & Poni (1997) and Greenspam *et al.* (1996), that evapotranspiration may be the main, if not sole, driving force of sugar accumulation in post véraison berries.

By combining these two models, a system is obtained that demonstrates how transpiration and photosynthesis generate water flow from the roots to the leaves where, because of the low water pressure formed by the increase in sugar concentration, a mass flow can form towards the grape berries. This causes an increase in cell and therefore berry size, as well as an increase in sugar accumulation. By osmoregulation and osmotic adjustment, the water potential remains low in the berry cells, causing a continuous flow of water, carrying sugar. This is supported by Leopold (1964), stating that although water movement is a passive process which is primarily governed by transpirational pull, it is also assisted by the water potential gradient.

2.3.2.2 Osmotically active substances

Stage III of berry development is characterized by the accumulation of soluble solids, which in the case of grapes are mainly sugars. The accumulation of osmotically active substance lowers the water potential in the cytosol, and subsequently results in the influx of water from the phloem (During *et al.*, 1987) into the berry cells, causing the turgor pressure to rise (Dreier *et al.*, 2000). This increase in turgor pressure can not be actively controlled by plant cells, but instead is controlled indirectly by regulating the osmotic potential. The latter is achieved by means of osmoregulation (Zhang *et al.*, 1999), which refers to osmotic adjustment with the aim of maintaining a constant intracellular osmotic potential independent of the extracellular water potential (Delauney & Verma, 1993), and osmotic adjustment. During osmotic adjustment, a cells osmotic potential is altered in a regulatory manner so that a decrease occurs in extracellular water potential, thereby preventing or minimizing a decrease in intracellular turgor pressure (Bernstein, 1961).

2.3.3 Berry ripening

The ripening of berries entails changes in the cell wall structure to allow for fruit softening. This in turn however, weakens the structure of the cell wall and exposes the ripening fruit to berry split at this very vulnerable stage in development.

With the onset of stage III of berry development, ie. véraison, the berry skin starts to loosen due to changes in the structural properties of the berry skin cell walls. This occurrs when the hemicellulose, pectin, and structural proteins intertwined with the network of cellulose micro-fibers, lose there structural stabilizing polysaccharides and Ca. During this time, structural protein incorporation and phenolic cross-linkage formation become active, especially in the walls of the epidermis and sub-epidermis cells (Huang *et al.*, 2005). The characteristic ultrastructural changes in the cell wall that takes place during this phase includes swelling of the cell walls in the epidermis and sub-epidermis, degradation of the middle lamellae in the inner cells, cell wall surfaces becoming wavy, and acidification of the apoplast causing breakage of the hydrogen bonds that holds the hemicellulose and micro-fibers together (Huang *et al.*, 2005). Note that acidification is also responsible for the breakage of Ca-bridges between pectin molecules, causing a reduction in wall-bound Ca (Brett & Waldron, 1990). Furthermore, there is a derease in insoluble pectin, hemicellulose, and cellulose; possibly due to active hydrolysis of structural polysaccharides in the cell wall and enzyme activity such as expansin (McQueen-Mason *et al.*, 1992), beta-1,4-endo-glucanase, endoxyloglucan transferase and hydrolase. Pectin is also hydrolyzed at this stage by pectinases such as polygacturonases, pectate lyase, beta-galactosidase, alpha-galactosidase, alpha-arabinofuranosidase and pectin methylesterase (Huber, 1983; Nunan *et al.*, 2001)). Sodium dodecyl sulfate-insoluble amino acids, hyperoxyproline, and activity of wall bound peroxidase increases, mainly in the walls of the of the outermost layers, including the epidermis and sub-epidermis (Huang *et al.*, 2005).

Huang *et al.* (2005) concluded that skin strength and elasticity is reduced after véraison, along with an increase of skin extensibility. This implies continuous cell wall loosening of the cells in the berry skin following véraison. They further reported that cell walls in the skin are compact with narrow cellular

cavities and thick protoplasm, while cells in the pulp were much bigger and highly vacuolated. Huang *et al.* (2005) concluded that these cells underwent tangential extension, while the inner cells expanded in both dimensions. This could explain findings that change of berries from spherical to oval promotes longitudinal cracking Considine & Kriedemann (1972) and the increases the incidence of micro-cracking of fruit during ripening (Knoche *et al.*, 2001).

2.3.4 Environmental factors

Environmental conditions are believed to play an important role in the occurrence of berry split, especially in the late season. In this way, environmental factors may lead to elevated turgor pressure in berries, followed by a period of low transpiration, in this way increasing the incidence of berry split. In the same way, elevated turgor pressure, in combination with cool humid days with little or no wind, also increases the susceptibility of grapes to berry split (Considine & Kriedemann, 1972; Yamamura & Naito, 1985).

Therefore, the way in which environmental conditions influence plant turgor pressure seems to be the key method by which environmental conditions influence berry split in grapes. Curry *et al.* (2002) observed that splitting coincided with high rainfall, even in plants that received irrigation and correlated berry susceptibility to split to berry TTS. As little as 10 mm rain during ripening and a relative humidity of more then 95 % is considered to be favorable conditions for of berry split (Avenant, 2000). The influence of rainfall on berry split further increases late in the season. This is due to the increased incidence of micro cracking during ripening (Knoche *et al.*, 2001)(work done on cherries), and the subsequent uptake of water from the environment, leading to elevated cell turgor pressure (Glenn & Poovaiah, 1989).

2.3.5 Viticultural practices

Traditional practices such as the use of growth regulators and scheduled irrigation can cause berry split if the environmental conditions are conducive to increasing plant water potential.

2.3.5.1 Pre-harvest practices

Gibberellic acid (GA_3), which is commonly used for the enlargement of grape berries, has been found to cause splitting on Thompson Seedless, Redglobe and Ruby Seedless, the use of GA_3 in conjunction with CPPU has been found to increased the incidence of berry split even more (Zoffoli *et al.*, 2008b).

Irrigation practices such as surface and overhead sprinklers have been found to increase the incidence of berry split on Thompson Seedless, as well as in rabbiteye blueberry, if followed by a period of high relative humidity (Christensen, 1975; Perez-Harvey, 2008; Marshall *et al.*, 2002).

Other viticultural practice, such as prevention of dense canopies, production in low laying areas, and excessive irrigation contribute to elevated free water or humidity, which is specially critical during harvesting, further increases the potential for split to take place.

2.3.5.2 Post-harvest practices

Influencing the micro-environment in which the grapes are packed, may have an effect on the occurrence of post-harvest berry split. This includes packing material such as carrier bags, moisture absorbing materials, SO_2 sheets, and outer (micro-, macro- or non-perforated) bag types. Packing material with low levels of breathing ability can change the moisture content, as well as the temperature in the boxes. This can affect the severity of berry split, as well as other physiological disorders (Fourie, 2008).

It was further found that an increase in SO_2 levels may lead to an increase in post-harvest hairline cracking in Thompson Seedless (Zoffoli *et al.*, 2008a). This can be attributed to an event of increased relative humidity, with the subsequent formation of water droplets on the surface of the grapes. The SO_2 then dissolves in the droplets form H_2SO_4 (Sulfuric Acid), thereby acidifying the grape skin and causing it to break open.

2.3.5.3 Prevention practices

The prevention of berry split can be divided in long- and short-term strategies. Long term prevention strategies include: *(i)* planting split resistant cultivars, *(ii)* production in suitable viticultural areas, such as areas where rain and especially thunder showers are not a frequent occurrence during the ripening stages of grapes (Sekse, 1998), as well as low lying areas where humidity can lead to a higher incidence of berry split.

Short term prevention strategies include: *(i)* maintaining a constant soil water content, *(ii)* covering of canopies where rain is predicted close to harvest, *(iii)* early harvest as soon as the required sugar concentration is achieved, and *(iv)* the use of foliar sprays (Avenant, 2000)

In the event of unfavorable environmental conditions, the following factors need to be taken into consideration as part of possible prevention strategies: *(i)* the wetting period, *(ii)* weather conditions prior, and immediately after rainfall, *(iii)* stage of harvesting on a particular cultivar and vineyard, as harvesting of immature grapes can also result in berry split (Burger, 2005), and *(iv)* the susceptibility of a particular cultivar to berry split and decay (Fourie, 2008)

2.4 General discussion

Highly osmotically active photosynthate (sugar) produced in the leaf or source cells, causes an influx of water into the cells through the phloem. This influx in turn causes an increase in intracellular turgor pressure, which generates the mass flow of photosynthate through the xylem to the plant sinks. During the ripening phase, the majority of photosynthate is directed to the berry cells, although some will also reach the roots and shoots for storage.

In the berry cells, the sugar is stored in vacuoles in order to keep the osmotic potential of the cell low, to ensure the inflow of more sugar to the berries. Although, at this stage, some water will leave the berries through transpiration, the turgor pressure exerted on individual cell walls in the berry increases. In the event of turgor pressure exceeding the opposing force of the cell wall, individual cell walls break open, an event referred to as berry split.

The splitting of berries are most prevalent in the early morning, when turgor pressure will be at its highest, while transpiration low.

The plant micronutrient B, plays an inadmissible structural role in plant cell wall strength, as it forms strong complexes with pectin. These complexes have the advantage of being indestructible by wall acidification or expansin. Although the plant macronutrient Ca also plays an important role in plant cell wall strength by forming complexes with pectin, these complexes can however be removed by wall acidification, a natural occurrence during ripening of fruit, and expansin, a natural occurring enzyme aiding in fruit softening (summarized in Fig 2.4.1).

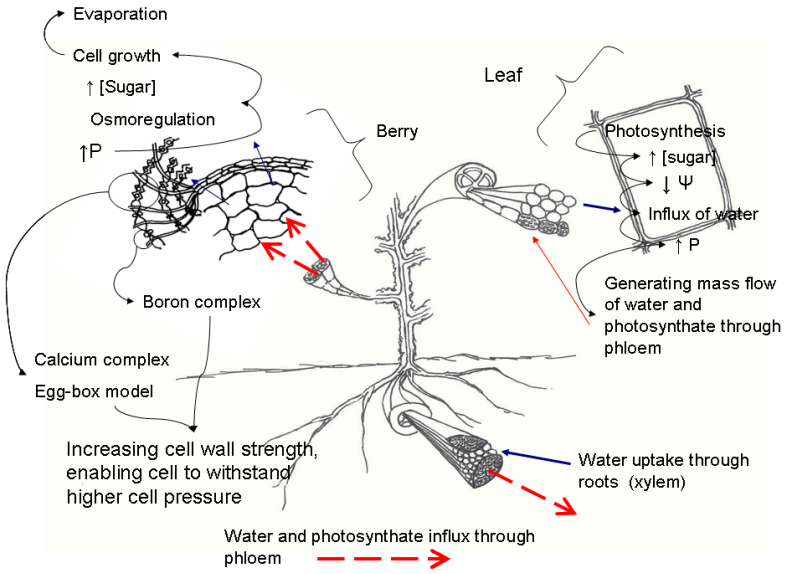

Figure 2.4.1: The proposed mechanism for the increase of pressure in berry cells, also showing the interaction of boron and calcium with pectin.

Viticulturists manipulate vines to prevent or reduce the occurrence of berry split in two ways: *(i)* increasing berry skin strength by addressing cell wall strength through the use of products such as B and Ca treatments, and *(ii)* by reducing the plant water status in such a way that growth is not negatively influenced, but to such an extend as to reduce the occurrence of berry split.

The aim of this study was to determine whether different plant water conditions, induced through irrigation frequencies, B and Ca, applied as bunch

directed sprays can influence the appearance of berry split, preventing financial losses for the producer.

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Chapter 3

The effect of different irrigation frequencies in combination with calcium and boron bunch applications on the susceptibility of SoutherngrapeOne to pre-harvest berry split.

3.1 Abstract

The novelty of table grapes justifies the demand for quality. The South African table grape export industry strives towards achieving this goal by the usage of integrated pre- and post-harvest management practices. Berry split is one of the main quality disorders since it diminishes the visual attraction of grapes. The sporadic occurrence of berry split makes it difficult to predict and hence to prevent.

The aim of this study was to determine whether different irrigation fre-

quencies, calcium (Ca) and/or boron (B) could play a significant role in the prevention of berry split. For two consecutive seasons (2006/07 to 2007/08) SoutherngrapeOne vines were subjected to irrigation frequencies based on typical irrigation scheduling used in the table grape industry, which comprised of a low, medium and high frequency. The low frequency was duplicated in order to demonstrate the effect that a heavy irrigation, just before harvest may have on berry split. The treatments were subdivided to accommodate four bunch spray treatments, i.e. Ca, B, combination thereof and water, applied from set to véraison.

Medium irrigation frequency resulted in the lowest amount of split in the first (2006/07) season but was not repeated for the 2007/08 season. Sub-treatments of Ca and B increased the amount of pre-harvest berry split.

3.2 Introduction

Prevention of berry split remains a challenge in the table grape industry. Split berries are easily infected by *Botrytis cinerea* resulting in rot and a reduction in value of the grapes. Split starts with the rupture of walls of individual cells of the skin (Considine & Kriedemann, 1972). Rupture of cell walls depends on two factors: *(i)* The mechanical strength of the cell wall which is determined by the cell wall composition and *(ii)* the pressure that is exerted on the cell wall by the inflow of water into the grape.

Water-flow into grapes is a passive process that is governed by transpirational pull (Leopold, 1964) and osmotically active solids accumulating in berry cells (Coombe, 1960, Zhang *et al* 1997; *teste* Huang *et al.* (2005)). Plant cells do not directly influence the turgor pressure, but can regulate it, by actively regulating the cell wall osmotic potential in two ways, i.e. osmoregulation, and osmotic adjustment (Morgan, 1984). In the third stage of berry development this is accomplished mainly by regulating the sugar concentration in the cytosol. Inflow of water increases pressure in the cell and thus on the cell wall as needed for cell expansion. When the pressure is too high for the cell wall to withstand, it ruptures.

It is generally assumed that vines exposed to low irrigation frequencies (**LIF**)as opposed to vines exposed to high irrigation frequencies (**HIF**), will

show more split berries when exposed to a sudden water availability. This may be because the vines undergoing **LIF**), would by means of osmotic adjustment, lower the water potential in the berry cells. A sudden increase in plant available water (PAW), due to rain or irrigation would then increase berry cracking (Avenant, 2000) and induce a higher percentage of pre-harvest split.

Cell wall strength is determined by the composition of hemicellulose, pectin and structural proteins that are intertwined with the network of cellulose micro-fibres (Brett & Waldron, 1990). Calcium (Ca) and boron (B) are termed *apoplastic elements*, mainly due to its localization in the cell wall where it plays a intricate role in cell wall strength (Matoh & Kobayashi, 1998). Cellulose micro fibres can form complexes with Ca (Ferguson, 1984; Jarvis, 1984) and B (Matoh & Kobayashi, 1998), giving these two elements unique cell wall structural support qualities.

Calcium is a macro-nutrient that has the ability to connect pectins to each other as well as pectines to polysaccharides by forming cross bridges (Wuytack and Gillet (1978) *teste* Demarty *et al.* (1984)). These bridges form calcium pectate gels that are regarded as strong cohesive networks, cross-linked by dimeric "egg-box" junctions between unesterified chain faces of cellulose (Walkinshaw & Arnott, 1981). These networks are strong enough to inhibit cell wall extensions (Tagawa & Bonner, 1957).

During ripening, acidification of the cell wall takes place (Huang *et al.*, 2005) and this can break the Ca bridge between pectin molecules (Brett & Waldron, 1990). The hemicellulose and micro-fibres that are held together by hydrogen bonds are broken by this acidification. Enzymatic activity such as expansin, which is naturally expressed at ripening to induce fruit softening (McQueen-Mason *et al.*, 1992) also weakens the cell wall. Furthermore, Ca flows from the skin into the flesh (Huang *et al.*, 2005) as ripening continues. The berry therefore becomes more and more suspectable to pre-harvest berry split.

Boron is an essential micro-nutrient for plants and is considered to perform a structural role in cell walls (Ishii *et al.*, 2001). Borate forms the most stable di-esters with cis-diols on furanoid rings, which makes these reactions limited to ribose and apiose (Loomis & Durst, 1992) on the cellulose micro-fibres forming boron-rhamnogalacturonan-II (Kobayashi *et al.*, 1996). This strengthens the

cell wall because the micro fibres are pulled together.

Sugar in grapes causes an inflow of water that results in a higher turgor pressure, at the same time cell wall weakening takes place through acidification and enzymatic activity, also known as turgor driven extension (Goldberg, 1975; Fry, 1989).

In this study, the susceptibility of grapes to split pre-harvest under different irrigation frequencies was investigated. The value of bunch applications of Ca and B to prevent berry split was also established. SoutherngrapeOne was used as a model cultivar because of its high susceptibility to berry split.

3.3 Materials and Methods

3.3.1 Experimental vineyards

SoutherngrapeOne, a newly released, late ripening, white seedless cultivar was grafted on pre-established Ramsey rootstocks. The individual cordon and vines were trained in alternative directions on a gable (double slanting) trellis system. Vines were spaced 3.5m x 1.0m on an Oakleaf soil (MacVicar *et al.*, 1977) on the farm Clovelly in the Hex River valley, near De Doorns (33°47'S 19°67'E). Soil textural analysis revealed variation in the block which has patches of sandy loam, loamy sand, sandy clayloam and sand (to a depth of 900 mm). Though the heavier soils could negate the effect of the irrigation, a randomized block design was used in the statistical analysis of the data.

3.3.2 Experimental layout and treatments

The experimental design was a split-plot with irrigation as main plot treatment and bunch spray treatments, Ca and B, as split plot factors during two consecutive growing seasons (2006/07 and 2007/08). The main plot design was a randomized complete block with 4 irrigation frequency treatments, 1) high irrigation frequency (HIF) irrigated 12.4 mm every second day, 2) medium irrigation frequency (MIF) irrigated 24.9 mm every fourth day, 3) low irrigation frequency (LIF) irrigated 50.9 mm every eighth day, and 4) a duplication of the low irrigation receiving an irrigation just before harvest (LIF+I), repli-

cated at random in 6 blocks. The treatment design of the split-plot factors was a 2 x 2 factorial with two Ca levels (without Ca, with Ca) and two B levels (without B, with B), plus a water control the second year, randomly allocated within each main plot treatment (Table 3.3.1). To obtain more information from the sub-plots effect treatments, single degree meaningful contrasts were drawn up to test the factorial effect and control effect. For the 2006/7 season each main plot consisted of eight experimental vines and each sub-plot consisted of two vines, the 2007/08 season consisted of ten vines per main plot. The experimental vineyard covered approximately 0,2 ha. Due to limited vines available there were no side rows in the block. To minimize overlapping treatment effects sprinklers with a narrow radius (75cm) was used in this trail. Soil capacitance probes (DFM Probe Utilities) were used to verify the soil water content change in response to the scheduled irrigations and to monitor for overlapping irrigations which did not occur (Fig. 3.3.1).

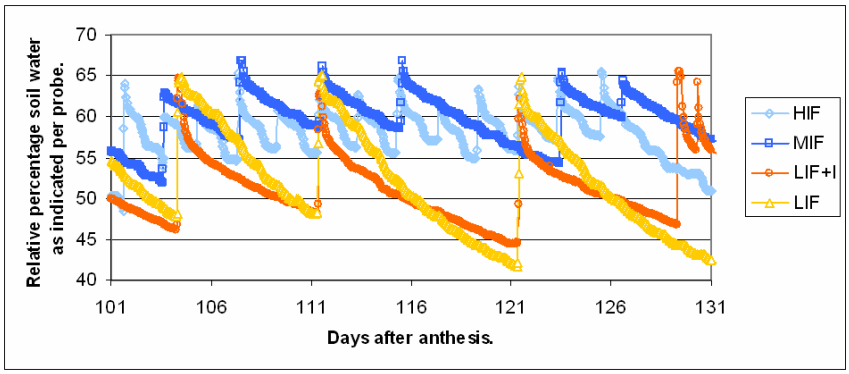


Figure 3.3.1: **Typical soil probe readings for 2007/08 indicating differences in scheduled frequencies. HIF with frequent irrigations, MIF with twice as long water withdrawal times. And LIF and LIF+I receiving the same irrigation with the exception that LIF+I received a heavy irrigation two and one days before harvest.**

Root studies revealed an average root depth of 700 mm. This was the depth used to calculate the plant available water (PAW), based on an estimation of the soil water holding capacity as deduced from the soil texture.

Table 3.3.1: **Bunch spray treatments (sub-treatments) applied during the 2006/07 and 2007/08 growing season. Treatments were applied as four bunch directed sprays from set to veraison at Clovalley in the Hex River Valley.**

Sub-treatment	Description
Control	Neither Ca nor B applied
Boron	1.5kg/ha Solubor[1]
Calcium	8L/ha Stopit®[2] and 5L/ha Caltrac®[3]
Ca + B	Combination of treatment B and Ca
Water[4]	Clean water spray

[1] 307.5g B/kg
[2] $CaCl_2$ at 160g Ca^{2+}/L
[3] $CaNO_3$ at 400g Ca^{2+}/L
[4] Applied only in the 2007/08 season

3.3.3 Statistical analysis

Analysis of variance was performed on all variables assessed using General Linear Models, Procedure of SAS statistical software version 9.1 (SAS Institute Inc., Cary, NC, USA)). Shapiro-Wilk test was performed to test for normality (Shapiro & Wilk, 1965). Student's t-least significant difference was calculated at the 5% level to compare treatment means. A probability level of 5% was considered significant for all significance tests (Snedecor & Cochran, 1980).

3.3.4 Measurements

3.3.4.1 Climatic data

Daily rainfall and mean daily relative humidity (2006/07 and 2007/08 seasons) for the area were obtained from an automatic weather station at the Hex Valley Experimental Farm.

3.3.4.2 Determining total soluble solids, titratable acidity and pH.

From each plot samples were taken (see section 3.3.4.5) and used to determine berry mass. Thereafter the berries were pressed by means of a small bag press,

and filtered. The filtrate was used for determining sugar concentration (total soluble solids), acid concentration (titratable acids) and pH of the juice. Total soluble solids (TSS) concentration was determined with an electronic hand refractometer (Model: ATAGO dbx 30) and is expressed as °Brix, while the acid concentration (g/L) and pH was determined with a Mettler DL21 titrator.

3.3.4.3 Calcium and boron analysis

After cold storage twenty undamaged berries were individually washed in lukewarm water with Teepol and rinsed twice in distilled water to remove any Ca or B residues remaining from the bunch sprays. The berries were then frozen at -20°C. The skins were removed after it was thawed in warm water for 20-30 seconds. The berry skins were rinsed once more in distilled water to remove any juice that may have washed onto the skins and were then allowed to air dry. Both skins and flesh were analyzed for Ca and B content at an accredited commercial soil and plant tissue laboratory by incinerating it for analysis using a ICP spectrometer.

3.3.4.4 Determining the effect that irrigation frequency has on plant water status

During the two consecutive growing seasons, stem water potentials were measured with a pressure chamber once a week over a two month period (February and March) up to harvest. This was done to establish whether the different irrigation frequencies affected plant water status. Five leaves per treatment were covered with polyethylene bags and aluminum foil, to prevent light and air movement, for a minimum of 50 minutes from 13:00 onwards. Thereafter it was cut from the shoot with a sharp blade, immediately removed from the bag and put into a pressure chamber where the reading were taken (as adapted from Begg & Turner (1970)).

3.3.4.5 Sampling for berry split

Bunches that displayed more that 40% split berries before harvest were removed from the vines. The bunches that had to be removed pre-harvest were

weighed and expressed as a percentage of the total crop weight.

The percentage of berry split over time for the 2006/07 season, from véraison to harvest, was calculated by randomly cutting five berries from the top to the bottom of each of five bunches on two vines, which made an experimental unit. A total of 50 berries were sampled. The results were expressed as percentage berry split per number of berries.

3.4 Results and discussion

The analysis of variance of the different parameters are presented in Tables A.0.3 to A.0.7. Only those variables that showed significant differences in the various seasons are discussed.

3.4.1 Climatic data

The focus of this study was on the influence of plant water status on berry split. Unfortunately humidity and rainfall are unpredictable in field trials and may have influenced the outcome of these results. During the 2006/07 season a total of 90.5 mm rain fell subsequent to anthesis with 51.9 mm that fell 21 days prior to harvest. The 2007/08 season received 1.4 mm of rain 21 days prior to harvest with a total of 50.2 mm from anthesis (Fig. 3.4.1). The bulk of the relative humidity stayed between 40 and 75%, with even smaller differences closer to date of harvest.

3.4.2 Total soluble solids accumulation

The TSS accumulation during the season was influenced by the treatments in different ways (Table 3.4.1). Overall, the application of Ca resulted in lower sugar levels (Fig. 3.4.2), whereas B succeeded in rising or maintaining the same sugar level as the control. However, these effects were diminished towards the end of the season, at which point there were no statistical differences in sugar levels at harvest.

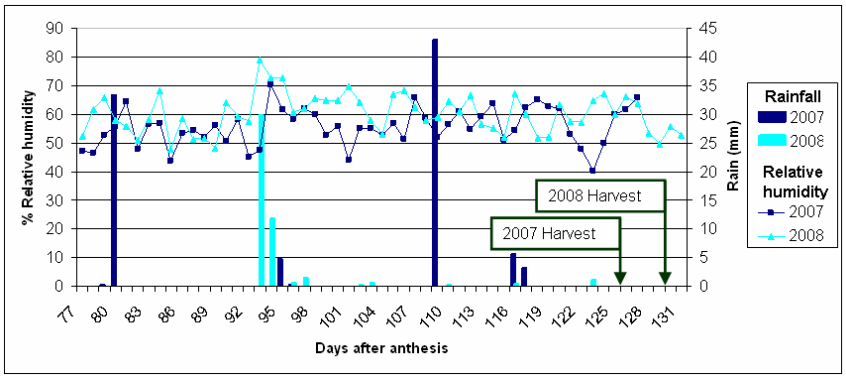


Figure 3.4.1: **Precipitation and mean daily relative humidity for the Hex River Valley Experimental Farm for 2007 and 2008 seasons.**

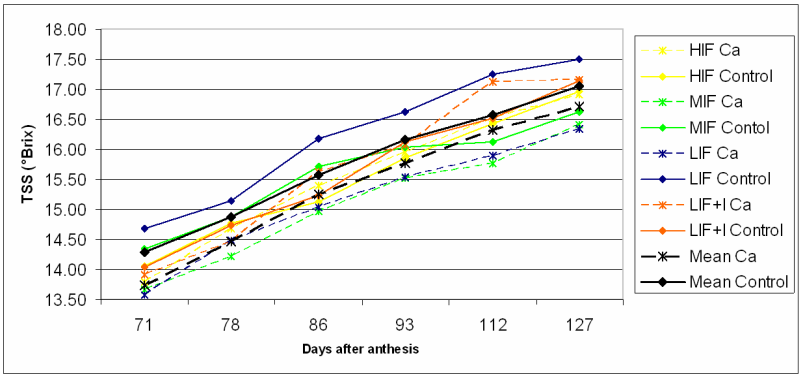


Figure 3.4.2: **Effect of Ca bunch spray under different irrigation frequencies on TSS accumulation of SoutherngrapeOne in the Hex River Valley (2006/07).**

3.4.3 Plant water status

Stem water potentials were measured in order to investigate whether the different irrigation frequencies effected plant water status. The removal of the bags from the leaves before a reading was taken might have resulted in leaves from the HIF treatment developing immediate stress, albeit very slight. In both seasons HIF and MIF resulted in similar readings (Table 3.4.2) which indicates the possible negating effects obtained by removal of the bags before each reading was taken (Dr. P.A. Myburgh: Personal communication, 2010). Higher accumulative stem water potentials in both LIF treatments indicates that vines in theses treatments experienced more water stress for the dura-

Table 3.4.1: **Treatments that affected TSS (°Brix) accumulation during ripening on SoutherngrapeOne (2006/07).**

DAA	Treatment	Mean	t-Grouping	P-value	LSD
71	Control	14.29	A	*<0.0001*	*0.289*
	Boron	14.34	A		
	Calcium	13.73	B		
	Boron + Calcium	13.80	B		
78	Control	14.88	A	*0.0003*	*0.3136*
	Boron	14.91	A		
	Calcium	14.46	B		
	Boron + Calcium	14.35	B		
86	Control	15.57	B	*<0.0001*	*0.3323*
	Boron	16.01	A		
	Calcium	15.25	B		
	Boron + Calcium	15.33	B		
93	Control	16.15	B	*0.0007*	*0.3585*
	Boron	16.53	A		
	Calcium	15.77	C		
	Boron + Calcium	15.95	BC		
112	Control	16.58	AB	*0.0338*	*0.4835*
	Boron	16.94	A		
	Calcium	16.33	B		
	Boron + Calcium	16.26	B		
127	Control	17.06	AB	*0.0093*	*0.4635*
	Boron	17.52	A		
	Calcium	16.71	B		
	Boron + Calcium	17.02	B		

Values with different letters are significantly different at specific days after anthesis.

tion of the trail than those vines subjected to the HIF and MIF treatments. The readings on the day of harvest for the LIF+I treatments during both seasons clearly shows increased (less negative) values, indicating stress relaxation before harvest.

A clear distinction therefore occurred in the average plant water status between the HIF, MIF and LIF treatment.

Table 3.4.2: **Stem water potentials during the 2006/07 and 2007/08 season as induced by different irrigation frequencies on SouthernGrapeOne.**

Season	Irrigation frequency	Accumulative stem water (MPa^2)	Stem water at harvest (KPa)
2006/07	HIF	39.7 A	-882.8 B
	MIF	41.2 A	-850.8 B
	LIF	47.2 B	-1092.8 A
	LIF+I	50.2 B	-920.0 B
	P-value	0.001	0.0003
	LSD	4.9	94.9
2007/08	HIF	35.7 A	-631.67 B
	MIF	36.0 A	-671.7 B
	LIF	38.9 AB	-908.3 A
	LIF+I	42.7 B	-640.0 B
	P-value	0.01	<0.0001
	LSD	6.31	98.5

3.4.4 Berry Ca and B content

None of the applications raised the B or Ca concentration in either the skin or the flesh of the grapes. No increase of Ca content could therefore be achieved in the skin by any of the treatments (Table 3.4.3). Neither did any of the treatments succeeded in raising the B content in the skin nor the flesh (Table 3.4.4). The fact that the Ca and B treatments showed lower concentrations in the flesh for Ca and B than the control, indicate to the fact that flesh Ca and B can be affected by other factors which cannot be overruled by bunch sprays. The lowering of the B and Ca does however not seem logical. However, this phenomenon is not unique to grape cultivars as it also occurs in apples, citrus and stone fruit (Dr. A. Kotze & Mr. M van Zyl: Personal communication, 2009)

There was also a possibility that the preparation of the flesh and especially the skins, by repeatedly rinsing in distilled water, could influence the Ca and B content. This however seems unlikely since all the samples were treated the same and only unsplit berries were used.

Table 3.4.3: **Effect of B and Ca bunch directed spray treatments on the Ca content of the berry skin and flesh of SoutherngrapeOne (2007/08).**

Treatment	**Skin Ca (mg/kg)**	**Flesh Ca (mg/kg)**
Control	1886.1 A	814.6 a
Boron [a]	1799.4 A	571.8 b
Calcium [b]	1900.4 A	500.6 b
Calcium + Boron	1697.0 A	636.2 ab
P value	0.4388	0.0248
LSD	293.52	204.87

[a] Solubor at 1.5kg/ha
[b] Stopit® at 8L/ha combined with
Values with different letters are significantly different
Caltrac® at 5L/ha

Table 3.4.4: **Effect of B and Ca bunch directed spray treatments on the B content of the berry skin and flesh of SoutherngrapeOne (2007/08).**

Treatment	**Skin B (mg/kg)**	**Flesh B (mg/kg)**
Control	0.31 A	0.49 a
Boron[a]	0.29 A	0.36 b
Calcium[b]	0.29 A	0.29 b
Calcium + Boron	0.29 A	0.40 ab
P value	0.5488	0.0205
LSD	0.0231	0.1195

[a] Solubor at 1.5kg/ha
[b] Stopit® at 8L/ha combined with Caltrec® at 5L/ha
Values with different letters are significantly different

3.4.5 Pre-harvest berry split

3.4.5.1 Pre-harvest berry split due to different irrigation frequencies

The danger of pre-harvest berry split lies in the degradation of the bunches and sporulation of *Botrytis cinerea*. Figure 3.4.3 illustrates the typical development

of bunch decay due to splitting if it is not removed from the vines.

(a) Grape bunch displaying berry split: *Pre-harvest.*

(b) Grape bunch displaying berry split with advanced signs of decay: *During harvest.*

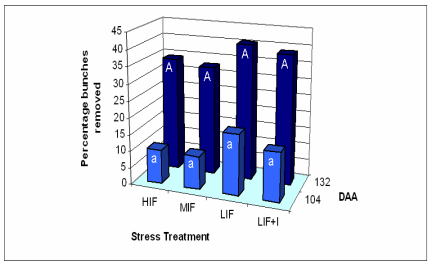
(c) Grape bunch with serious decay: *Post-harvest.*

Figure 3.4.3: **Progressive degradation of SoutherngrapeOne bunches that displayed early symptoms of berry split (2007/08).**

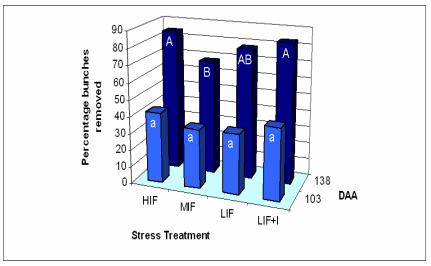
In the 2006/07 season there was no difference in the amount of split generated by the irrigation frequencies (Fig. 3.4.4). This result is similar to work done on Alphonse Lavalee (Combrink *et al.*, 1982), Barlinka (Fourie, 1989),as well as on Sunred Seedless and Muscat Supreme (Myburgh & Howell, 2007), where neither irrigation level nor period of withdrawal during ripening affected the amount of berry split.

Only the 2007/08 season showed treatment differences in pre-harvest berry split (Fig. 3.4.4). Up to 21 days prior to harvest, the 2006/07 season received 51.6 mm rain, where the 2007/08 season was only subjected to 1.4 mm of rain in the same time (Fig. 3.4.1). The drier later season may have led to the irrigation treatments having a larger effect on the PAW and therefore berry split.

From 2007/08 it seems that *(i)*, vines exposed to low irrigation frequencies (**LIF** and **LIF+I**), similar to results found by Myburgh (2005) and *(ii)*, heavily irrigated (**HIF** treatment) vines both increase pre-harvest berry split (Fig. 3.4.4). Investigating the effect of plant water stress and irrigation cut off time, 40% of PAW withdrawal terminated at 15°Brix resulted in the best overall berry quality for Sunred Seedless, while 20% of PAW withdrawal, irrespective of being cut off at 15°Brix, was found to be the best for Muscat Supreme (Myburgh & Howell, 2007). This illustrates that cultivar differences do exist. Medium irrigation frequency seems to be the safest irrigation approach to minimize the appearance of pre-harvest berry split on SoutherngrapeOne, which is in accordance with previous research results on other cultivars (Myburgh & Howell, 2007).



(a) Effect that different irrigation frequencies had on bunches that had to be removed in the 2006/07 season. *(104 DAA with P=0.4659, LSD=12.42 and 132 DAA with P=0.6952, LSD=15.76).*



(b) Effect that different irrigation frequencies had on bunches that had to be removed in the 2007/08 season. *(103 DAA with P=0.476, LSD=12.82 and 138 DAA with P=0.047, LSD=12.34).*

Figure 3.4.4: **The effect of irrigation frequencies on the amount of bunches that had to be removed during the both 2006/07 and 2007/08 growing season on an experimental block of Southerngrape-One in the Hex River Valley. Bars with different letters are significantly different.**

The critical split point[2] for SouthergrapeOne is not yet known. From Fig. 3.4.5 it seems that this point may lie above 15.5°Brix, since this is the point where there was a major increase in berry split.

[2]Point of sugar accumulation where a specific cultivar is most suspectable to berry split (Mr. J.H. Avenant: Personal communication, 2007)

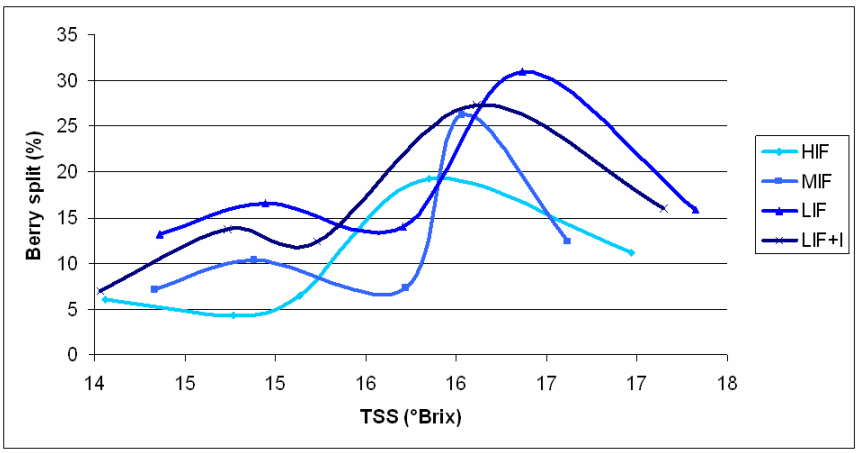
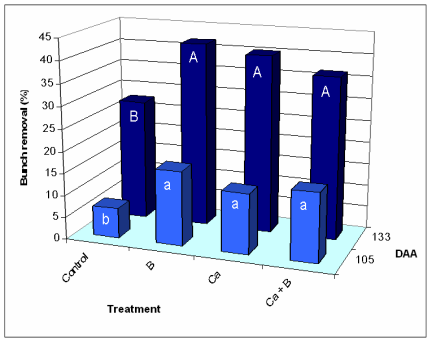


Figure 3.4.5: **The relation between irrigation frequency and TSS to berry split, indicating a possible critical split point of Southern-grapeOne above 15.5°B (2006/07).**

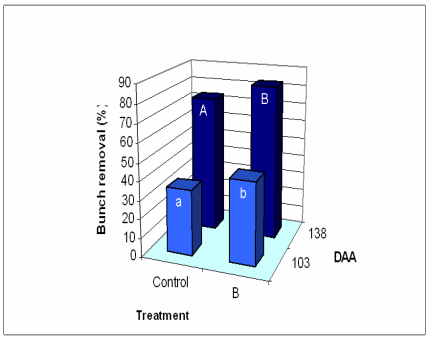
3.4.5.2 Pre-harvest berry split as affected by bunch applied calcium and boron.

In the 2006/07 season, Ca and B treatments showed a higher occurrence in split compared to the control (Fig. 3.4.6a) resulting in more bunches that had to be removed. The decline of the Ca and B content in the flesh (Tables 3.4.3 and 3.4.4), possibly due to the diluting effect after véraison (Mr. P. Raath: Personal communication, 2009), may render the grapes more suspectable to berry split. Combrink *et al.* (1982) also found that neither Ca nor B bunch applications reduced the occurrence of berry split on Alphonse Lavallee. It was speculated that the extra water that was put on the grapes might have raised the humidity around the grapes, thereby increasing the berries susceptibility to split, since it possibly occurs due to the direct uptake of water from the environment (Glenn & Poovaiah, 1989). In the following season (2007/08) a fifth sub-treatment of only a water spray on the grapes was therefore introduced. The water bunch treatment however, did not show any effect on the occurrence of split. Bunch applied B, again, caused an increased incidence of split (Fig. 3.4.6b) in the 2007/08 season, but not the Ca or combination application. Calcium may however be helpful in the prevention of berry split early in the season (Table 3.4.5). This may possibly be due to a higher con-

centration of skin Ca early in the season, since there is a movement of Ca from the skin into the flesh post véraison (Huang *et al.*, 2005).



(a) Effect of calcium and boron bunch treatments on pre-harvest berry split at 105 and 133 DAA in the 2006/07 season *(105 DAA with P=0.0123, LSD=4.4845 and 133 DAA with P=0.0002, LSD=6.3945).*



(b) The amount of pre-harvest berry split as induced by boron in the 2007/08 season *(103 DAA with P=0.0211, LSD=8.0983 and 138 DAA with P=0.0162, LSD=7.6504).*

Figure 3.4.6: **Effect of B and Ca bunch applications on the number of bunches that had to be removed due to pre-harvest berry split in 2006/07 and 2007/08. Bars with different letters are significantly different.**

3.4.5.3 Split as induced by the applied treatments during ripening

Berry split was found to be influenced separately by irrigation and bunch spray treatments (Table 3.4.5). At 86 DAA, the **LIF** resulted in the highest incidence of berry split. Similarly, vines exposed to low irrigation frequency displayed a higher incidence of berry split throughout the season. This trend continued until 112 DAA, when the highest incidence of berry split was observed for the season, closely relating to the supposed critical split point (Fig. 3.4.5) for SoutherngrapeOne. Following this critical split point, no further differences were generated by the applied treatments. Calcium and B had very little effect on split during the season. However, at 93 DAA the Ca and combination spray of Ca plus B reduced the amount of split substantially. Later in the season this effect may have been overcome by the diluting effect due to the swelling of grapes after véraison.

Table 3.4.5: **Effect of irrigation and bunch spray treatments with B and Ca on percentage berry split SoutherngrapeOne (2006/07).**

DAA	Treatments	Mean (%)	t-Grouping	P-value	LSD
86	HIF	6	B	*0.033*	*5.1466*
	MIF	7	B		
	LIF	13	A		
	LIF+I	7	B		
	Control	7	A	0.4189	2.4462
	Boron	9	A		
	Calcium	8	A		
	Boron + Calcium	9	A		
93	HIF	4	B	*0.0091*	*6.7816*
	MIF	10	AB		
	LIF	17	A		
	LIF+I	14	A		
	Control	12	AB	*0.0147*	*4.4316*
	Boron	15	A		
	Calcium	9	B		
	Boron + Calcium	9	B		
105	HIF	6	C	*0.0522*	*5.6828*
	MIF	7	BC		
	LIF	14	A		
	LIF+I	12	AB		
	Control	12	AB	0.5994	5.7449
	Boron	12	A		
	Calcium	6	C		
	Boron + Calcium	7	BC		
112	HIF	19	B	*0.0135*	*6.6504*
	MIF	26	A		
	LIF	31	A		
	LIF+I	27	A		
	Control	22	A	0.5883	7.48
	Boron	27	A		
	Calcium	27	A		
	Boron + Calcium	28	A		

Values with different letters are significantly different

3.5 Conclusion

Different stem water potentials induced by means of irrigation frequencies did not result in reproducible data over the two growing seasons. Frequent and seldom irrigated vines (that was established by the **HIF**, **LIF** and **LIF+I** treatments) resulted in the same number of bunches that had to be removed due to excessive berry split. This is contradicting to the belief in the grape industry that irrigation close to harvest will result in an increase of berry split. The MIF treatment resulted in the lowest number of bunches that had to be removed pre-harvest. This was found to be the case for only the 2007/08 season. A lack of similar results may have been caused by late rain in the 2006/07 season. Berry sampling over time indicated that **HIF** and **MIF** will be the safest strategy to prevent berry split up to the critical split point. From these results, the **MIF** seems to be the best prevention strategy of pre-harvest berry split on SoutherngrapeOne.

None of the bunch applications of B, Ca or the combination thereof, resulted in an increased concentration of B or Ca content in the berries. Furthermore, non of these bunch treatments result in a decrease of berry split over time. The wide belief that B and Ca treatments can prevent berry split, is proving inconsistent with the results presented in this study. However, the approach to increase the structural B and Ca content in the berry skin, could still prove to be advantages in the prevention of berry split.

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Chapter 4

The effect of different irrigation frequencies in combination with calcium and boron bunch sprays on post-cold storage physiological disorders on SoutherngrapeOne.

4.1 Abstract

The geographic placement of the South African table grape industry forces the use of shipment which may take up to 7 weeks to reach Europe and the Far East. This time delay exposes grapes the to a range of physiological disorders.

The effect irrigation frequency, combined with boron (B) and calcium (Ca) applications on post cold storage quality of SoutherngrapeOne, was investigated over a 2 year period in the Hex River Valley. Different irrigation frequencies were chosen, based on based on typical irrigation scheduling used in the table grape industry, which comprised of a low, medium and high frequency. The low frequency was duplicated in order to demonstrate the effect that a

heavy irrigation, just before harvest may have on berry physiology post-cold storage. These treatments were sub-divided to allow for B, Ca, combination of B and Ca and water as bunch directed sprays.

Irrigation frequency affected the stem colour of packed grapes after cold storage. Boron reduced post-cold storage berry split. Calcium led to an reduced loose berries and forced berry drop. It also caused an increase in berry cracking and decay. No clear trend were observed in the incidence of post-harvest cracking in relation to differences in irrigation frequency.

4.2 Introduction

Physiological disorders which develop during cold storage, can result in financial loss for the producer. These disorders include rachis browning, loose berries, sulfur burning, internal and external browning, as well as split berries.

Rachis browning is a factor of water loss. It is mainly dependant on maturity of the rachii and its thickness. A 2 % water loss is sufficient to show browning on Flame Seedless, Thompson Seedless, Ruby Seedless and Fantasy Seedless stems (Kader, 2002). More woody and mature Thompson Seedless stems tend to brown slower than immature stems during cold storage. Stems with a pedicel diameter of 4 mm or greater show less browning during cold storage (Villalta *et al.*, undated).

Loose berry or abscission, is the separation of organs from the mother plant. It is brought about by changes in gene expression causing the loosening of adjacent cell walls (Taylor & Whitelaw, 2001). **Berry shatter**, refers to berry drop from the cap stem due to fragile stalk tissue structure, **wet drop** is the separation due to a short and thin berry brush and **dry drop** is the separation due to the formation of a abscision zone between the pedicel and berry (Deng *et al.*, 2007).

Sulfur burn. Two stage sulfur dioxide systems are generally used for the control of *Botrytis cinerea* during cold storage (Nelson & Gentry, 1966). It can however cause bleaching of berries, premature browning of grape stems (Marois *et al.*, 1986) and induce hairline cracking (Palou *et al.*, 2002), due to the high initial SO_2 released in the box combined with high relative humidity

(Opperman *et al.*, 2007). For successful shipment, grapes need to be able to withstand the SO_2 levels that inhibit the growth of *Botrytis cinerea*.

Split berries are one of the main quality defects that occur in the export of table grapes. They can easily become infected by *Botrytis cinerea* resulting in decay. Split starts with the breaking of individual cell walls of the skin (Considine & Kriedemann, 1972). Rupture of cell walls depends on two factors: *(i)* the mechanical strength of the cell wall and *(ii)* the pressure that is exerted on the cell wall.

The strength of cell walls are influenced by the network of hemicellulose, pectin and structural proteins inter-knitted with cellulose micro-fibers (Brett & Waldron, 1990). These micro fibres can form complexes with calcium (Ca), (Jarvis, 1984; Ferguson, 1984) and boron (B) (Matoh & Kobayashi, 1998), both of which have been shown to have a structural role in the cell wall. Calcium forms "egg-box" junctions between cellulose fibers to form calcium pectate gels that are regarded as strong cohesive networks (Walkinshaw & Arnott, 1981), while B has the ability to pull these fibers closer together with the formation of boron-rhamnogalacturonan-II (Kobayashi *et al.*, 1996) and doing so strengthening the cell wall. Due to the low osmotic potential, caused mainly by sugar accumulation (Coombe, 1960) before harvest there is an inflow of water causing the pressure to rise (Dreier *et al.*, 2000), which may later lead to berry split. This is emphasized by the importance that pre-harvest water management has on post-harvest quality (Fourie, 2008).

In this study, the susceptibility of grapes to post-harvest berry split when exposed to different irrigation frequencies and bunch applied B and Ca, was investigated on SoutherngrapeOne as a model cultivar because of its high susceptibility to split.

4.3 Materials and Methods

The experimental vineyard and layout is described in Chapter 3.

4.3.1 Statistical analysis

Analysis of variance was performed on all variables assessed using General Linear Models, Procedure of SAS statistical software version 9.1 (SAS Institute Inc., Cary, NC, USA). Shapiro-Wilk test was performed to test for normality (Shapiro & Wilk, 1965). Student's t-least significant difference was calculated at the 5 % level to compare treatment means. A probability level of 5 % was considered significant for all significance tests (Snedecor & Cochran, 1980). For the stem colour (See 4.4.2.5) the Chi-Square test was used, to determine relationships between groups and the Cramer's V test was used to determine associations between treatments.

4.3.2 Packing and cold storage

In each experimental unit, consisting of two vines, four of the best bunches per vine were selected and marked with different coloured tape. All eight bunches were packed in individually marked crates and transported to the packing facility on the farm. Damaged berries were removed and weighed separately from the bunches that were packed. Bunches were packed in a 9 kg box in a micro-perforated bag with a Uvasys® SO_2 sheet on top of the grapes for postharvest fungal control, moisture absorbing paper-sheets were placed at the bottom of the box, and between the grapes and the SO_2 sheet. The bags were then folded and sealed with tape. Each box represented a main irrigation treatment replication with its sub-treatments. The grapes were stored at -0.5°C for 5 weeks followed by 1 week at 10°C at Infruitec-Nietvoorbij Stellenbosch, to simulate transport and sale conditions.

4.3.3 Measurements

Climatic data for both seasons along with stem water potentials, induced by different irrigation frequencies, as well as berry B and Ca analysis are specified in Chapter 3.

4.3.3.1 Physiological disorders

Grape quality after cold storage was determined by sorting all the berries and grouping them as described in Table 4.3.1. These groups were weighed separately and expressed as a percentage of total berry weight. If more than one disorder occurred on the same berry it was prioritized as follows, first the loose and forced berry drops were grouped, then; 1) Split, 2) Hairline cracking, 3) Decay, 4) Internal browning, 5) External browning and 6) SO_2 damage.

Stems were classified according to level of discolouration, into five groups. *Group 1*: green as if just harvested, *group 2*: more green that brown, *group 3*: green/brown, *group 4*: more brown than green and *group 5*: fully brown stems. All the replications were evaluated on the same day with the help of the ARC Infruitec-Nietvoorbij's trained staff.

Table 4.3.1: **Post storage sorting characteristics of SouthrengrapeOne for seasons 2006/07 and 2007/08.**

Berry Disorder	**Characteristics**
Loose berry	Loose berries in the carry bag.
Forced berry drop	Berries that fell off after four tucks on the main rachis.
Split	Berries with splits into the flesh.
Hairline Cracking	Berries that were only cracked through the skin.
SO_2 damage	Slight whitening of the skin (bleaching in circular forms).
Decay	Mould growing on the berries.
Internal browning	Brown colouring of the berry, when cut open, the flesh is discolored.
External browning	Brown colouring of the berry, only the skin is discolored.

4.4 Results and discussion

Irrigation frequency lead to different stem water conditions (Chapter 3). Boron and Ca bunch sprays lead to a decrease in B and Ca content in the flesh when compared to the control, where the content in the skin remained unchanged.

4.4.1 Total soluble solids at harvest

The sugar content at harvest is presented in Table 4.4.1. Calcium affected the sugar content in both seasons, lowering it. The high percentage of bunches that had to be removed in the 2007/08 season (Chapter 3), compelled the harvest at a lower sugar concentration.

Table 4.4.1: **Total soluble solids at harvest as affected by bunch sprays of SoutherngrapeOne at Clovelly (2006/07 and 2007/08).**

Season	Bunch spray treatment	Mean	t-Grouping	P-value	LSD
2006/07	Control	17.06	AB	0.0379	0.4635
	Boron	17.5	A		
	Calcium	16.7	B		
	Boron + Calcium	17.0	B		
2007/08	No Calcium	15.0	A	0.015	0.3419
	Calcium	15.0	B		
	No Boron	15.0	A	0.8561	0.3419
	Boron	15.0	A		

Means with different letters are significantly different.

4.4.2 Post cold storage physiological disorders

The analysis of variance are presented in Table B.0.1, B.0.2 and B.0.3. Only those variables that showed significant differences in various seasons are discussed. Tables B.0.4 and B.0.5 contains the mean percentage disorders as expressed per weight of berries.

4.4.2.1 Loose berry, and forced berry drop

Different irrigation frequencies did not result in differences of loose berries or forced berry drop in the 2006/07 season. The rain in that season could result in irrigation having less of an effect. This was not the case for the 2007/08 season during which the occurrence of loose berries, showed a complex interaction with irrigation and bunch spray treatments, with no repeatable pattern emerging (Table 4.4.3). In the 2006/07 season, neither B nor Ca sub-treatments resulted in significant differences concerning berry drop compared to the control and each other (Table 4.4.2).

Table 4.4.2: **The effect of bunch directed sprays on forced berry drop of SoutherngrapeOne after cold storage (2006/07).**

Bunch spray treatment	% Forced berry drop	t-Grouping
Control	2.4	AB
Boron	2.8	A
Calcium	1.8	B
Boron + Calcium	1.6	B

Means with different letters are significantly different (P= 0.0205, LSD=0.8483)

4.4.2.2 Berry split

Even though the 2006/07 season received more rain than the 2007/08, non of the irrigation treatments influenced berry split. This result is in contrast to the belief that irrigation close to harvest will result in split during cold storage. In the 2006/07 season however, the bunch directed sprays resulted in differences. The B application significantly reduced the percentage of berry split (Table 4.4.4).

Table 4.4.3: **The effect of irrigation frequency combined with bunch directed sprays of Ca and B on loose berries of SoutherngrapeOne after cold storage (2007/08).**

Irrigation frequency	Sub-treatment	% Loose berries	t-Grouping
HIF	Control	1.6	d
	Water	3.7	abcd
	Boron	4.4	ab
	Calcium	2.1	bcd
	Calcium + Boron	1.7	d
MIF	Control	2.9	bcd
	Water	2.4	bcd
	Boron	2.4	bcd
	Calcium	5.4	a
	Calcium + Boron	3.1	abcd
LIF	Control	2.0	bcd
	Water	1.5	d
	Boron	3.9	abcd
	Calcium	2.9	abcd
	Calcium + Boron	1.4	d
LIF+I	Control	4.4	ab
	Water	4.2	abc
	Boron	1.8	cd
	Calcium	2.7	bcd
	Calcium + Boron	2.9	abcd

Means with different letters are significantly different. (P= 0.0399, LSD= 2.2735)

Table 4.4.4: **The effect of bunch spray treatments on split that occurred during cold storage on SoutherngrapeOne (2006/07).**

Bunch spray treatment	% Split	t-Grouping
Control	14.8	A
Boron	6.9	B
Calcium	14.0	A
Boron + Calcium	12.3	A

Means with different letters are significantly different. (P= 0.0036, LSD= 4.5277)

4.4.2.3 Hairline cracking

Hairline cracking was induced by all the bunch spraying treatments in 2007/08 (Table 4.4.5). Hairline cracks may be due to pre-harvest conditions when these cracks can form but also other factors such as, fruit quality, post-harvest handling or storage conditions (Palou *et al.*, 2002). The control (un-sprayed grapes) developed the least hairline cracking.

Table 4.4.5: **The effect of bunch directed sprays on hairline cracking of SoutherngrapeOne (2007/08).**

Bunch spray treatment	% Hairline cracking	t-Grouping
Control	0.3	B
Boron	0.7	A
Calcium	0.6	AB
Boron + Calcium	0.3	AB

Means with different letters are significantly different. (P= 0.0058, LSD= 0.3363)

4.4.2.4 Decay

Statistical differences between the bunch sprayed treatments were only found in the 2006/07 season. This may have been due to a higher concentration of spores in the 2006/07 season, the presence of which is random at best. Calcium showed an increase in decay after cold storage (Table 4.4.6). This may however not have been a direct effect of the Ca spray, but rather a secondary effect due to the pre-harvest berry split it may cause (Chapter 3). Post-harvest decay on grapes is mainly due to *Botrytis cinerea* that can sporulate and grow in boxes where free water, sustained by a high relative humidity (Fourie, 2008) and low sulfur dioxide levels (Zoffoli *et al.*, 2008) can be found. Calcium applications may also increase the occurrence of micro-cracks in the skin (Myburgh, 2005) due to uneven uptake of Ca, resulting in differences in skin flexibility (J.P. Zoffoli: Personal communication, 2009).

Table 4.4.6: **The effect of bunch sprays on post cold storage decay of SoutherngrapeOne (2006/07).**

Bunch spray treatment	% Decay	t-grouping
Control	1.3	B
Boron	1.9	AB
Calcium	2.2	A
Boron + Calcium	2.4	A

Means with different letters are significantly different.
(P= 0.0720, LSD= 0.8202)

4.4.2.5 Stem color

The stem colour is related to water loss during cold storage (Kader, 2002) and was related to the frequency of irrigation. Irrigation treatment **HIF**, **MIF** and **LIF** resulted in most of the stems being placed in group 2, declining towards group 5. Where most of treatment **LIF+I**'s stems were placed in group 3, also declining towards group 5 (Table 4.4.7).

Table 4.4.7: **The effect that the applied irrigation treatments had on stem color of SoutherngrapeOne (2007/08).**

Irrigation frequency	Group 1	Group 2	Group 3	Group 4	Group 5
HIF	0	40	39	18	3
MIF	0	41	31	21	7
LIF	0	48	34	11	7
LIF+I	0	25	41	17	17

Chi-Square probability of 0.0106
Cramer's V of 0.1421

4.5 Conclusion

The applied irrigation frequencies resulted in different stem water conditions. These conditions, combined with B and Ca bunch sprays were generally disappointing regarding fruit quality after cold storage.

A **LIF+I** resulted in browner stems after cold storage, with little differences between the rest of the irrigation treatments. The interaction that irrigation frequency showed with bunch sprays in affecting loose berries is inconclusive since there is no pattern. Calcium reduced the percentage of forced berry drop, but increased split and decay substantially. Boron led to a decrease in berry split but produced the most hairline cracks. None of these results could be reproduced for both growing seasons putting the "supposed" positive effects of Ca and/or B on post storage physiological disorders on SoutherngrapeOne in question.

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Chapter 5

Berry skin cell size and wall thickness of SoutherngrapeOne as affected by different irrigation frequencies in combination with bunch directed boron and calcium sprays

5.1 Abstract

Claims have been made towards the benefits that calcium and boron may have towards the improvement of fruit quality as it influences plant cell wall strength by reducing it in diameter. These claims were tested by bunch applications of boron (B) and calcium (Ca) applied to SouthergrapeOne grapes from set to véraison. The effect that different irrigation frequencies may have was also investigated.

As expected irrigation frequency showed an inverse correlation to cell size.

Calcium however resulted in an increase in cell wall diameter.

5.2 Introduction

Fruit ripening causes changes in the cell wall structure of fruit (Brett & Waldron, 1990). These changes lead to fruit softening, making fruit more susceptible to physiological disorders and microbial attack. Plant cell walls consist primarily of three groups of polysaccharides, namely cellulose, hemicellulose and pectins (Jamet *et al.*, 2008). These macro molecules are an interwoven network forming the skeleton of each individual cell.

Cellulose are extremely long chains of B(1-4)linked glucose. These chains forms groups called micro-fibers (Himmel *et al.*, 2007). Hemicellulose is a diverse group of carbohydrate polymers. They are mostly B(1-4)linked glycosidic linkages but, in addition to glucose contains various other monomer units such as mannose, xylose, arabinose, galactose and 4-O-methyl glucuronic acid. They are also shorter than cellulose chains and have side chains attached to them. Pectin is a chemically diverse group, consisting of alfa(1-4)linked D-galactoronic acid residues, making them acidic. When their side chains are attached to rhamnose residues (L-arabinose and D-galactose), they are referred to as rhamnogalactorans. Rhamnogalacturonan II (RG-II) is a pectin with a homogalacturonan backbone with several structurally different oligosaccharide side chains, making out 10% of cellular pectin (Ridley *et al.*, 2001).

Boron was found localized in cell walls, where it is structurally important. It is also associated with pectins within the cell wall (Hu & Brown, 1994). Rhamnogalacturonan II can form a complex with B, forming boron-rhamnogalacturan-II (RG-II-B) (Kobayashi *et al.*, 1996), forming very stable complexes or di-esters with cis-diols on furanoid rings, limiting these reactions to ribose and apiose (Loomis & Durst, 1992). Hu *et al.* (1996) concluded that in plant cell walls, the primary function of B is as a structural component. Absence of B in pumpkin tissue inhibited growth, which was accompanied by the swelling of cell walls (Ishii *et al.*, 2001). An inverse correlation between the quantity of di(RG-II-B) and cell wall thickness of pumpkin leaves was observed. Redgwell *et al.* (1997) observed similar cell wall swelling in ripening tomato and kiwi fruit and contributed it to a lack of cross-linking borate es-

ters. This is of particular importance to this study, since there is three times more RG-I and RG-II in berry skin cells than in the flesh and three fould more RG-I than RG-II in berry cell walls (Vidal *et al.*, 2001).

Pectin molecules contains a unique three dimensional structure, that makes hydrogen bonds to other polysaccharides impossible (Preston, 1979). The pectin chain interactions with cations are also relatively weak because the chain is uncharged (Grant *et al.*, 1973). Polygalacturonic acid residues on pectins provide a negative charge for interactions with proteins and cations such as Ca (Cosgrove, 2005) this may allow for the formation of the "egg box model" (Powell *et al.*, 1982) strengthening the cell wall.

During ripening, skin loosening along with cell wall acidification and loss of structural calcium, takes place (Huang *et al.*, 2005a). Excessive post-harvest softening of fruit is unwanted as it may lead to physical damage during storage and rendering the fruit susceptible to microbial attack (Fischer & Bennett, 1991). In accordance with this, calcium deficiency has been associated with post-harvest fruit cracking in cherries (Brown *et al.*, 1995).

Literature focuses on the effect of nutrient deficient and toxic levels on plant growth (Brown *et al.*, 1995; Ishii *et al.*, 2001). Little is known about the effect that the addition of boron and calcium will have on grapes grown in non-deficient conditions. In this study we attempted to raise skin-B and- Ca content, and in doing so tried to increase the formation of these complexes in order to strengthen the berry skin as a whole. This work was done to establish whether the treatments resulted in a change in cell wall thickness, berry skin thickness and berry skin cell size, that is to be linked to fruit quality.

5.3 Materials and Methods

The experimental vineyard is described in Chapter 3.

5.3.1 Experimental layout

The most extreme of irrigation frequency treatments (treatment LIF and HIF, Chapter 3) were selected for microscopy to determine whether irrigation frequency, along with bunch spraying treatments of B and Ca affected cell wall

diameter or cell size in the berry skin. Three of the repetitions were chosen from the six as described in Chapter 3. For the cell wall thickness only the 2007/08 season's grapes were used. Both seasons grapes were used for the berry skin and cell size measurements.

5.3.2 Measurements

Measurement of berry-B and- Ca levels, climatic conditions, and statistical analysis are shown in Chapter 3.

5.3.2.1 Microscopy

The Diakou & Carde (2001) method for transmission electron microscope (TEM) preparation was adapted. One cubic millimeter blocks were cut from grapes, so that the skin and epidermis were intact. These samples were taken after cold storage to determine whether the treatments had an effect on the grape skins until after that time. These samples were immediately fixed in 2.5% glutaraldehyde[6] for 16 hours. Afterwards they were washed twice in a 0.1 M phosphate buffer, pH 7.4 for 5 minutes each time. These samples were then stained using a 1 % tannic acid[6] solution for 30 minutes and washed again twice[6], thereafter rinsed twice in distilled water for 5 minutes. The samples were then dehydrated by placing it in *(i)* 50 % ethanol for 10 minutes, *(ii)* 70 % ethanol for 10 minutes, *(iii)* 90 % ethanol for 10 minutes, *(iv)* 95 % ethanol for 10 minutes, *(v)* twice in 100 % ethanol (EM grade) for 10 minutes and, *(vi)* twice in acetone for ten minutes. The process of infiltration and embedding started with a *(i)* 50:50 solution of Spurr's resin:acetone for 16 hours, followed by *(ii)* 75:25 resin:acetone for 8 hours, *(iii)* 100 % resin for 16 hours, *(iv)* samples were finally placed in fresh 100 % resin for two hours. Samples were then orientated in molds, filled with resin and placed in a 60°C oven for 24 hours.

5.3.2.2 Cell wall thickness

To determine the cell wall thickness, a Leo Omega 912 transmission electron microscope was used. Samples were prepared as described in section 5.3.2.1,

[6]In 0.1 M phosphate buffer, pH 7.4

cut to 120 nm thickness with a Reichert ultra S ultramicrotome and collected on 200 mesh copper grids, before staining it with 2 % uranyl acetate and Reynolds lead citrate. Foto's were taken with a Proscan CCD camera mounted on the LEO 912 TEM and analyzed with EasiVision Pro software which was developed by Soft Imaging System GmbH, allowing for measurements like these to be taken. The distance was measured between two adjacent cells' cell walls, including the middle lamellae. This distance represented two cell walls. Two berries were used in each of the three repeats, on each of these berries five reading were taken for one representative value.

5.3.2.3 Berry skin thickness and cell size

In an attempt to verify whether these treatments can result in differences in cell size and more importantly berry skin thickness, samples, as described in section 5.3.2.1, were cut with a Reichert ultra S ultramicrotome to 1 um thickness and stained with 1 % (w/v) toluidine blue and washed under water. Samples were then enhanced 10 fold under a light microscope. All images were printed on A5 size paper. The cells in the skin were counted and cut out of the paper. Skin and flesh cells were separated from one another as described by Huang *et al.* (2005b), that post véraison skin cells underwent tangential extension, resulting in longer and thinner cells, with swollen cell walls, where the inner cells expanded in both directions, making them rounder, bigger, with thinner cell walls and highly vacuolized. A 1 mm^2 size of paper to the same scale was also cut. Both pieces of paper were weighed so that the surface area of the skin cells could be calculated. The skin thickness was calculated similarly by using the same scale. This technique was adapted from "Topographic Map Interpretation" (Locke, 1998). For the calculation of the cell radius, all cells were considered to be round.

5.4 Results and discussion

The irrigation frequency treatments resulted in different stem water potentials, B and Ca bunch sprays lead to a decreased B and Ca content in the flesh when compared to the control, where the content in the skin remained unchanged. °Brix at harvest is presented in Chapter 4.

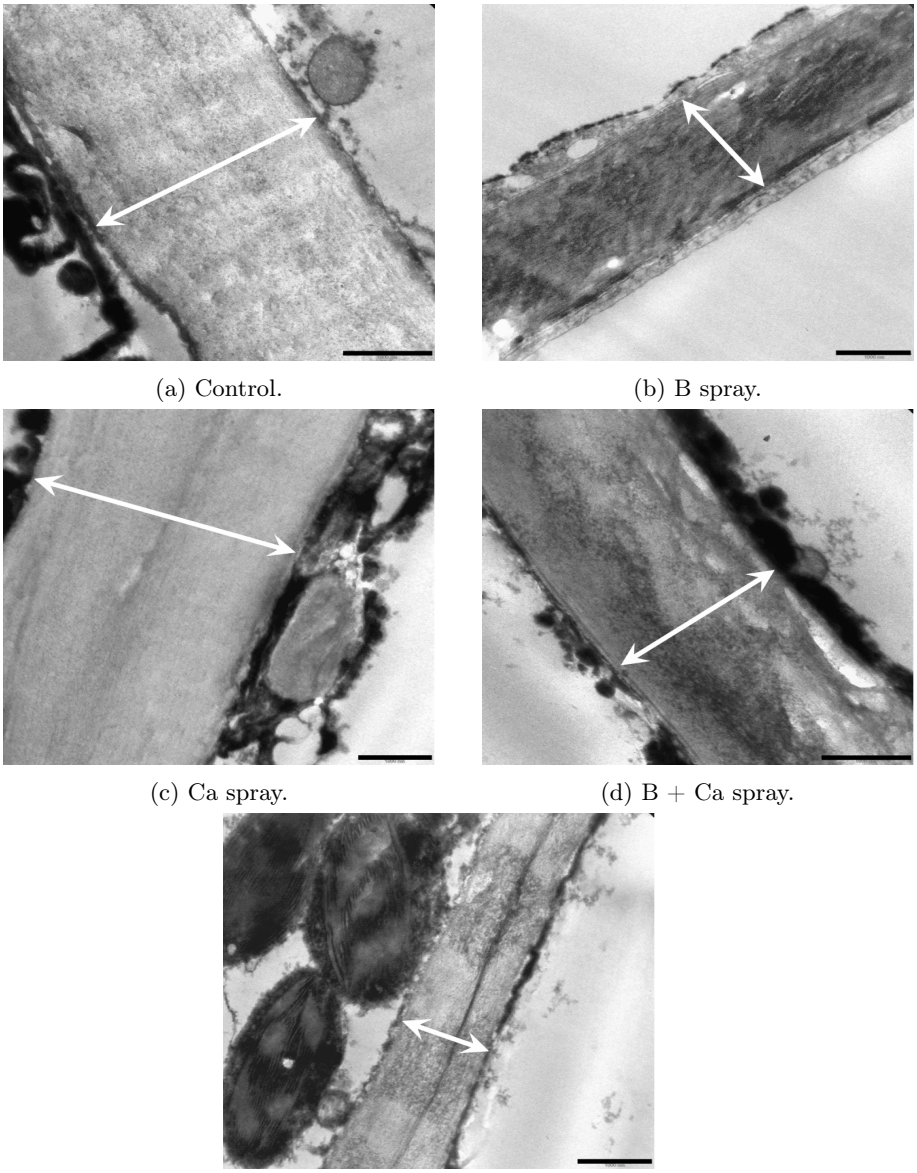
The analysis of variance summary of variable are presented in Table C.0.1, and C.0.2. Only those variables that showed significant differences are discussed. Table C.0.3 depicts the mean values and standard deviations of the berry skin thickness, cell surface area and cell radius of skin cells for both the 2006/07 and 2007/08 season. Table C.0.4 depicts the mean and standard deviation for the cell wall thickness of 2007/08.

5.4.1 Cell wall thickness

Cell wall thickness was mostly affected by Ca, for a P value of 0.07, increasing cell wall thickness instead of decreasing it (Table 5.4.1). This is a surprisingly strong correlation taking into consideration that there is no real change in Ca content in the berry skins (Chapter 3). It is possible that both B and Ca may have had positive effects on the cell wall strength throughout the season. This effect may have been masked by the ripening effect (Huang *et al.*, 2005b), resulting in naturally thickening of cell walls in grapes. Irrigation frequency had no effect on the diameter of the cell walls (Table C.0.1). Figure 5.4.1 shows cell walls that was produced by the **HIF** treatment, and Figure 5.4.2 shows typical cell walls from the **LIF** treatment in this study.

Table 5.4.1: **The effect of Ca treatment on cell wall diameter in the berry skin of SoutherngrapeOne. (P= 0.0748, LSD= 583.37).**

Treatment	**Cell wall diameter (nm)**
Control	1936.3a
Calcium	2529.5b



(a) Control.

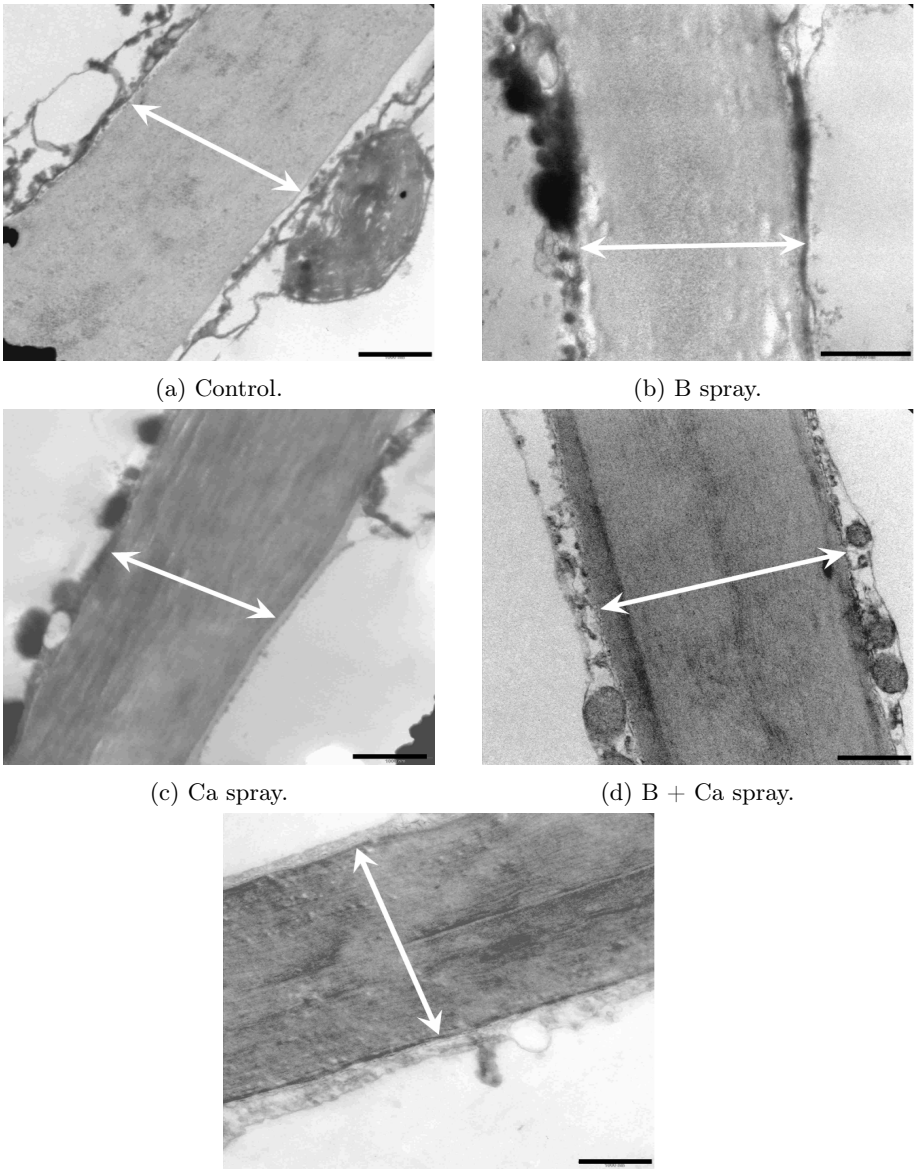
(b) B spray.

(c) Ca spray.

(d) B + Ca spray.

(e) Water spray.

Figure 5.4.1: **Transmission electron micrographs of sections of the berry skin of SoutherngrapeOne under high irrigation frequency, with bunch spray treatments as indicated. White arrow indicate measured distance of two adjacent cell walls including the middle lamellae. Bar = 1000nm.**



(a) Control.

(b) B spray.

(c) Ca spray.

(d) B + Ca spray.

(e) Water spray.

Figure 5.4.2: **Transmission electron micrographs of sections of the berry skin of SoutherngrapeOne under low irrigation frequency. White arrow indicate measured distance of two adjacent cell walls including the middle lamellae. Bar = 1000nm.**

5.4.2 Berry skin thickness and cell size

Vines subjected to low irrigation frequency showed smaller cells in the skins than the vines subjected to high irrigation frequency (Fig 5.4.3). It is important to point out that these differences could have been due to the last period of withdrawal of irrigation (just before harvest). For the **LIF** treatment, irrigation was terminated 10 days before harvest, while for the **HIF**, irrigation was terminated 6 days before harvest. Irrigation frequency had the biggest effect on the skin thickness. Though not statistically significant, the **LIF** treatment reduced berry skin diameter compared to the **HIF** treatment. It is important to remember that due to the experimental procedure, these findings are only preliminary indicators. If more precise procedures were used, such as the software that was used for the cell wall thickness measurements, the results might have been more accurate.

Table 5.4.2: **Effect that irrigation frequency has on cell surface area, cell radius and berry skin thickness of SoutherngrapeOne (2006/07 and 2007/08).**

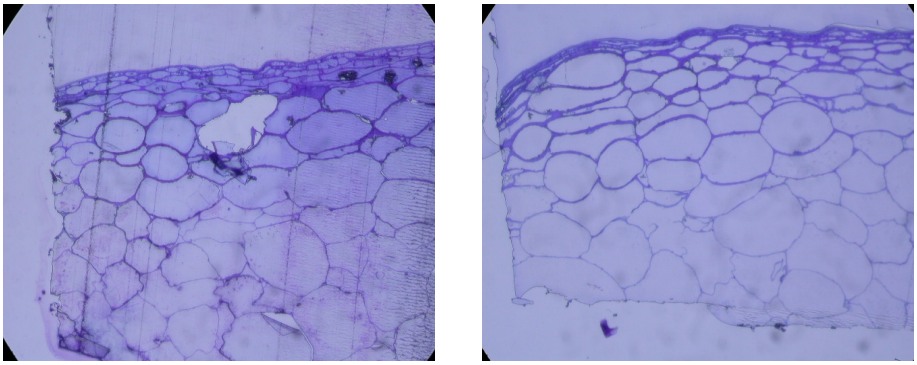
Irrigation treatment	**Cell surface area[a](mm^2)**	**Cell radius[b] (mm)**	**Berry skin thickness[c](mm)**
HIF	$111.50x10^{-4}$A	$18.73x10^{-2}$A	$4.54x10^{-1}$A
LIF	$86.85x10^{-4}$B	$16.54x10^{-2}$B	$3.85x10^{-1}$A

Values with different letters are significantly different.

[a] P= 0.0056, LSD= $1x10^{-3}$

[b] P= 0.005, LSD= 1.210^{-2}

[c] P= 0.0660, LSD= $8x10^{-2}$



(a) High irrigation frequency treatment (b) Low irrigation frequency treatment

Figure 5.4.3: **Extreme examples that different irrigation frequencies has on berry skin thickness. Light microscope sections of the skin (exocarp) and flesh (mesocarp) of SoutherngrapeOne after cold storage, subjected to high and low irrigation frequencies.**

5.5 Conclusion

It is clear that irrigation frequency did not influence cell wall diameter. Calcium was the only treatment that showed a trend to increase the cell wall diameter, and also relates to the increase of berry split and decay during cold storage (Chapter 4). Cell size in the berry skin was reduced by a low irrigation frequency. It is however not clear whether these changes are caused by a difference in the final period of water withdrawal prior to harvest, or to the total effect of water withdrawal frequency throughout the season.

A microscopy study focusing on berry skin and flesh during berry development from set until after cold storage, may provide more answers to the response of cell wall thickness, and sell size in the berry skin, from these treatments and how it relates to fruit quality. A method to increase the amount of structural boron and calcium within berry cell walls is still required.

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Chapter 6

Conclusion

Each year the table grape industry faces financial losses due to quality related defects. These include loose berries, SO_2 damage, browning of the skin and stalk, decay, as well as berry split. Berry split contributes to financial loss by causing a decrease in both yield, and value if split occurs during shipment as it diminishes the visual attractiveness of grapes.

The aim of this study was to determine the influence that irrigation frequency, in combination with B and Ca bunch directed treatments have on the occurrence of pre- and post-harvest berry split. To this end, Southerngrape-One was chosen as a model cultivar, due to its high natural susceptible to berry split.

It was found that medium irrigation frequency resulted in the best strategy for pre-harvest berry split prevention. High and low irrigation frequency resulted in similar amounts of berry split. This is in contrast to the belief that irrigation before harvest will increase berry split. Furthermore, vines exposed to low irrigation frequencies gave rise to a higher percentage brown stems. There was also a change observed in berry skin cell size, as brought about by the irrigation frequency; where a low irrigation frequency resulted in a decrease in cell size.

Surprisingly, instead of preventing the occurrence, or decreasing the amount of pre-harvest berry split, bunch directed B and Ca treatments seem to have caused an increase in the amount of pre-harvest berry split. Boron and Ca further influenced post-cold storage disorders in the following way: Treatment

with Ca seem to decrease loose berries, but increased decay. Boron reduced berry split only in the 2006/07 season. All treatments resulted in an increase of hairline cracking, with B increasing it substantially in the 2007/08 season. None of the applied treatments had the desired effect of decreasing the cell wall diameter of cells in the berry skin. A possible explanation for these unwanted effects following Ca treatment could be the increased cell wall diameter. Together these morphological changes could account for the hypersensitivity of Ca-treated grapes to produce pre-harvest and post-cold storage berry split.

Badly managed irrigation regimes, as well as the unnecessary use of B and Ca, may result in an increase of berry split and other physiological disorders that may lead to financial losses for the producer.

Appendix A

Effects of irrigation frequency, boron and calcium on pre-harvest berry split.

Averages of the stem water potentials are given in Table A.0.1 and A.0.2. The analysis of variance of the different parameters are presented in Tables A.0.3 to A.0.7.

Table A.0.1: **Averages of raw pressure chamber readings (KPa) for the 2006/07 season.**

Irrigation treatment	Repeat	71 DAA	78 DAA	84 DAA	91 DAA	112 DAA	119 DAA	127 DAA
HIF	1	-660	-660	-610	-600	-650	-670	-687
	2	-900	-850	-800	-675	-750	-700	-800
	3	-870	-950	-1000	-940	-790	-650	-825
	4	-1112	-1170	-1220	-900	-740	-710	-1025
	5	-1020	-1060	-1200	-920	-800	-830	-980
	6	-940	-1180	-940	-762	-840	-740	-980
MIF	1	-600	-840	-670	-780	-740	-690	-620
	2	-600	-810	-660	-550	-925	-687	-820
	3	-820	-1000	-1040	-570	-770	-1020	-925
	4	-690	-790	-700	-700	-800	-680	-820
	5	-1020	-1140	-1080	-780	-840	-925	-970
	6	-1080	-1020	-1160	-800	-900	-960	-950
LIF	1	-750	-680	-710	-850	-950	-870	-987
	2	-770	-812	-930	-810	-990	-975	-930
	3	-890	-900	-1100	-1020	-1000	-860	-980
	4	-1010	-1020	-1260	-1000	-990	-1020	-1170
	5	-1060	-1080	-1220	-1140	-1140	-1320	-1350
	6	-1030	-1200	-1220	-950	-1075	-1070	-1140
LIF+I	1	-730	-770	-775	-1162	-1000	-825	-740
	2	-920	-990	-1160	-1020	-1020	-1000	-940
	3	-860	-800	-860	-1000	-1000	-910	-860
	4	-1080	-1140	-1260	-1060	-1090	-1150	-980
	5	-990	-1270	-1166	-883	-987	-910	-960
	6	-1200	-1270	-1310	-1070	-1137	-1150	-1040

Table A.0.2: **Averages of raw pressure chamber readings (KPa) for the 2007/08 season.**

Irrigation treatment	Repeat	82 DAA	89 DAA	97 DAA	104 DAA	110 DAA	124 DAA	130 DAA
HIF	1	-440	-630	-560	-540	-560	-520	-580
	2	-880	-920	-700	-860	-690	-770	-660
	3	-1070	-960	-710	-870	-820	-780	-680
	4	-820	-490	-710	-680	-550	-610	-620
	5	-930	-1040	-810	-900	-810	-800	-710
	6	-820	-880	-810	-710	-780	-600	-540
MIF	1	-700	-800	-590	-820	-660	-650	-610
	2	-490	-610	-560	-650	-560	-710	-610
	3	-960	-800	-840	-740	-720	-780	-760
	4	-720	-670	-580	-680	-530	-600	-680
	5	-870	-960	-880	-800	-780	-800	-660
	6	-260	-1020	-910	-750	-1080	-760	-710
LIF	1	-870	-540	-800	-900	-860	-850	-860
	2	-820	-890	-980	-770	-620	-830	-920
	3	-790	-790	-790	-650	-520	-740	-790
	4	-1000	-1130	-1010	-1070	-820	-950	-1080
	5	-1040	-1010	-1060	-1000	-980	-980	-980
	6	-830	-990	-1080	-850	-910	-780	-820
LIF+I	1	-610	-680	-790	-580	-620	-600	-590
	2	-730	-850	-800	-660	-610	-760	-680
	3	-840	-950	-940	-790	-650	-860	-790
	4	-840	-1110	-1090	-940	-920	-1010	-650
	5	-890	-990	-810	-730	-640	-810	-560
	6	-930	-1060	-970	-860	-700	-830	-570

Table A.0.3: **Analysis of variance for bunches that had to be removed from SoutherngrapeOne, subjected to different irrigation frequencies and treated with four bunch spraying treatments in the Hex River Valley (2006/07).**

Variable	**Removed bunches 105DAA**			**Removed bunches 133DAA**		
Source	DF	MS	P	DF	MS	P
Block	5	1053.42	0.3181	5	2392.32	0.1687
Irrigation (I)	3	729.88	0.4659	3	641.37	0.6952
Error (a)	15	814.37		15	1312.08	
Bunch Spray Treatment (S)	3	961.23	0.0001	3	1975.10	0.0001
I x S	9	116.71	0.4757	9	275.93	0.3597
Error (b)	60	120.63		60	245.27	
Corrected Total	45479.93			85150.41		
Non-Normality (P<W)	0.57571			0.96865		

DF= Degrees of freedom
MS= mean Square
P= Probability of F-ratio test

Table A.0.4: **Analysis of variance for bunches that had to be removed from SoutherngrapeOne, subjected to different irrigation frequencies and treated with four bunch spraying treatments in the Hex River Valley (2007/08).**

Variable	**Removed bunches 103DAA**			**Removed bunches 138DAA**		
Source	DF	MS	P	DF	MS	P
Block	5	749.75	0.2860	5	890.47	0.1794
Irrigation (I)	3	474.67	0.4760	3	1690.42	0.0470
Error (a)	15	542.57		15	502.76	
Bunch spray treatment (S)	4	641.73	0.1787	4	539.91	0.2036
Control vs res	1	324.55	0.3689	1	11.56	0.8572
Ca	1	6.26	0.9005	1	7.89	0.8818
B	1	2199.79	0.0211	1	2140.13	0.0162
Ca x B	1	36.33	0.7632	1	0.06	0.9896
I x S	12	468.53	0.3125	12	309.99	0.5760
Error (b)	80	397.44		80	354.69	
Corrected Total	119			119		
Non-Normality (P<W)	0.053749			0.22097		

DF= Degrees of freedom
MS= mean Square
P= Probability of F-ratio test

Table A.0.5: **Analysis of variance for berry skin and flesh's boron and calcium content of SoutherngrapeOne, subjected to different irrigation frequencies and treated with four bunch spraying treatments in the Hex River Valley (2007/08).**

Variable	**Berry skin Ca**			**Berry skin B**			**Berry flesh Ca**			**Berry flesh B**		
Source	DF	MS	P	DF	MS	P	DF	MS	P	DF	MS	P
Block	5	871717.98	0.0078	5	0.00388	0.3620	5	132469.50	0.6692	5	0.07	0.7405
Irrigation (I)	1	161617.36	0.1914	1	0.00031	0.7540	1	703107.22	0.1901	1	0.31	0.2532
Error (a)	5	70885.39		5	0.00278		2	184344.73		2	0.12	
Bunch spray treatment (S)	3	112364.76	0.4388	3	0.00054	0.5488	3	162823.08	0.0248	3	0.06	0.0205
I x S	3	291162.09	0.0872	3	0.00181	0.0856	3	21447.20	0.6802	3	0.01	0.5933
Error (b)	29	120831.40		29	0.00075		20	42093.14		20	0.01	
Corrected Total	46			46			34			34		
Non-Normality (P<W)	0.75888			0.13433			0.76543			0.7363		

DF= Degrees of freedom
MS= mean Square
P= Probability of F-ratio test

Table A.0.6: **Analysis of variance for sugar accumulation over time of SoutherngrapeOne subjected to different irrigation frequencies and treated with four bunch spraying treatments in the Hex River Valley (2006/07).**

Variable	**71 DAA**			**78 DAA**			**86 DAA**			**93 DAA**			**112 DAA**			**127 DAA**		
Source	DF	MS	P	DF	MS	P	DF	MS	P	DF	MS	P	DF	MS	P	DF	MS	P
Blok	5	0.66	0.6256	5	1.32	0.2213	5	1.75	0.0946	5	3.09	0.1127	5	5.00	0.0783	5	7.28	0.0283
Irrigation (I)	3	0.44	0.7076	3	0.30	0.7846	3	0.57	0.5351	3	1.32	0.4515	3	4.00	0.1583	3	2.87	0.2933
Error (a)	15	0.93		15	0.83		15	0.75	0.0129	15	1.42		15	2.01		15	2.11	
Bunch Spray Treatment (S)	3	2.37	<.0001	3	2.10	0.0003	3	2.78	<.0001	3	2.47	0.0007	3	2.14	0.0338	3	2.67	0.0093
I x S	9	0.29	0.3396	9	0.19	0.7568	9	0.77	0.0263	9	0.51	0.2390	9	0.76	0.3772	9	0.51	0.6207
Error (b)	59	0.25		59	0.29		60	0.33		59	0.38		59	0.69		59	0.64	
Corrected Total	94			94			95			94			94			94		
Non-Normality (P<W)	0.46368			0.74771			0.9277			0.46652			0.42012			0.20033		

DF= Degrees of freedom
MS= mean Square
P= Probability of F-ratio test

Table A.0.7: **Analysis of variance for percentage split over time of SoutherngrapeOne subjected to different irrigation frequencies and treated with four bunch spraying treatments in the Hex River Valley, South Africa, 2006/07.**

Variable	**86 DAA**			**93 DAA**			**105 DAA**			**112 DAA**			**127 DAA**		
Source	DF	MS	P	DF	MS	P	DF	MS	P	DF	MS	P	DF	MS	P
Block	5	64.79	0.4657	5	75.70	0.6846	5	100.36	0.0483	5	322.54	0.0563	5	524.38	0.0185
Irrigation (I)	3	253.05	0.0330	3	675.11	0.0091	3	109.13	0.0522	3	576.79	0.0135	3	143.44	0.3939
Error (a)	15	66.63		15	121.48		11	30.94		15	115.57		15	134.87	
Bunch spray treatments (S)	3	20.90	0.3109	3	223.67	0.0147	3	53.01	0.2227	3	150.43	0.4433	3	135.95	0.2351
I x S	9	16.32	0.4881	9	42.11	0.6931	8	45.64	0.2714	9	57.31	0.9555	9	70.89	0.6529
Error (b)	56	17.13		60	58.90		8	29.23		59	165.88		60	93.25	
Corrected Total	91			95			38			94			95		

DF= Degrees of freedom
MS= mean Square
P= Probability of F-ratio test

Appendix B

Effect of irrigation frequencies, boron and calcium on post-cold storage grape physiology.

The analysis of variance summery of variables are presented in Table B.0.1, B.0.2 and B.0.3. Tables B.0.4 and B.0.5 contains the mean percentage disorders as expressed per weight of berries.

Table B.0.1: **Analysis of variance for physiological disorders of SoutherngrapeOne after cold storage subjected to different irrigation frequencies and bunch spraying treatments at Clovalley in the Hex River Valley, South Africa (2006/07).**

Variable	**Berry drop**			**Forced berry drop**			**Split**			**Hairline cracking**		
Source	DF	MS	P	DF	MS	P	DF	MS	P	DF	MS	P
Block	5	12.15	0.4107	5	6.33	0.4396	5	191.73	0.0838	5	89.63	0.5464
Irrigation (I)	3	9.85	0.4757	3	14.70	0.1111	3	19.22	0.8646	3	75.97	0.5633
Error (a)	15	11.26		15	6.19		15	78.91		15	107.64	
Bunch Spray Treatment (S)	3	9.79	0.1204	3	7.58	0.0205	3	308.42	0.0036	3	15.26	0.1086
I x S	9	5.99	0.2903	9	0.74	0.9566	9	90.86	0.1771	9	5.99	0.5934
Error (b)	60	4.84		60	2.16		60	61.48		60	7.24	
Corrected Total	95			95			95			95		
Non-Normality (P<W)	0.54998			0.001643			0.47308			$4.441 \text{x} 10^{-5}$		
Variable	**$S0_2$ damage**			**Decay**			**Internal browning**			**External browning**		
Source	DF	MS	P	DF	MS	P	DF	MS	P	DF	MS	P
Block	5	13.01	0.002	5	8.47	0.588	5	162.28	0.035	5	343.71	0.2858
Irrigation (I)	3	2.41	0.3392	3	2.03	0.9061	3	14.07	0.8381	3	59.04	0.8689
Error (a)	15	1.99		15	11.05		15	50.03		15	248.63	
Bunch Spray Treatment (S)	3	3.46	0.1156	3	4.95	0.072	3	14.96	0.3304	3	7.13	0.7272
I x S	9	1.73	0.4307	9	2.30	0.3499	9	7.66	0.7946	9	19.07	0.3305
Error (b)	60	1.68		60	2.02		60	12.84		60	16.30	
Corrected Total	95			95			95			95		
Non-Normality (P<W)	0.089647			0.011422			$7.419 \text{x} 10^{-4}$			$3.988 \text{x} 10^{-5}$		

DF= Degrees of freedom; MS= Mean Square; P= Probability of F-ratio

Table B.0.2: **Analysis of variance for physiological disorders of SoutherngrapeOne after cold storage subjected to different irrigation frequencies and bunch spraying treatments at Clovalley in the Hex River Valley, South Africa (2007/08).**

Variable	**Berry drop**			**Forced berry drop**			**Split**			**Hairline cracking**		
Source	DF	MS	P	DF	MS	P	DF	MS	P	DF	MS	P
Block	5	2.51	0.9775	5	1.61	0.9811	5	837.35	0.2146	5	0.56	0.61
Irrigation (I)	3	5.52	0.8065	3	5.67	0.6995	3	736.52	0.2744	3	0.51	0.584
Error a	15	16.92		15	11.75		15	516.57		15	0.76	
Bunch spraying treatment (S)	4	4.46	0.4482	4	2.45	0.3758	4	114.97	0.4956	4	0.78	0.0673
Control vs res	1	0.62	0.7195	1	0.70	0.5823	1	5.22	0.8444	1	0.16	0.4999
Ca	1	6.89	0.2331	1	4.57	0.1609	1	116.58	0.355	1	0.00	0.9056
B	1	0.85	0.6742	1	0.63	0.6008	1	175.26	0.2574	1	0.15	0.5131
Ca x B	1	9.77	0.1565	1	4.15	0.1813	1	166.29	0.2699	1	2.75	0.0058
I x S	12	9.33	0.0399	12	3.84	0.0866	12	217.72	0.1038	12	0.47	0.199
Error b	79	4.78		79	2.28		79	134.68		79	0.34	
Corrected Total	118			118			118			118		
Non-Normality (P<W)	0.12397			0.66665			0.9212			0.06372		
Variable	**$S0_2$ damage**			**Decay**			**Internal browning**			**External browning**		
Source	DF	MS	P	DF	MS	P	DF	MS	P	DF	MS	P
Block	5	0.88	0.0643	5	204.98	0.0143	5	8.14	0.0128	5	5.96	0.3247
Irrigation (I)	3	0.26	0.5158	3	71.74	0.2663	3	2.02	0.3937	3	1.15	0.8622
Error a	15	0.33		15	49.26		15	1.90		15	4.67	
Bunch spraying treatment (S)	4	0.12	0.6435	4	13.09	0.7016	4	0.10	0.9878	4	1.21	0.7885
Control vs res	1	0.03	0.6895	1	10.18	0.5162	1	0.18	0.7011	1	0.42	0.7006
Ca	1	0.20	0.3073	1	34.09	0.2362	1	0.13	0.7448	1	3.44	0.2738
B	1	0.05	0.5976	1	2.75	0.7353	1	0.03	0.8722	1	0.98	0.5584
Ca x B	1	0.20	0.3064	1	0.11	0.9472	1	0.07	0.8158	1	0.01	0.9498
I x S	12	0.30	0.1277	12	35.12	0.1545	12	0.50	0.9586	12	1.16	0.9553
Error b	79	0.19		79	23.93		79	1.24		79	2.84	
Corrected Total	118			118			118			118		
Non-Normality (P<W)	0.023265			0.000000178			0.000000199			0.000000157		

DF= Degrees of freedom; MS= Mean Square; P= Probability of F-ratio

Table B.0.3: **Analysis of variance for Stem colour disorders of SoutherngrapeOne after cold storage, subjected to different irrigation frequencies and bunch spraying treatments at Clovally in the Hex River Valley, South Africa (2006/07 and 2007/08).**

		Stem class 1			Stem class 2			Stem class 3			Stem class 4			Stem class 5		
Season	Source	DF	MS	P	DF	MS	P	DF	MS	P	DF	MS	P	DF	MS	P
2006/07	Block	5	0.00	.	5	5028.41	0.4163	5	4536.54	0.2276	5	1058.72	0.3047	5	26.04	0.7008
	Irrigation (I)	3	0.00	.	3	572.46	0.946	3	265.54	0.9633	3	350.93	0.7273	3	17.36	0.755
	Error (a)	15	0.00	.	15	4709.08		15	2885.73		15	796.41		15	43.40	
	Bunch Spray Treatment (S)	3	0.00	.	3	1823.99	0.0045	3	1474.62	0.0462	3	256.48	0.554	3	52.08	0.272
	I x S	9	0.00	.	9	477.26	0.2757	9	537.50	0.4275	9	392.44	0.394	9	34.72	0.5405
	Error (b)	60	0.00		60	377.84		60	522.00		60	364.91		60	39.06	
	Corrected Total	95			95			95			95			95		
	Non-Normality (P<W)	0				0.44		0.32857			$1.194x10^{-5}$			$1.5449x10^{-13}$		
2007/08	Block	5	81.73	0.5353	5	3329.26	0.2908	5	1418.60	0.5057	5	734.27	0.7412	5	1264.58	0.0587
	Irrigation (I)	3	73.23	0.5326	3	2058.13	0.4901	3	766.39	0.6966	3	564.11	0.7433	3	827.03	0.1904
	Error (a)	15	96.05		15	2433.37		15	1574.68		15	1352.46		15	459.41	
	Bunch spraying treatment (S)	4	79.06	0.501	4	432.44	0.7883	4	592.60	0.5878	4	362.34	0.3977	4	139.13	0.6976
	Control vs res	1	35.10	0.542	1	1001.33	0.3228	1	43.46	0.8201	1	5.37	0.9021	1	135.99	0.4645
	Ca	1	184.26	0.1645	1	240.67	0.627	1	1033.59	0.2693	1	748.17	0.1489	1	322.67	0.261
	B	1	46.76	0.4817	1	176.04	0.6777	1	1060.01	0.2633	1	560.67	0.2108	1	88.17	0.5557
	Ca x B	1	46.76	0.4817	1	287.04	0.5957	1	189.84	0.6348	1	140.17	0.53	1	287.04	0.5957
	I x S	12	96.86	0.4261	12	804.68	0.6536	12	1438.55	0.0775	12	385.84	0.3757	12	113.26	0.9372
	Error (b)	79	93.58		79	1011.44		79	835.16		79	352.19		79	251.72	
	Corrected Total	118			118			118			118			118		
	Non-Normality (P<W)	0			0.21978			0.90533			0.32987			$1.545x10^{-5}$		

DF= Degrees of freedom; MS= Mean Square; P= Probability of F-ratio

Table B.0.4: **Effect of irrigation frequency and bunch spraying treatments on the after cold storage disorders of SoutherngrapeOne at Clovelly (2006/07 and 2007/08).**

Season	Treatment	Loose berry (%)	Forced berry drop (%)	Split (%)	Hairline cracking (%)	$S0_2$ damage (%)	Decay (%)	Internal browning (%)	External browning (%)
2006/07	HIF	2.8	1.5	11.3	2.2	1.0	1.8	2.7	4.8
	MIF	3.1	1.6	11.6	6.1	1.5	2.4	3.2	7.4
	LIF	4.3	3.2	11.7	2.5	1.7	1.7	3.1	3.9
	LIF+I	3.5	2.2	13.3	3.9	1.3	1.9	4.5	4.2
	Control	3.7	2.4	14.8	2.8	1.8	1.3	2.6	4.5
	B	4.2	2.8	6.9	3.4	0.8	1.9	2.9	5.8
	Ca	2.7	1.8	14.0	3.7	1.5	2.2	4.4	5.1
	CaB	3.1	1.6	12.3	4.7	1.4	2.4	3.6	4.9
2007/08	HIF	2.7	2.4	37.2	0.6	0.2	4.2	0.6	1.4
	MIF	3.2	3.0	42.8	0.3	0.3	2.8	0.5	1.1
	LIF	2.3	2.1	30.7	0.4	0.2	2.5	0.7	1.2
	LIF+I	3.2	2.1	37.8	0.5	0.1	5.9	1.1	0.9
	Control	2.8	2.3	37.8	0.3	0.2	4.6	0.7	1.1
	Water	2.9	2.5	38.0	0.3	0.2	3.2	0.8	1.3
	B	3.4	2.7	38.1	0.7	0.3	2.9	0.8	1.5
	Ca	3.1	2.5	33.2	0.6	0.2	4.5	0.8	0.9
	CaB	2.2	1.9	38.5	0.3	0.1	4.1	0.7	1.1

Table B.0.5: **Stem color after cold storage as affected by irrigation frequency and bunch spray treatments of SoutherngrapeOne (2006/07 and 2007/08).**

Season	Treatment	Stem class 1 (%)	Stem class 2 (%)	Stem class 3 (%)	Stem class 4 (%)	Stem class 5 (%)
2006/07	HIF	0.0	37.6	47.4	14.0	1.0
	MIF	0.0	42.0	50.7	7.3	0.0
	LIF	0.0	44.7	42.8	10.5	2.1
	LIF+I	0.0	49.3	45.5	5.2	1.0
	Control	0.0	34.9	51.9	13.1	1.0
	B	0.0	55.6	36.7	7.7	0.0
	Ca	0.0	42.5	43.9	10.5	3.1
	CaB	0.0	40.6	53.8	5.6	0.0
2007/08	HIF	0.0	39.7	40.5	18.0	3.4
	MIF	1.1	37.8	29.6	20.8	7.2
	LIF	3.3	45.3	34.2	10.5	6.6
	LIF+I	0.0	25.8	40.2	15.5	15.7
	Control	0.0	42.0	34.7	16.6	6.5
	Water	0.0	34.0	35.0	22.5	8.3
	B	0.0	34.7	44.4	15.3	5.5
	Ca	1.4	40.6	31.2	14.5	11.1
	CaB	4.2	34.4	35.0	12.1	10.1

Appendix C

Effect of irrigation frequency, boron and calcium on berry morphology

The analysis of variance summary of variable are presented in Table C.0.1, and C.0.2. Table C.0.3 depicts the mean values and standard deviations of the berry skin thickness, cell surface area and cell radius of skin cells for both the 2006/07 and 2007/08 season. Table C.0.4 depicts the mean and standard deviation for the cell wall thickness of 2007/08.

Table C.0.1: **Analysis of variance for cell wall thickness of SoutherngrapeOne subjected to cold storage after being exposed to different irrigation frequencies, and treated with four bunch spraying treatments of B and Ca in the Hex River Valley, South Africa, 2006/07 to 2007/08.**

Variable	**Cell wall thickness (nm)**		
Source	DF	MS	P
Block	2	403078.14	0.4677
Irrigation (I)	1	1149317.81	0.2134
Error a	2	354213.36	
Bunch spraying treatment (S)	4	799172.75	0.1702
Control vs res	1	34330.23	0.7814
Ca	1	1576370.33	0.0748
B	1	467647.71	0.3135
Ca x B	1	954002.17	0.157
I x S	4	394175.99	0.4796
Error b	15	429920.53	
Corrected Total	118		
Non-Normality (P<W)	0.96339		

DF= Degrees of freedom
MS= Mean Square
P= Probability of F-ratio

Table C.0.2: **Analysis of variance for light microscopy analysis of SoutherngrapeOne subjected to cold storage after being exposed to different irrigation frequencies, and treated with four bunch spraying treatments of B and Ca in the Hex River Valley, South Africa, 2006/07 to 2007/08.**

Variable	**Berry skin thickness**			**Cell surface area**			**Cell radius**		
Source	DF	MS	P	DF	MS	P	DF	MS	P
Year (Y)	1	0.00034259	0.0876	1	0.000000012	0.4911	1	0.00000071	0.5298
Year (Rep)	4	0.00004511	0.6481	4	0.00000000283	0.3875	4	0.00000169	0.4563
Irrigation (I)	1	0.00042669	0.066	1	0.0000000611	0.0056	1	0.00004723	0.005
Error a	4	0.00006767		4	0.00000000209		4	0.0000015	
Bunch spraying treatment (S)	3	0.00007422	0.2314	3	0.00000000547	0.4276	3	0.00000435	0.3782
IxS	3	0.0000697	0.2532	3	0.00000000514	0.4528	3	0.00000383	0.4296
YxS	3	0.00009337	0.1602	3	0.00000000412	0.5401	3	0.00000353	0.4619
YxIxS	3	0.00006307	0.2895	3	0.00000000894	0.2389	3	0.00000625	0.2412
Error b	10	0.00004388		10	0.0000000054		10	0.0000038	
Corrected Total	32			32			32		
Non-Normality (P<W)	0.30597			0.18411			0.39752		

DF= Degrees of freedom
MS= Mean Square
P= Probability of F-ratio

Table C.0.3: **Differences in berry skin thickness, cell surface area and cell radius of skin cells as affected by irrigation frequency and bunch spray treatments on SoutherngrapeOne at Clovalley in the Hex River Valley, South Africa (2006/07 and 2007/08).**

Variable		**Skin thickness (mm)**		**Cell surface area (mm^2)**		**Cell radius (mm)**	
Source	n	Mean	Std Dev	Mean	Std Dev	Mean	Std Dev
Year							
2006/07	18	0.04476256	0.00833401	0.00097061	0.0002189	0.01746904	0.00200234
2007/08	15	0.03829169	0.00860633	0.00100885	0.00029806	0.0177635	0.00244624
Irrigation treatment							
HIF	16	0.04539246	0.00967832	0.00111501	0.00026045	0.01873135	0.00207944
LIF	17	0.03846012	0.00688816	0.00086845	0.0001857	0.0165408	0.00173625
Bunch directed spray treatment							
Control	9	0.04586474	0.00769648	0.00100642	0.00025006	0.01777705	0.00220687
Boron	7	0.04457664	0.00822172	0.00094318	0.00016056	0.01727527	0.00144468
Calcium	9	0.03810291	0.0078084	0.00110512	0.00032001	0.01860529	0.00251309
Boron + Calcium	8	0.03904451	0.01073484	0.0008747	0.00022453	0.0165659	0.00213694

n= Number of Samples
Std Dev= Standard Deviation

Table C.0.4: **Berry skin cell wall thickness as affected by irrigation frequency and bunch directed sprays on SoutherngrapeOne at Clovalley in the Hex River Valley, South Africa (2007/08).**

Variable		**Cell wall thickness (nm)**	
Source	n	Mean	Std Dev
Irrigation treatment			
HIF	15	2083.08809	739.977844
LIF	14	2464.17896	630.536737
Bunch directed spray treatment			
Control	6	2348.59067	918.294596
Water	5	1788.73175	322.28324
Boron	6	2059.33	315.827344
Calcium	6	2881.42622	665.014338
Boron + Calcium	6	2177.51444	746.831832

n= Number of samples
Std Dev= Standard Deviation