THE EFFECT OF DIFFERENT WATER AND NUTRIENT MANAGEMENT STRATEGIES ON THE CALCIUM CONTENT IN APPLE FRUIT

BY

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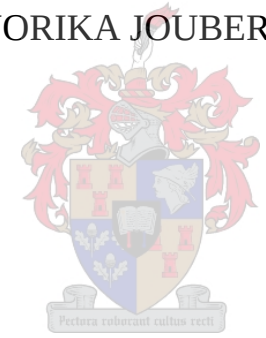

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DECLARATION

I, the undersigned, hereby declare that the work contained in this thesis is my own original work and has not previously, in its entirety or in part, been submitted at any university for a degree.

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SUMMARY

Production of quality fruit is the main aim in horticultural crops. Numerous research reports stress the important role of calcium (Ca) in maintaining firmness and preventing the development of physiological disorders in fruit. This study focused on the effect of water and nutrient management strategies, rootstocks and foliar Ca applications on fruit Ca content.

Final Ca content/concentration in apple fruit at harvest did not differ significantly between treatments water with micro jets (hand nutrition), water and nutrients with fertigation, or water and nutrients with pulsating drip when applied to 'Brookfield Gala' trees in third leaf, on two rootstocks (M793 and M7).

In the second trial, three Ca levels were applied to 'Brookfield Gala' trees through a pulsating drip system during three phenological periods to evaluate the effect on Ca uptake of the fruit. During the second season, application of high Ca levels for the period full bloom to harvest gave a higher fruit Ca concentration than with applications of standard or low Ca.

In the rootstock trial, there was a tendency towards more vigorous rootstocks experiencing stronger vegetative growth and probably higher leaf:fruit ratios, with bigger fruit. More dwarfing rootstocks had more favourable leaf:fruit ratios, but smaller fruit. Thus the rootstocks could have played a direct role in fruit size and this could have resulted in the differences observed in the fruit Ca content/concentrations.

When Ca products were applied to 'Golden Delicious' trees according to supplier specifications, eight applications of $Ca(NO_3)_2$ and Calcimax® between 40 and 80 dafb were

as effective in controlling bitter pit as three applications (once a month, from six dafb) of Ca acetate and Ca fulvate. In the second experiment, indications were that late applications (starting at 80 dafb) of $Ca(NO_3)_2$ and Ca acetate were more effective in increasing the Ca content of fruit at harvest than mid and early season applications (40-80 dafb and shortly after full bloom respectively). Late $Ca(NO_3)_2$ (80dafb) gave the highest Ca content in fruit at harvest. For effective control of bitter pit, results pointed towards early (shortly after full bloom) and mid (40 dafb to 80 dafb) season applications with foliar Ca. No satisfactory conclusions regarding bitter pit control could be drawn due to a low bitter pit incidence (< 1%).

Evaluation of two pre-harvest prediction methods (one season) indicated that magnesium infiltration may be preferred to ethylene forcing, due to its higher correlation with actual bitter pit. The low bitter pit incidence in 2005/06 could have influenced the reliability of the results.

Results pointed toward adequate natural Ca uptake into 'Brookfield Gala' fruit if soil physical and chemical conditions (C.E.C, pH ect.), soil aeration and drainage, fine root development and management practices (irrigation, fertilization, pruning, tree training and thinning) are ideal. In both trials, fruit Ca levels at harvest were relatively high during the second season (6.96 to 8.85 $mg.100g^{-1}$ fresh weight). With 'Golden Delicious' foliar applications of Ca, especially $Ca(NO_3)_2$, from 80 dafb effectively increase Ca content in fruit at harvest to maintain fruit quality, but not necessarily reduce bitter pit incidence.

OPSOMMING

Die effek van verskillende water- en voedingsbestuurstrategieë op die Ca-inhoud in appelvrugte

Die produksie van kwaliteit vrugte is die hoof doelwit vir enige produsent. Die kritiese impak van kalsium (Ca) in vrug fermheid en by die voorkoms van fisiologiese afwykings, is al bewys in die literatuur. Hierdie studie het gefokus op die effek wat water- en voedingsbestuur strategieë, asook onderstamme en blaar-Ca toedienings op die Ca-inhoud van vrugte het.

Die Ca-inhoud van die appelvrugte tydens oes het nie betekenisvol verskil tussen toedienings van water deur mikrospuite (voeding met hand), water en voeding deur sproeibemesting, of water en voeding deur pulserende drup aan 'Brookfield Gala' bome (draend en in die derde blad) op twee onderstamme (M793 en M7) nie.

In 'n verdere proef op dieselfde perseel, is drie Ca-toedieningsvlakke op drie fenologiese stadiums aan appelbome toegedien deur 'n pulserende drup sisteem en die effek daarvan op Ca-inname in die vrugte is ge-evalueer. Vrugte van bome wat onderhewig was aan hoë Ca-vlakke in die voedingsoplossing gedurende die volle periode van volblom tot oes het hoër Ca-konsentrasies in die vrugte getoon as bome wat onderhewig was aan standaard of lae vlakke van Ca-voedingsoplossings.

In die onderstamproef was daar 'n tendens dat meer groeikragtige onderstamme, wat sterk vegetatiewe groei en ook moontlik hoër blaar:vrug verhoudings ondervind het, groter vrugte gehad het. Meer dwergende onderstamme, met meer aanvaarbare blaar:vrug verhoudings, het kleiner vrugte gehad. Dus blyk dit of die onderstam 'n direkte rol kon speel op vruggrootte en as gevolg daarvan, was daar 'n verskil in vrug-Ca.

Toediening van verskillende Ca-produkte, volgens verskaffers se riglyne, het getoon dat agt $Ca(NO_3)_2$ en Calcimax® toedienings, tussen 40 dnvb en 80 dnvb, net so effektief was in die beheer van bitterpit as drie toedienings Ca-asetaat en Ca-fulfaat (een keer ‘n maand, vanaf ses dnvb). Laat toedienings (beginnend teen 80 dnvb) van $Ca(NO_3)_2$ en Ca-asetaat was meer effektief in die toename van Ca-inhoud van vrugte tydens oes, as mid-seisoen en vroeë seisoen toedienings (40-80 dnvb en kort na volblom onderskeidelik). Laat (80 dnvb) $Ca(NO_3)_2$ het die hoogste Ca-inhoud in vrugte tydens oes gelewer. Uit die resultate blyk dit of bitterpit effektief beheer kan word deur vroeë (kort na volblom) en middel (40 dnvb) seisoen Ca-blaarspuite. Daar kon egter geen bevredigende gevolgtrekkings betreffende bitterpit beheer gemaak word nie, weens lae voorkoms van bitterpit (< 1%).

Evaluasie van vooroes voorspellings metodes gedurende die seisoen het aangedui dat magnesium-infiltrasie meer akkuraat was as etileenforsering by die korrekte voorspelling van werklike bitterpitvoorkoms na opberging. Die lae voorkoms van bitterpit gedurende 2005/06, kon egter ‘n effek gehad het op die betroubaarheid van die resultate.

Resultate toon dat genoegsame Ca-opname natuurlik sal plaasvind na ‘Brookfield Gala’ appels as fisiese en chemiese grond toestande (katioon uitruil kapasiteit, pH ens.), grond-deurlugting en -dreinering, fyn wortelontwikkeling, asook bestuursaspekte (besproeiing, bemesting, snoei, boomopleiding, uitdun en lighuishoudings-bestuur) optimaal is. Vrug Ca-vlakke was hoog tydens oes in die tweede seisoen (6.96 tot 8.85 $mg.100g^{-1}$ vars massa) in beide studies. Vir ‘Golden Delicious’ appelbome is blaar-bespuitings met Ca, veral met $Ca(NO_3)_2$, na aan oes (van 80 dnvb), steeds die beste manier om vrug Ca-inhoud te verhoog om sodoende vrugkwaliteit te optimaliseer, maar dit sal nie noodwendig bitterpit voorkoms verminder nie.

Dedicated to my father, Hannes and my mother, Marinda

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CONTENTS

CHAPTER 1

INTRODUCTION

Sufficient levels of calcium (Ca) in fruit at harvest are necessary to maintain high fruit quality throughout long term storage (Siddique & Bangerth, 1993; Casero et al., 2002; Conway et al., 2002). Adequate Ca helps to maintain apple fruit firmness (Conway et al., 2002). It is especially important in apples and pears, because these fruits are stored for extended periods (Faust, 1989). Furthermore, low fruit tissue Ca are associated with a number of physiological disorders of which bitter pit is the most important disorder of apples (Martin et al., 1975; Sharples, 1980; Terblanche et al., 1980; Broom et al., 1998; Faust, 1989; Yuri et al., 2002; Jackson, 2003). Under South African conditions Terblanche et al. (1980) recommended that the minimum Ca concentration at harvest for 'Golden Delicious' apples for bitter pit control is 5.4 and 6.6 mg.100g^{-1} fresh weight for unsprayed and Ca-sprayed fruit, respectively.

Ca shows a very low mobility in the phloem (Zocchi & Mignani, 1995). Therefore Ca is considered to move preferentially upwards in the xylem sap (Vang-Peterson, 1980; Jackson, 2003) which is dependent on the Ca exchange adsorption on the xylem walls, the transpirational flux (Bangerth, 1979; Banuelos et al., 1987; Faust, 1989; Jackson, 2003), and on the xylem functionality of the fruit (Dichio et al., 2003), and is not simply a matter of mass flow (Jackson, 2003). As a result of Ca mobility in the transport vessels, the major challenge associated with Ca application is that Ca must not only be absorbed by the tree, but Ca must also be transported into the fruit. Another difficulty with Ca uptake is that its mobility in the soil is also low and therefore Ca must be applied and mixed during preplant soil preparation in a quantity that will supply the tree for most of its life (Faust, 1989). It seems that Ca uptake by plant roots is directly related to Ca availability in the soil as well as the soil

volumetric water (Ernani et al., 2002). A high level of Ca in the cation exchange capacity (C.E.C.) of the soil is necessary. Ca should constitute 70-80% in the C.E.C. of the soil (Terblanche et al., 1980). Maintaining the correct soil water status is also effective in improving root growth, which is vital because roots must function efficiently for optimum uptake of nutrients (Faust, 1989). Generally most Ca uptake occurs through young, white, nonsuberized roots (Jackson, 2003).

Intensive cell division provides a considerable sink for Ca, mainly during the first 35-50 day period of exponential fruit growth following fertilization (Palmer et al., 2003). The extent of this stage may be crucial to the final Ca status of the fruit (Ferguson & Watkins, 1989). This indicates the importance of early xylem transport for the supply of Ca to the developing fruit (Casero et al., 2002). Unfortunately other elements may negatively influence Ca uptake and translocation to the fruit. Timing of nitrogen (N) application may have an influence on the incidence of bitter pit, because early season applications may compete with Ca translocation when Ca influx into the developing fruit is critical (Ferguson & Watkins, 1989). Potassium (K) reduces Ca uptake by ion antagonism (Failla et al., 1990), and the balance between these two elements is important in the susceptibility of fruit to bitter pit (Ferguson & Watkins, 1983). As a result of high levels of K in fruit, a high K/Ca ratio is associated with increased wastage of fruit due to rotting and bitter pit (Sharples, 1980).

Ca deficiencies are not necessarily alleviated by raising soil Ca levels (Faust, 1989). Therefore the direct application of Ca to the fruit by foliar sprays of Ca salts is still the most effective method of ensuring adequate fruit Ca levels during fruit growth (Bramlage et al., 1980; Conway et al., 2002; Schlegel & Schönherr, 2002), especially to bitter pit susceptible cultivars. Ca uptake from spray applications can happen only directly via the fruit skin; little,

if any Ca is transported from the leaves to the fruit (Saure, 2002). In South Africa, $Ca(NO_3)_2$ is preferred above $CaCl_2$ as it is less likely to cause leaf scorch, especially in sensitive cultivars such as 'Granny Smith' (Wooldridge et al., 1998). It seems that the most effective time of application of Ca sprays to reduce bitter pit incidence successfully is still uncertain. Results obtained by Lötze & Theron (2006) on the effectiveness of pre-harvest Ca application for bitter pit control in 'Golden Delicious apples', under South African conditions, indicate that foliar applications during the first 40–70 days after full bloom effectively increases fruit Ca content early in the season and also reduces the incidence of bitter pit. Neilsen et al. (2005) showed that in order to achieve fruit with maximum Ca concentration at harvest, $CaCl_2$ should be applied close to harvest.

The main objective of this study was to increase the Ca content in apple fruit in order to reach sufficient amounts of the element at harvest to ensure good fruit quality. Three water and nutrient application systems were evaluated as well as the effect that three Ca application levels applied directly to the soil may have on the uptake and translocation of Ca to fruit. Additionally, root studies were also done in these trials, since active root tips are needed for Ca uptake. Possible effects of six rootstocks on the uptake of Ca into 'Reinder Golden Delicious' apple fruit were evaluated. The effect of pre-harvest foliar Ca applications to increase fruit Ca content and reduce bitter pit incidence of 'Golden Delicious' apples were evaluated firstly for different Ca formulations, and secondly for applications commencing at three different times. Finally, ethylene forcing and magnesium infiltration were evaluated as pre-harvest methods to predict bitter pit incidence for 'Golden Delicious' apples.

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CHAPTER 2

CHALLENGES ASSOCIATED WITH CALCIUM SUPPLY, UPTAKE AND TRANSPORT INTO APPLE FRUIT TO ENSURE OPTIMUM FRUIT QUALITY

Introduction

Apples and pears are two deciduous fruit types that are stored for extended periods after harvest and adequate fruit firmness is one of the key parameters of fruit quality at the final destination after export. Calcium (Ca) is perhaps the most important mineral element determining fruit quality and adequate Ca helps maintain apple fruit firmness (Conway et al., 2002).

Ca concentration in apple fruit is important because low Ca concentrations are associated with bitter pit (Broom et al., 1998), dating as far back as 1936 (Bramlage et al., 1980). Bitter pit, a physiological disorder of apple fruits, remains a problem in apple storage in many parts of the world, even after decades of research (Martin et al., 1975). Many factors contribute to the development of bitter pit. Climate and soil, plant nutrition and orchard management, internal relationships between vegetative and generative growth, and storage conditions are factors that may contribute to the development of bitter pit in some way (Saure, 1996).

2.1 Calcium physiology

2.1.1 The occurrence and role of Ca in fruit tissues

As an integral part of the cell wall, Ca is involved with the stabilising and strengthening of the cell walls and/or the cell membranes. In membrane structure and function Ca is of paramount

importance for the integrity of the membrane (Zocchi & Mignani, 1995). One of the functions of the cell walls is to provide structural rigidity and physical protection to the cell (Wooldridge, 2001). The structure of the cell wall consists of cellulose microfibrils, hemicelluloses, pectin and protein. Ca is involved with the structural rigidity in the middle lamella which lies between the cell walls of cells.

Ca appears to act as an intermolecular binding agent, cross linking and stabilising the pectin-protein complexes in the middle lamella, thereby increasing the rigidity of the cell wall (Zocchi & Mignani, 1995; Fallahi et al., 1997; Wooldridge, 2001). Ca ions have been shown to bind pectin molecules within the cell wall (Bramlage et al., 1980; Jackson, 2003).

Ca ions are essential for the activity of a number of enzymes, of which some are components of membranes (Bramlage et al., 1980). Another principle characteristic assigned to Ca is the protection Ca ions provide against the damaging effects of toxic ions (heavy metals), salinity and low pH (Zocchi & Mignani, 1995).

2.1.2 Influence of Ca on storage quality of fruit

Ca is perhaps the most important mineral element determining fruit quality (Conway et al., 2002) and the post harvest storage life of fruit (Siddiqui & Bangerth, 1993). It is especially important in apples and pears, because these fruits are stored for extended periods and the effect of Ca on storage quality cannot be substituted by other factors (Faust, 1989).

Firmness is one of the parameters of fruit quality. Most deciduous fruit derive their firmness from the structural integrity and chemical composition of the cell walls and membranes (Zocchi & Mignani 1995). As pectic compounds degrade during the course of ripening, or by

invasion of pathogens, the fruit softens (Faust, 1989). Ca deficiency may facilitate cell membrane deterioration with subsequent loss of turgor and leakage of cell fluids (Saure, 2002). Therefore, adequate Ca helps to maintain apple fruit firmness (Conway et al., 2002).

2.1.3 Effect of Ca deficiency on the development of physiological disorders

Although a number of physiological disorders of apples are associated with low fruit tissue Ca (Faust, 1989; Jackson, 2003), bitter pit is the most important disorder (Yuri et al., 2002; Jackson, 2003). Terblanche et al. (1980) found a negative relationship between the incidence of bitter pit and fruit Ca. Sharples (1980) found that the susceptibility of 'Cox's Orange Pippen' apples to rotting and bitter pit closely correlated with each other, and both were negatively correlated ($p<0.01$) with fruit Ca over three consecutive seasons. Martin et al. (1975) subjected 'Cleopatra' apple trees to a wide variety of treatments related to nutrient supply. Evidence from their experiments supported the hypothesis that bitter pit development is primarily a response to low fruit Ca content.

Bitter pit has been described as follows: (by various authors). The primary symptom is the discrete pitting of the cortical flesh, and the collapse of the outermost cells causes small depressions (Jackson, 2003). Plasmolysis of the cytoplasm has occurred by the time that pitting is visible to the naked eye (Jackson, 2003). The localized brown pits are accentuated by the localized decrease in Ca (Hopfinger et al., 1984). The skin over these depressions usually takes on a deeper green colour than the surrounding skin (Faust & Shear, 1968). Steenkamp & de Villiers (1983) reported that a much higher concentration of Ca, potassium (K) and magnesium (Mg) was found in the pitted tissue than in the sound tissue. One possible explanation is that the high metabolic activity associated with the disorder attracts nutrients (e.g. Ca, Mg and others) to the affected tissues (Faust, 1989). Steenkamp & de Villiers

(1983) argued that bitter pit tissue has a higher concentration of oxalic and citric acid, but a lower concentration of malic and succinic acid, than sound tissue.

Pitting may even appear deep in the flesh and only become visible when the fruit is cut (Jackson, 2003). Pitting appears to be more towards the calyx end of the fruit (Faust & Shear, 1968; Ferguson & Watkins, 1989), as a result of localized deficiency of Ca (Saure, 2002), and the calyx end is usually lower in nutrients than the stem end (Faust, 1989). However, bitter pit may develop in the orchard or only become evident after a period of storage, and the surface appearance, especially at harvest, may fail to reflect the severity of the disorders after storage (Jackson, 2003).

Under South African conditions Terblanche et al. (1980) recommended that the minimum Ca concentration for 'Golden Delicious' apples for bitter pit control is 5.4 and 6.6 $mg.100g^{-1}$ fresh weight (FW) for unsprayed and Ca-sprayed fruit, respectively. Levels of 5 $mg.100g^{-1}$ FW and above are also necessary to maintain high quality fruit throughout long-term storage.

2.2 Ca nutrition

2.2.1 Ca uptake from the soil and translocation to the fruit

Although much research has been done on the relationship between fruit Ca concentrations and the occurrence of bitter pit, very little is known about the relationship between orchard nutrition and fruit eating quality (Faust, 1989). To ensure acceptable fruit quality at harvest and for long term storage, sufficient levels of fruit Ca must exist (Casero et al., 2002). Unfortunately, Ca nutrition is complex and the major challenge associated with Ca application is that Ca must not only be absorbed by the tree, but also be transported into the fruit.

2.2.1.1 Supply by the soil

Most nutrients are applied yearly to the surface of the soil. Some nutrients are not so mobile in the soil and therefore must be applied and mixed before the tree is planted, in a quantity that will supply the tree for most of its life (Faust, 1989). One of those elements is Ca.

Ca deficiency is a common phenomenon in all major apple-growing areas, even on calcareous soil (Wojcik & Szwonek, 2002). Under field conditions, the relationship between nutrient uptake and quantities available in the soil are difficult to establish (Terblanche et al., 1980). Ca redistribution within plants is limited, and the presence of adequate levels of Ca in the soil solution, does not ensure sufficient uptake or translocation to the tree and especially to the fruit (Shear, 1980). However, a high level of Ca in the C.E.C. of the soil is necessary. Ca should constitute 70-80% in the C.E.C. of the soil (Terblanche et al., 1980).

The major source of Ca for soil application is limestone (Korcak, 1980). The two limestone types well known by most South African producers are calcitic and dolomitic limestone. Ca should be applied before planting because subsurface liming is usually impossible thereafter (Kotzé & Joubert, 1981) because Ca applied to the soil surface basically remains in the soil surface and penetration through the soil is very slow. The result is that Ca is not present throughout the complete root zone for efficient Ca uptake by roots. However, when Ca is applied to the soil before planting, Ca can be deposited into all the soil horizons for efficient root uptake. Limestone is also added to the soil if the soil pH is too low and the soil is acidic. According to Faust (1989) all soluble aluminum (Al) exists as Al^{3+} on acid soils below pH 4.0. In such a case half of the cation exchange sites may be occupied with Al and as a result of that Ca, Mg, K, P and other nutrient uptake in the root zone decreases (Kotzé et al., 1977). Limestone increases the pH by reducing the amount of free $[H^+]$ in the soil solution. Since Ca

is less available in acid soils, regular liming programmes are essential in areas of low soil pH (Bramlage et al., 1980).

Ca occurs in soils predominantly as the divalent cation (Ca^{2+}) held on exchange sites and to a lesser extent in chelated forms, insoluble phosphates, sulphates or silicates, as ion pairs, or in microbes (Korcak, 1980). The first step in Ca uptake involves movement of Ca in the soil towards the roots (Jackson, 2003). For this process to proceed with maximum efficiency, the ion concentration in the soil water must be high enough to enable the nutrients needed by the plant to reach the root by mass flow (Jackson, 2003). It seems that the amount of Ca taken up by plant roots is directly related to Ca availability in the soil and to the soil volumetric water (Ernani et al., 2002). Shear (1980) agreed that although Ca uptake and translocation is generally accepted to be an ion exchange phenomenon, it is dependent on optimum soil moisture. Under proper orchard soil management however, the exchange complex of the soil is dominated by Ca (Korcak, 1980).

2.2.1.2 Uptake by the roots

The root is the unique higher plant organ responsible, amongst other functions, for mineral uptake (Jeschke & Hartung, 2000). Many factors can contribute to low nutrient uptake by roots, for example, poor soil aeration when oxygen levels are limited, low moisture conditions of soil, or low metabolic activity (Faust, 1989). High nutrient uptake can reflect optimum moisture conditions, large, well-developed root systems with healthy root tips or high photosynthetic rates that supply the root with sufficient carbohydrates for optimum root metabolism (Faust, 1989). Furthermore, roots anchor the tree, absorb, transport and occasionally store nutrients and water and synthesize compounds essential for regulation of above-ground activities of the tree (Faust, 1989). The effect of any manipulation of the

above-ground parts of the tree may have on the functioning of the roots of the tree must always be considered. Even a pre-plant decision like planting density may affect the root development. Atkinson & Wilson (1980) explains that intense planting density affects root distribution in such a way the roots tends to grow deeper as lateral growth is restricted when trees are planted closer together.

Depending on soil form, texture, structure and limitations, most roots occupy the soil volume between 0 and 80 cm in depth, with the most active portion of the roots between 0 and 30 cm depth (Atkinson & Wilson, 1980; Faust, 1989). This is especially true for South African apple producing areas. Soil aeration is often the determining factor in how deep the majority of roots penetrate (Faust, 1989).

For optimum uptake of nutrients by roots, roots must function efficiently, and competition within the tree must be altered in favour of the target organ (Faust, 1980). Periodicity in root growth depends mainly on shoot growth and fruit load of the tree (Faust, 1989). Root growth commences in early spring when the soil reaches the appropriate temperature. Apple root growth starts at 4-5°C in the Northern hemisphere (NH) (cit. Kolesnikov, 1971 in Faust, 1989). Growth ends at the beginning of active shoot growth (Head, 1967). The next peak in root growth starts when shoot growth ceases around August in the NH (Head, 1967).

Neilsen & Neilsen (2003) listed three pathways through which nutrients are taken up by roots: i), nutrient uptake by direct root interception; ii), uptake by mass flow of dissolved nutrients in water absorbed and iii), by diffusion if a concentration gradient for the specific ion develops around the absorbing root.

Uptake by the root itself seems to be complex. In their study on the ion transport and endodermal suberization in the roots of *Zea mays* Ferguson & Clarkson (1975) found that maximum Ca translocation took place 12 cm from the maize root tip. They mentioned that lateral roots are initiated in this region in the pericycle and that the structure of the endodermis may change transiently in this region. Generally most Ca uptake occurs through young, white, nonsuberized roots (Faust, 1989; Jackson, 2003). Researchers reported that the endodermis caused a major barrier for Ca uptake in apple and pear. Work by Atkinson & Wilson (1980) showed that this was not the case in apples and pears. According to them, the phellogen of woody roots fails to act as a barrier to Ca, because the deposition of the suberin takes place on the inside of the cell walls rather than within the phellogen, so the apoplastic pathway remains viable.

The uptake and transport of Ca by apple seedlings is affected by the nutrient balance in the solution (Kotzé, 1979). According to Faust (1980) K and P are taken up over the whole length of the root which suggests that these ions move via the symplast but, Ca is not transported effectively by the symplast (Faust, 1989). Therefore active root growth is likely to be more important in Ca uptake than in the uptake of K or P (Faust, 1980).

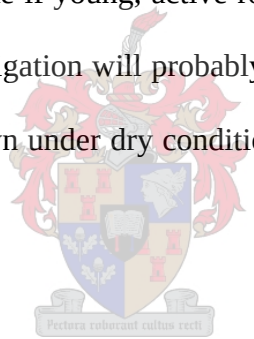
2.2.1.3 Factors influencing root growth and may affect nutrient uptake

Some of the most important factors that have an influence on the effectiveness of root growth and the ability of roots to take up nutrients to fulfil the need of the tree include:

Irrigation

Maintaining the correct soil water status is vital in improving root growth. Upward movement of Ca is associated with the transpiration rate of the tree (Faust, 1989). Therefore,

soil water must be at optimum to fulfil the water requirement of the plant as well as the nutrients lost in the transpiration process. Generally, micro-irrigation and drip irrigation are used for applying water to fruit trees. The wetted soil volume in an orchard irrigated by drippers is about 30-50 percent of that irrigated by surface irrigation (Levin et al., 1980). The root distribution pattern of trees irrigated by drippers depends mainly on the wetted soil volume under the dripper (Pijl, 2001). The advantage of this is more accurate management over plant processes because nutrients and water can be applied during certain phenological periods as needed, (Stassen et al., 1999) but also when evapotranspiration is high. Furthermore, the application of fertilizers through the drip system increase fertigation efficiency when nutrients are applied only to the restricted root zone (Bar-Yosef, 1999) in order to ensure availability for uptake if young, active roots are present. After storage, fruits from trees that received adequate irrigation will probably show less susceptibility to bitter pit than fruits from trees that were grown under dry conditions due to adequate fruit size and Ca in fruit.



Mulch

Mulching increases root growth at the soil surface (Faust, 1989). In young apple trees (under mulch) the root growth was higher in all root diameters, particularly at 0-8 cm depth (White & Holloway, 1967).

Root restriction

Root restriction could result if trees are planted in a container, in ridges over heavy clay, which restricts downward penetration, or too close in a high-density orchard (Faust, 1989). Low oxygen levels caused by poor soil aeration, low metabolic activity and low moisture in

soils are all factors that can influence root activity negatively and therefore also nutrient uptake.

Rootstock

Effects of rootstock on scion yield, growth, and uptake of minerals has been reported (Lockard & Schneider, 1981; Sharma & Schaunan, 1991). The effect of rootstock on the absorption and transport of mineral elements is complex to such an extent that an interaction between rootstock and cultivar seems to exist for accumulation, utilization and redistribution of nutrients (Oukabli & Lahlou, 2005). Therefore it can influence Ca uptake and transport to the fruits.

Nutritional status of the tree

Root growth is greatly influenced by the general nutritional status of the tree (Faust, 1989). Ca is essential for the growth of shoot tips and where Ca deficient conditions are present in the tree, even if not yet observed above ground, root tips may have already died (Faust, 1989).

2.2.1.4 Upward movement of Ca in the plant transport systems

The xylem is the primary pathway for supplying shoots with the mineral elements essential for growth (Jeschke & Hartung, 2000). Ca is considered to move preferentially in the xylem sap (Vang-Peterson, 1980; Jackson, 2003), upward with the transpiration stream (Faust, 1989). Upward movement is dependant on the Ca exchange adsorption on the xylem walls, the transpirational flux (Bangerth, 1979; Banuelos et al., 1987; Jackson, 2003), the xylem functionality of the fruit (Dichio et al., 2003) and is not simply a matter of mass flow (Jackson, 2003). The xylem appears to provide the only route for the major distribution of Ca

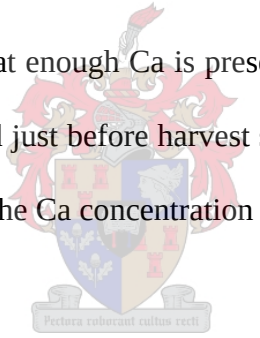
in plants, and any permanent loss of this element during movement in the xylem must be of significance in the overall Ca nutrition of the plant (Ferguson & Bollard, 1976).

The main reason of Ca deficiency in fruit can be attributable to the low mobility of the ion in the plant transport systems (Zocchi & Mignani, 1995). Ca shows a very low mobility in the phloem (Zocchi & Mignani, 1995). This indicates the importance of the early xylem transport for the supply of Ca to the developing fruit (Casero et al., 2002). According to Lang (1990), xylem flow reverses at times, in other words, it flow from the fruit to the tree and it occurs particularly during periods of high evaporative demand. Xylem sap flows into the fruit during the night but frequently flows out to the tree during the day. This process of xylem reversal is of importance to the overall water economy of the tree (Lang, 1990). As mineral elements are carried with the water in the xylem, this reversal may have an important effect on the final mineral composition of the fruit.

2.2.1.4 Uptake into the fruit

The final stages of movement into organs and tissues, or the flux into the fruits, are partly under metabolic control (Jackson, 2003). Accumulation of Ca and other elements occurs rapidly during the first period of fruit growth. Palmer et al. (2003) classified the first period as the initial 35-50 days of exponential growth following fertilization. This first period of rapid Ca uptake is accompanied by the cell division stage of fruit. Ferguson & Watkins (1989) note in their review on bitter pit that intensive cell division provides a considerable sink for Ca, and the extent of this stage may be crucial to the final Ca status of the fruit. Thereafter, because fruit growth continues, the Ca concentration of the fruit decreases with the rate of final expansion of the fruit. Schlegel & Schönherr (2002) stated that rapid penetration into young fruits can take place through trichomes and stomata, but after 45 days

after full bloom (dafb) fruit lose them and then penetration of Ca can only take place through lenticels. Therefore foliar Ca is applied from 70 dafb until harvest to increase fruit Ca. During the second phase, the period of cell enlargement, Ca uptake into fruit still continues, but at a slower rate than initial Ca uptake. Witney et al. (1986) concluded that although Ca concentrations peak at about six weeks after fruit set in avocado and thereafter decline, total fruit Ca content appears to increase steadily except for a short period of six to eight or ten weeks after fruit set. This plays an important role in determining the storage quality of fruit. Large fruit, with high Ca dilution rates, usually have a very poor storage quality in contrast with small fruit with a high Ca concentration and good storage potential (Faust, 1989). Perring (1979) showed that soil conditions with adequate soil moisture enhances Ca uptake, but dry conditions may cause the outflow of Ca from the fruit. Irrigation in dry areas would therefore be appropriate to ensure that enough Ca is present in the fruit at harvest. However, excessive irrigation during the period just before harvest should be avoided as far as possible, as it will result in further dilution of the Ca concentration in the fruit.



Lang (1990) studied the xylem: phloem balance in two apple cultivars, one susceptible to bitter pit and the other one resistant, and found that the xylem and phloem made almost equal contributions to growth of fruit early in the season. Mid-season phloem transport tended to dominate fruit growth. Toward the end of the season, fruit growth was almost totally phloem dominated, and this dominant supply of nutrients by the phloem might shift the balance of fruit mineral composition with time. This might explain higher K concentrations and lower Ca concentrations in fruit at harvest, especially when supply of K is more than needed by the tree, and Ca supply to fruit are reduced by ion antagonism.

The early season spur canopy also plays a role in the final Ca concentration of the fruit. Proctor & Palmer (1991) concluded that any damage to young leaves from factors such as low temperature, or inadvertent spray injury, could have drastic effects on fruit set, flowering, fruit Ca level at harvest and the storage potential of such fruit due to carbohydrate transport.

Another important aspect of Ca translocation is the role that hormones play. Ca is translocated preferentially toward the shoot apex in growing plants. The auxin, indole acetic acid (IAA) that is synthesized in the shoot apex induces the transport to the growing tips (Faust, 1989). During shoot growth the growing tip becomes a centre for Ca accumulation, because an IAA-stimulated proton efflux pump in the elongation zones of the shoot apex increases the formation of new cation exchange sites. Basipetal IAA-transport forces Ca to be translocated acropetally (Bangerth, 1979). This may explain why Ca transport into the fruit decreases at the same time when IAA synthesis in the fruit subsides (Faust, 1989), but this downward movement of Ca in the phloem, is only to a very small extent.

2.2.1.5 Movement within the fruit

The apple core usually has the highest Ca concentration, followed by the inner cortex, which has a higher Ca concentration than the outer cortex (Jackson, 2003). It is generally agreed that Ca-related disorders mostly arise from internal Ca distribution problems, since the cytosol has very low levels of free Ca^{2+} (Witney et al., 1986).

2.2.2 Influence of specific elements on Ca nutrition and bitter pit incidence

In the following paragraphs the most important elements that play a role in the life cycle of apple trees will be discussed. The effect these elements might have on storage disorders and especially bitter pit will also be mentioned.

2.2.2.1 Nitrogen

N is the most regularly deficient and most commonly applied fertilizer in orchards (Neilsen & Neilsen, 2003). In order to achieve early yield, nitrogen has been over-applied, but even when nitrogen application is reduced with initiation of cropping, there may still be high levels of nitrogen stored in the soil, grass, sod and tree that is utilised (Bramlage et al., 1980). Young trees receive high quantities of N to stimulate growth of the trees (Bramlage et al., 1980). These high rates may continue after cropping begins, since high N levels can increase fruit yields, but at harvest, high-N fruit tend to be larger, greener, softer, more subject to pre-harvest drop and more likely to be affected with cork spot and bitter pit (Bramlage et al., 1980).

N nutrition in apples must be well regulated (Faust, 1980). Ca and N are both essential nutrients for the growth and well-being of all plants (Korcak, 1980). According to Korcak (1980), interactions between Ca and N occur, and therefore the nutritional balance of the tree must always be considered when fruit quality is low. The supply of N can have a negative effect on the uptake of Ca.

Ferguson & Watkins (1989) mentioned that the timing of N application can influence the incidence of bitter pit, because early season applications may compete with Ca translocation when Ca influx into the developing fruit is critical. Bramlage et al. (1980) stated that the total amount of N applied to fruit trees is more important for fruit quality than the form in which N is applied. Ca oxalate forms when N is supplied as a nitrate (NO_3^-). When it is supplied as ammonia (NH_4^+), Ca uptake is reduced and this reduces the Ca supply to the tree (Faust, 1980). According to Bramlage et al. (1980) the use of Ca nitrate as a source of nitrogen

fertilizer is often recommended. It may be easier to prevent Ca disorders by reducing the N concentration of the tree, rather than increasing the Ca concentration of the fruit (Faust, 1989).

2.2.2.2 Phosphorus

Kotzé & du Preez (1988) stated that the P requirement of apple trees under South African conditions appears to be low. Basso & Wilms (1988) commented on the nutritional status of apple trees in Southern Brazil, and found that their soils are naturally poor in available P. Apple trees do not respond to P fertilization and P does not move into the soil easily (Faust, 1989). Therefore P should be applied to the soil, as with Ca, before planting to ensure that it is available throughout the soil profile.

Most fruits need P to maintain its firmness and P functions as a structural element in DNA and RNA. Furthermore P is involved in the energy transfer mechanism and has a regulatory function in many enzymatic processes. When Ca levels and P levels are low, break down may develop. The threshold value for P concentration for Cox is 11 $mg.100g^{-1}$ FW (Waller, 1980). The P content of 'Golden Delicious' apple generally varies between 6.0 and 12.0 $mg.100g^{-1}$ FW (Terblanche et al. 1980). Terblanche et al. (1980) also stated that P fertilization experiments have failed to prove that P has any negative effects on bitter pit incidence. In conclusion, from the literature on nutrient applications, it seems that high levels of P has no direct effect on the uptake and transport of Ca in the plant or on the incidence of bitter pit.

2.2.2.3 Potassium

Potassium (K) is a major nutrient that needs to be supplied in relatively large quantities to crop plants and to fruit trees in particular (Faust, 1989). Faust (1989) further stated that the K

requirement of fruit trees is nearly equal to the requirement for N and Ca. One of the most important roles of K in plants is that it acts as an osmoticum maintaining the water status of cells and thus K is important in the opening and closing of stomata. Stomatal closure leads to reduced transpiration, and Ca transport is to a large extent dependent on the transpiration stream (Faust, 1989).

K reduces Ca uptake by ion antagonism (Failla et al., 1990) and the balance between these two elements is important in the susceptibility of fruit to bitter pit (Ferguson & Watkins, 1983). The risk of bitter pit increases as the levels of K in the fruit increases (Waller, 1980) as a result of its direct inhibiting effect on the fruit supply of Ca (Terblanche et al., 1980). In a study by Martin et al. (1975) on the incidence of bitter pit where 'Cleopatra' apple trees were supplied with different levels of K, Ca and N, an increase in K increased the incidence of bitter pit only under conditions of low Ca supply. When Ca and N supplies were at the same time high, K was not increased. According to Sharples (1980), a high ratio of K/Ca is associated with increased wastage of fruit due to rotting and bitter pit. Tomala (1997) stated that a K/Ca ratio above 22:1 is likely to be associated with commercial losses due to bitter pit. Both leaf and fruit K are positively related to bitter pit in apples and K therefore is an important factor in the induction of bitter pit (Terblanche, 1985). Increases in fruit K can also lead to an increase in titratable acidity. The middle lamella of the cell walls contains pectin that can be converted by these acids from insoluble protopectin to a water soluble condition, thereby causing disintegration of cells (Terblanche, 1985). Poor fruit quality can be the result.

2.2.2.4 Magnesium

Magnesium (Mg) is required and taken up by fruit trees in smaller quantities than Ca (Faust, 1989). Unlike Ca deficiency in trees, Mg deficiency is easy to address, because Mg is a mobile ion. Mg is easily transported from old to young leaves.

Fruit that is high in Mg is usually low in Ca (Faust, 1989). Susceptibility of fruit to bitter pit increases as Mg levels increase, especially where Ca levels are marginal (Waller, 1980). Apart from a high K/Ca ratio, a high Mg/Ca ratio is also associated with increasing bitter pit (Sharples, 1980).

2.2.2.5 Boron

Ca and Boron (B) are both essential elements for optimal growth of apple trees and fruit quality (Deyton et al., 2002). According to Peryea et al. (2003) B deficiency is a widespread problem in apple orchards. According to Bramlage et al. (1980) B deficiency occurs over most of North America and periodic applications of borax to the soil are a standard practice in many areas. Alternatively to that, one or two applications of a soluble form of B at or shortly after blossoming are recommended.

High Ca supplies reduced the transport of B to fruits in the early expansion phase, while a simultaneous high supply of Ca and N increases both Ca and B transport into young fruits (Bengtsson & Jensén, 1997). There appears to be a “barrier” for transport of Ca to the fruit. Therefore transport of Ca to fruit compared to leaves is much more restricted than transport of B to fruit compared to leaves (Bengtsson & Jensén, 1997). The transport of B seems to be much more freely.

Deyton et al. (2002) stated that soil treatments of B decreased the incidence of bitter pit and internal break down while foliar applications increased sensitivity to the two disorders. Excessive levels of B in fruit can cause earlier maturation and increased incidences of internal break down and decay after storage. B deficiency is reported to interact with Ca deficiency in the promotion of cork spot and bitter pit, since these disorders can be reduced by B application.

2.3 Practices to improve fruit Ca status

2.3.1 Foliar sprays

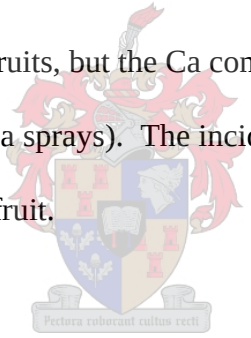
Foliar applications of nutrients are common in fruit production. Neilsen & Neilsen (2003) define this method clearly as the direct supply of nutrients to trees via spray application of dilute concentrations of minerals to foliage, buds and even bark.

Ca deficiencies are not necessarily alleviated by raising soil Ca levels (Faust, 1980), so the direct application of Ca to the fruit by foliar sprays of Ca salts is the most effective method of ensuring adequate fruit Ca levels during fruit growth (Bramlage et al., 1980; Conway et al., 2002).

As mentioned earlier, Ca is only transported to a very small extent in the phloem (Zocchi & Mignani, 1995), therefore it should be applied directly on to the fruit. Faust (1989) and Saure (2002) described that Ca uptake from spray applications can happen only directly via the fruit skin; little, if any Ca is transported from the leaves to the fruit.

Foliar Ca applications are reported to be highly successful in some cases, but in a substantial number of cases, results showed little effect on Ca content of fruit or bitter pit control (Hewett

& Watkins, 1991; Le Grange et al., 1998a). Van Goor (1971) reported on the effect of frequent sprays with $Ca(NO_3)_2$ solutions on the occurrence of bitter pit of 'Cox's Orange Pippen' apple. In most cases, bitter pit was reduced by Ca sprays and adequate commercial control of the disorder was obtained (reduction 74-94%). According to Terblanche et al. (1980), a series of three to five sprays with either $Ca(NO_3)_2$ (0.65 percent) or $CaCl_2$ (0.5 percent) can control bitter pit potential of about 16 percent in the orchard effectively, applied at fortnightly intervals from middle of December onwards under South African conditions, provided that proper fruit coverage is obtained with each spray. Rease & Drake (2000) applied foliar Ca at high rates either early (early June, late June and mid-July) or late (mid-July, early August, late August) in the season (Wenatchee, WA, Northern Hemisphere) and improved fruit quality of 'Red Delicious' and 'Golden Delicious' apples. Trees sprayed with $CaSO_4$ had healthy leaves and large fruits, but the Ca concentration in the fruit peel and cortex was equal to that of the control (no Ca sprays). The incidence of bitter pit was also very high, especially in the 'Golden Delicious' fruit.



Rease & Drake (2002) found that foliar sprays with $CaCl_2$ as Ca supply had the lowest bitter pit incidence and the highest Ca concentration in the fruit cortex when compared to other Ca sources. In South Africa, $Ca(NO_3)_2$ is preferred above $CaCl_2$, as $CaCl_2$ is more likely to cause leaf scorch, especially in sensitive cultivars such as 'Granny Smith' (Wooldridge et al., 1998).

Questions about the most effective time of application of foliar Ca to reach the highest Ca content in fruit at harvest have been asked for many years. Results of numerous spray rate application experiments have been confounded by application at different times (Le Grange et al., 1998b). A study by Casero et al. (2002) showed that early (starting at 6 dafb) foliar Ca applications did not increase Ca accumulation in 'Golden Delicious Smoothee' apples, while

late (starting at 70 dafb) Ca sprays increased the Ca absorption rate and accumulation in fruit. Their reasoning was, that during the first period of fruit growth, Ca is provided mainly by root absorption, but in the latter part of the season, when fruit Ca absorption is limited, foliar applications are more effective and results in an increase in fruit Ca content. As previously mentioned, Schlegel & Schönherr (2002) reasoned that rapid penetration into young fruits can take place through trichomes and stomata, but after 45 dafb fruit lose them and then penetration of Ca can only take place through lenticels and as a result foliar applications are effective. Results of Lötze & Theron (2006) on the effectiveness of pre-harvest Ca application for bitter pit control in 'Golden Delicious apples', under South African conditions, indicated that foliar applications during the first 40–70 dafb effectively increased fruit Ca content early in the season and also reduced the incidence of bitter pit. That is in contrast with present recommendations to start Ca applications later in the season (after 70 dafb) for a high fruit Ca at harvest. However, the aim of Lötze & Theron (2006) was primarily to reduce bitter pit prior to harvest and not necessarily to increase the final fruit Ca content. Work by Neilsen et al. (2005) on 'Braeburn' apples agrees with their findings. Five weekly sprays of $CaCl_2$ commencing the first week of June (NH) were as effective as a similar treatment applied later (commencing end of August) in the season, to reduce bitter pit incidence. This was despite a minimal impact on whole fruit Ca concentration at harvest (Neilsen et al., 2005).

2.3.2 Soil applications

As mentioned earlier, Ca is not very mobile in the soil and generally is applied during preplant soil preparation to fulfil the amount needed by plants for its entire life. Stassen et al. (1999) mentioned that with fertigation and hydroponics Ca applications must, especially, be made in the early fruit-developing phase as Ca uptake by fruit is optimal during the first six

weeks of fruit development. Increased fruit Ca concentration in 'Jonagold' apple fruit in the 2nd year after planting was associated with the use of $Ca(N0_3)_2$ and P fertigation. Kotzé & Joubert (1981) reported that the incidence of bitter pit was significantly reduced by application of lime on the soil surface, but it could not be eliminated completely. Nevertheless, Conway et al. (2002) stated that soil treatments with Ca to increase fruit Ca concentration have often achieved very little success. Furthermore, Neilsen et al. (2005) found that an annual liquid application of Ca thiosulphate which applied 35 g $Ca.tree^{-1}$ to the surface of the soil over two years was ineffective at increasing fruit Ca concentration. In their study Ca applications via the soil was also ineffective in reducing bitter pit incidence, however, fruit firmness was increased in the fruit which had received the soil thiosulphate application despite Ca concentration being unaffected.

2.3.3 Post-harvest dips

To maintain fruit quality, post-harvest dipping, vacuum infiltration and pressure infiltration of Ca solutions have been used with varying degrees of success (Sams & Conway, 1984). Vacuum infiltration of 'Anna' apple fruit with $CaCl_2$ resulted in firmer fruit than dipping fruit in 3% $CaCl_2$ (El-Ansary et al., 1994). Vacuum infiltrated fruit also had the lowest respiration rates after storage. Hewett & Watkins (1991) sprayed 'Cox's Orange Pippen' apple fruit with 0.6% $Ca(NO_3)_2$ at 2 week intervals and vacuum infiltrated the fruit with $CaCl_2$. After six weeks of storage, their results showed that when fruit were vacuum infiltrated alone it reduced bitter pit to a lesser extent than Ca sprays and was more effective in reducing external than internal bitter pit. They suggested that Ca applications over the growing season are superior to post-harvest vacuum infiltration with Ca in the prevention of bitter pit.

Conway et al. (2002) found that Ca concentrations resulting from pressure infiltration exceeded the required amounts for maintaining fruit firmness and reducing decay. Conway et al. (2002) warned on the other hand that several problems are inherent in using this procedure commercially. Some of the problems include cultivars that absorb different amounts of $CaCl_2$, as fruit of the same cultivar of different maturities or from different orchards and growing seasons.

2.4. Other factors influencing Ca nutrition in apples

2.4.1 Temperature

Temperature sets the boundaries on apple production areas (Palmer et al., 2003). Temperature during the 4-6 weeks immediately preceding harvest can influence the quality of the fruit at harvest and its storage potential (Palmer et al., 2003). The intake of Ca by fruit during actual fruit development is affected by temperature (Tromp, 1975). An important factor seems to be the rate of fruit growth, particularly early in the season (Tromp, 1986). Tromp (1986) noted that pre-bloom temperatures appeared to influence the level of Ca, but that was completely unexplained.

2.4.2 Humidity

When young seedlings of ‘Cox’s Orange Pippen’ were cultivated in growth chambers high humidity during the first eight weeks resulted in a higher P content in the leaves, whereas Mg and particularly Ca contents were higher in plants grown between 45% and 55% relative humidity (Naumann & Plancher, 1976).

2.4.3 Light

Light can influence photosynthesis (Faust, 1980). When light interception and light distribution through the tree is at its optimum, fruit quality factors such as fruit size, skin colour, and fruit storability should be affected positively. Ca uptake can be influenced by light. Faust (1980) indicated that energy is needed for Ca uptake independently from that needed for root growth. The rate of photosynthesis and carbohydrate partitioning within the apple tree may influence Ca uptake either by root growth or by supplying the energy needed for the ion to be taken up (Faust, 1980).

2.4.4 Crop load

At whole tree level, crop load is an important determinant of fruit size, with heavy crop loads associated with smaller average fruit size (Broom et al., 1998). Heavier crops of small apples usually have high Ca concentrations (Perring, 1979). Thus, fruit size is inversely related to the number of fruits per tree or total yield (Palmer et al., 1997). It has been shown that in non-bearing years leaf N, Ca and P content was lower while K and Mg was higher than in bearing years (Sadowski et al. 1995). Levels of K and to a lesser degree Mg, and the incidence of bitter pit and breakdown are all part of a complex related to mean fruit weight per tree (Martin et al., 1975).

Leaf to fruit ratio can be used to express crop load. A high leaf to crop ratio usually means fewer, larger fruit on the tree with higher incidence of bitter pit. Perring (1979) stated that vigorous extension growth near an apple might also lower its Ca concentration.

2.4.5 Fruit position

The position of fruit on the tree is one of the major sources of variation in mineral contents and storage potential in fruit (Ferguson et al., 1993). Ferguson et al. (1993) found that terminal fruit have higher Ca concentrations that suggested an enhanced input of Ca during fruit development in this position.

Primary spur leaves is the earliest supplier of carbon to the fruitlets, support and following of the initial growth of fruit during cytokinesis, when cell division sets the potential for subsequent fruit development (Corelli-Grappadelli & Lakso, 2004). He further states that fruit distribution within the canopy is highly correlated with light distribution, primarily because of the reduced number of flower buds that are differentiated under low light, but also because of the lower fruiting potential of the spurs that develop under reduced light conditions.

2.4.6 Fruit size

There is a strong relationship between fruit size and Ca concentration, with large fruit having less Ca and a much higher risk of bitter pit (Ferguson et al., 1993). Broom et al. (1998) tested the variability for individual fruit within 'Braeburn' trees, and found that a high degree of variability was present in the relationship between fruit weight and Ca concentration. Also, for all trees used in their study, fruit weight at harvest and fruit Ca concentration were greater with increasing number of full seeds in individual fruit and fruit weight at harvest and fruit Ca concentration were lower with increasing number of flat seeds from zero to six flat seeds. An inverse curvilinear relationship between fruit Ca and fruit diameter showed that fruit Ca concentration decreased as the fruit size increases (Perring & Jackson, 1975). Factors resulting in fruit expansion lead to lower Ca concentration by dilution (Perring, 1979).

To ensure good quality of ‘Gala’ fruits, Ernani et al (2002), stated that when large fruits and a high leaf/fruit ratio are expected to occur, Ca should be applied. Terblanche et al. (1980) stated that a fruit diameter threshold to ensure complete freedom from bitter pit would require a maximum fruit diameter of 61 mm.

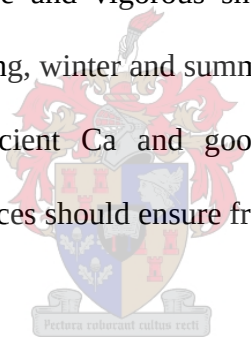
2.5 Conclusions

Ca is involved in physiological and biochemical mechanisms in the plant. Ca mainly occurs in cell walls, and in the middle lamella it binds to pectin-proteins to supply the rigidity of the cell. For most deciduous fruit that are stored for extended periods after harvest, firmness is one of the important quality parameters. Since the tree itself does not always absorb enough Ca to provide adequate amounts for excellent quality fruit, Ca applications are needed. Still, there are challenges involved with applications of Ca to ensure enough of the mineral in fruit at harvest, when it is most critical. Ca is not very mobile in the soil and therefore Ca is mostly applied preplant, but after application the amount of Ca in the soil must be correct to supply the tree for the rest of its life. This application has additional benefits as it can also correct the soil pH if necessary. However, active root growth, optimum soil moisture and the correct amount of the other minerals that contributes to the C.E.C. of the soil are needed to ensure uptake of Ca by roots. Nevertheless, with correct soil and root conditions, it is not certain that enough Ca will reach the fruit. Ca shows low mobility in the phloem and is primarily transported in the xylem, but when other sinks on the tree become stronger (mostly from 6 weeks after full bloom) transport of Ca to fruit may be less. Early xylem transport of Ca to fruit is of great importance. The supply of other elements (N, K, Mg ect.) in higher amounts than needed by a tree can also negatively influence the transport of Ca to fruit as the transport of those ions is preferred. The ratio of some of these ions to Ca in fruit at harvest may play an important role in the prediction of bitter pit incidence, especially where

susceptible cultivars are used. For instance, a high ratio (mostly above 22:1) of K/Ca in fruit at harvest may correctly predict bitter pit incidence of fruit.

Until present the best way to increase the Ca content in fruit seems to be by foliar applications. One of the most recent studies by Neilsen et al. (2005) have proved that direct soil applications of Ca were ineffective to achieve fruit with maximum Ca concentration at harvest.

Finally, unfavourable climatic conditions may have a negative influence on the Ca transport to fruit and these are factors that cannot be managed by fruit producers. In contrast with that, factors such as crop load, fruit size and vigorous shoot growth, can be controlled with management practices such as thinning, winter and summer pruning and fertilization to ensure that all fruit produced have sufficient Ca and good quality. In conclusion, correct management of all agricultural practices should ensure fruit with sufficient Ca levels and good quality in fruit production.



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CHAPTER 3

EFFECT OF WATER AND NUTRIENT APPLICATION STRATEGIES ON CALCIUM UPTAKE INTO APPLE FRUIT

Abstract

To optimise fruit production and quality, irrigation and fertilization are two of the common practices on commercial farms in South Africa. Optimum fruit Ca levels generally ensure good quality fruit. Three application systems viz. micro jets, fertigation drip and pulsating drip were used to apply water and nutrients to young 'Brookfield Gala' apple trees on two rootstocks, viz., M793 and M7 planted in a loamy sand soil. Fruit and leaf samples were collected at harvest for two consecutive seasons as well as fruit samples at 40 days after full bloom (dafb) in the second season. There were significant differences in fruit Ca concentration between the micro jet and fertigation treatments versus the pulsating drip treatment at harvest in 2004/05 when trees were still in a more vigorous growth phase. At 40 dafb during 2005/06 the interaction between treatment and rootstock was significant for Ca concentration. Results at harvest for 2005/06 showed no significant differences for fruit Ca concentrations or Ca contents between the treatments. Furthermore, the fruit mass did not differ significantly between the three systems for both seasons. Yield from the fertigation drip treatment (29.93 $t.ha^{-1}$) and pulsating drip treatment (33.63 $t.ha^{-1}$) was significantly higher than that of the micro jet treatment (19.64 $t.ha^{-1}$) during 2005/06. There was no significant difference between the yields on the two rootstocks. It was observed that the fine root volume of the trees on M7 was denser than for trees on M793. Overall chemical and physical soil conditions were ideal for active root development, after preplant soil preparation was done. Ca uptake into fruit of 'Brookfield Gala' apple trees was high and not dependent on the application strategies.

Introduction

In fruit production (especially in South Africa with its hot summers) irrigation is one of the most important factors in orchard management. Generally, micro-irrigation and drip irrigation are used for application of water to fruit trees. It is known that irrigation scheduling is usually based on the extraction of available plant water to maintain a favourable soil water content in the root zone (Koumanov et al., 1997). Micro-irrigation offers a large degree of control of water application to meet water requirements of trees. Koumanov et al. (1997) stated that micro-sprinkler irrigation has advantages over drip irrigation, because irrigation water is applied over a surface area larger than under drip irrigation. Therefore a larger root volume develops which minimises a lot of the risks and mistakes that can be made with irrigation. Drip irrigation differs from conventional irrigation methods in that water is applied to plants at more frequent intervals (Elfving, 1982). Furthermore, water application is localised as it is applied to a portion of the plant's potential root zone (Elfving, 1982) which results in the development of a restricted root zone in which moisture stress can be prevented by frequent water application (Haynes, 1985). The root distribution pattern of trees irrigated by drippers depends mainly on the wetted soil volume under the dripper (Pijl, 2001). The advantage of this is more accurate and timely management over plant processes because nutrients and water can be applied during certain phenological periods as needed, and can be adapted at short notice (Stassen et al., 1999). In general, the wetted soil volume in an orchard irrigated by drippers is about 30-50% of that irrigated by surface irrigation (Levin et al., 1980). Levin et al. (1972) made a survey in an apple orchard in the southern part of the Hula Valley in Israel. They found that over 80% of the root system and the water extraction were restricted to the upper 60 cm soil layer.

Fertilization is another important practise by which producers can manage the nutrient requirements of trees to produce quality fruit. Fertigation (the application of fertilizers through irrigation) brought new possibilities to control nutrient supplies to the plant (Pijl, 2001). The application of fertilizers through the drip system increases fertigation efficiency by applying the nutrients only to the restricted root zone to ensure availability for uptake by young, active roots (Bar-Yosef, 1999). This detail management of fertilizer application means that Ca and K can be supplemented during periods when it is most important for fruit development (Stassen et al., 1999) and it is known that sufficient levels of fruit Ca must exist to ensure acceptable fruit quality at harvest for long term storage (Casero et al., 2002). Sufficient levels of fruit Ca at harvest helps to maintain apple fruit firmness (Conway et al., 2002). Levels of 5.4 mg.100 g^{-1} fresh weight (FW) Ca and above are necessary to maintain high quality fruit throughout long-term storage and also for effective bitter pit control (Terblanche et al., 1980).

The presence of adequate levels of Ca in the soil solution, does not assure sufficient uptake or translocation to the tree and especially to the fruit (Shear, 1980). For optimum uptake of nutrients by roots, these must function efficiently, and competition within the tree must be altered in favour of the target organ (Faust, 1980). Generally most Ca uptake occurs through young, white, nonsuberized, actively growing roots (Faust, 1980; Faust, 1989; Jackson, 2003). With detail management of irrigation, a correct soil water status can be maintained that is not only effective in improving root growth, but also increases Ca uptake by the roots and transport of Ca to the fruits.

Within plants, Ca redistribution is limited and the main reason for Ca deficiency may be attributable to the low mobility of the ion in the plant transport systems (Zocchi & Mignani,

1995). Ca shows a very low mobility in the phloem (Zocchi & Mignani, 1995). Therefore, Ca is considered to move preferentially in the xylem sap (Vang-Peterson, 1980; Jackson, 2003), upward with the transpiration stream (Faust, 1989). Upward movement is dependant on the Ca exchange adsorption on the xylem walls, the transpirational flux (Bangerth, 1979; Banuelos et al., 1987; Jackson, 2003), and the xylem functionality of the fruit (Dichio et al., 2003) and is not simply a matter of mass flow (Jackson, 2003). Therefore, soil water must be at an optimum to fulfil the water requirement of the plant lost in the transpiration process. With a pulsating drip system this can be possible as water and nutrients are continuously applied to the root system. However, excessive irrigation during the period just before harvest should be avoided as far as possible as it will result in further dilution of the Ca concentration in fruit (Faust, 1989).

The objective of this study is to comment on the influence that different water and nutrient application strategies may have on the Ca uptake into apple fruit. The hypothesis is that frequent water and nutrient (especially Ca) supply through a pulsating drip system will increase fruit Ca. As a result of the more prolific root branching and the stimulation of feeder roots, Ca may easily be absorbed by the roots. Root distribution through the soil profile was studied as well, because of the important role actively growing roots play in Ca uptake.

Materials and methods

The study was done over two consecutive seasons on the commercial farm Snyerskraal, Genadendal in the Villiersdorp fruit production area (33º59´S; 19º18´E). This area normally experiences chilling of approximately 600 Infruitec units. 'Brookfield Gala' apple trees on two rootstocks were planted in August 2003, the standard rootstock M793 and a more dwarfing rootstock, M7. The trees were subjected to micro jet (one to two times a week),

fertigation drip (once a day) and pulsating drip (several times a day) irrigation systems. Micro jets wetted continuous areas within the rows, while the fertigation and pulsating drip systems wetted a restricted root area at both sides of the tree trunk. The pulsating drip is sometimes also referred to as open hydroponics because the root surface is continuously surrounded by a film of water and nutrients. All three systems were thus managed as near as possible to the way these systems are operated under commercial farm circumstances. Water and nutrients were applied through fertigation drip and pulsating drip, but only water was applied through micro jets, and for the latter treatment nutrients were applied by hand. Micro jets (32.0 $l.h^{-1}$) were spaced 1 m from each other and Netafim 2.3 $l.h^{-1}$ pressure compensated drippers (drippers every 600 mm) were used for the fertigation and pulsating drip systems. All standard commercial orchard practices were performed, except no additional foliar Ca sprays were applied to the trees. 'Granny Smith' trees were planted as cross-pollinators.

Soil preparation:

Trees were planted on a well drained, well aerated Tukulu soil. It is classified as loamy sand and the percentages clay, silt and sand as well as the water holding capacity of the soil are listed in Table 1. Preplant soil preparation was done by cross-ripping to a depth of 800 mm. It was first ripped in the direction which trees were to be planted and then 45º across. To achieve an ideal pH of about 5.5, calcitic lime was mixed into soil horizons. In addition, soil preparation was done to assure the correct percentages of Na, K, Ca and Mg expressed as exchangeable cation capacity in the soil (Table 2). The organic fraction of the soil was improved by applying sawdust to the soil before the first ridging. Before the second ridging, rotten straw was applied to the soil.

In the middle of each two rows of trees a trench was dug, 1200 mm deep and 500 mm wide. The trench was laid out with a double layer, black, gunplast plastic (150 micron) and filled with orchard soil in between to prevent lateral movement of water and nutrients.

Tree training, pruning and thinning:

Trees were received from a nursery and at planting they were cut back to 1.2 m because some trees were longer than that and others just reached 1.2 m height. The latter were cut back approximately 2 cm. Tree spacing was 4 m x 1.25 m. Trees were trained to a central leader spindle with lateral shoots bent according to the solaxe system. In the first year shoots were bent too far beyond the horizontal which resulted in water shoot development on the shoots. The water shoots were cut out during November that year. Thereafter, for the duration of the trial, light pruning was done only if necessary to improve spur development and fruit bearing positions. During 2004/05 approximately 10 fruit were left per tree. During the 2005/06 season, an average of 10 fruit were left per lateral branch during fruit thinning, depending on branch thickness and length. Bearing positions were mostly spurs, but also horizontal short shoots of approximately 30 cm long. One fruit per flower cluster was left on the inner 50% of the branch and two fruit per flower cluster on the outside 50% of the branch. The number of fruit was thus determined by the growth (number of lateral branches and bearing positions on the branches).

Water and nutrient management:

This study was done on the same site as a PhD study where the influence of water and nutrient management strategies on horticultural and physiological aspects was researched. Only essential aspects related to this aspect will be summarised.

Water management:

Long term (± 25 years) evaporation data from a nearby weather station and crop factors (Greene, 1985) for the Villiersdorp area (33º59´S; 19º18´E) were used to estimate annual and monthly water usage of apple trees. Previous information of a farm near Villiersdorp with 'Royal Gala' apple trees on a sandy loam soil was also taken into account to predetermine monthly water application. In the orchard this predetermined data was adapted according to weather data and soil water measurements (see soil and plant monitoring) (Table 3). Trees received an annual amount of 2607 $m^3.ha^{-1}$ water through the drip systems in their first and slightly more (3073 $m^3.ha^{-1}$) in their second year after planting. The annual amount of water for the micros was 25% more than for the drip systems. Trees in their third leaf received an annual amount of 4623 $m^3.ha^{-1}$ water through the drip systems and approximately 25% more through the micro jets.

Nutrient management:

The annual nutrient requirement was adapted from the studies of Stassen & North (2005) on pears (Table 4). These annual amounts were divided percentage wise according to phenological demand as explained by Stassen et al. (1999). The different phenological stages were:

1) Winter (Jun, Jul & August)
2) Budswell – full bloom (± September)
3) Full bloom – 40 dafb (October – ¾ November)
4) 40 dafb – 4 weeks before harvest (¾ November & December)
5) 4 weeks before harvest - harvest (January & February)
6) After harvest (March - May)

With the aid of a computer programme the water and nutrient data were used to prepare a balanced solution from a list of available fertilizers. Delivery capacity of micros or drippers, system efficiency, % leaf coverage, soil composition and predicted yield was also entered into the programme that then determined the application of water (and nutrients in the case of drippers) according to Table 3. The amount and kind of fertilizer that had to be mixed for application to the trees at different stages as well as the amount of water for each application was determined.

The computer programme was compiled by Gerhard Mostert, a private irrigation consultant. In the case of the micro jet treatment, hand application of N and P was done six times during the year at the commencement of each phenological period using the same amount as for drippers, but only six times per year. Their macro and micro elements were given according to standard procedures but also using the same annual amounts. During the first year after planting a young tree solution prepared by Omnia Fertilizers (Epsom Downs Business Park, 13 Sloane Street, Bryanston, South Africa) was used. For trees in their second leaf the nutrient solution was prepared as described for a fruit yield of 10 ton.ha^{-1} plus an additional 30% of all nutrients to allow for vegetative growth. In the third season the solution was prepared for a fruit yield of 25 ton.ha^{-1} (Table 4).

Water and nutrient application:

A 10 000 L tank was placed on a hill, 35 m higher than the orchard level (Fig. 1 of Appendix). Water and nutrients for the fertigation drip and pulsating drip treatments were mixed in the tank according to the computed calculations. The water and nutrients ran gravitationally downhill at 2.2 bar. At the orchard an AQ 516 Aquarius, 5 programme 16 zone controller was programmed to give signals to a Netafim Aqua Pro DC solenoid that

opened and closed the electronic valves. Calculations from the computer using the Gerhard Mostert programme were used to programme the controller monthly for a certain phenological period and adapted as needed with the time an application should start and the duration thereof (Table 3). Water and nutrients were filtered with an Arkal filter (120 mesh and 130 micron). Water for the micro jet treatment was received from water that was pumped from a nearby river and flowed through an irrigation pipe that was pressurised. The irrigation could be regulated from a regulation point as for the rest of the orchards on the commercial farm. The irrigation times and durations of the micro jets were also determined by the computer programme, but it was regulated by hand simply to open and close the valve of the solenoid whenever water had to be applied or application ended. Systems were flushed every six weeks with water to prevent nutrient build up in the root area.

Soil and Plant Monitoring:

Three A723 C-probes (capacitive multilayer soil moisture probes) were installed in the soil, near a tree trunk, one each per system and data were directly signalled to the computer for soil moisture at 20, 40, 60 and 80 cm. Three A723 PlantSense sensors were also installed on a tree trunk, again one per system to indicate stress conditions that the tree might have experienced. The probes and sensors were used to adapt the predetermined water applications to prevent over or under supply of water throughout the 60 cm root area. In July (SH) in the second season after 100 mm rain a soil profile was made deeper than 1.5 m to check the existence of a water table, from a nearby, but lower lying river and no water table was found. A water table was however observed at 1 m at the end of the rain period in October 2006.

Fruit, leaf and soil samples:

In the first season (2004/05), there were only enough fruit on the trees to collect a sample at harvest (110 dafb) for mineral analysis. In the second season (2005/06), fruit samples were collected at 40 days after full bloom (dafb) and at harvest (110 dafb) for mineral analyses. Three fruit per replicate per treatment were picked to represent one sample. For uniformity, fruit samples were collected from spurs on two-year-old shoots. During the second season all fruit from the five trees per replicate per treatment were picked and weighed at harvest to determine the yield of the trees. Leaf samples were collected for both the seasons at the end of January (SH) per system. The leaves on the middle of a 20–30 cm long one-year-old shoot, positioned at shoulder height on a tree, were picked for mineral analyses. Soil samples were collected during March 2005 at depths of 0-30 cm and 30-60 cm. Six samples from each depth were collected at random spots per system. Mineral analyses on fruit, leaves and soil were performed by a commercial laboratory (Bemlab Pty Ltd, Strand) on each fruit sample just after it was collected. The whole fruit without the pips and core was analysed.

Root volume and distribution:

Root studies were done on the trees on both rootstocks and all three water and nutrient application systems. A soil profile was made for each of the sites. Each profile was approximately 1.5 m wide (0.75 m on either side of the trunk) and 1 m deep and 0.5 m broad. Profiles were dug approximately 50 cm away from the tree trunk. A grid was designed with dimensions of 80 cm wide and 80 cm long and each square was 20 cm x 20 cm (Fig. 1). Once the profile was dug, the roots were sprayed with white paint. The soil around the roots was cleaned by using a soil hammer so that only the white roots remained. Photographs were taken of these profiles (Fig. 2a-f). The photographs were used to determine root distribution and thickness.

Statistical analysis:

The trial was laid out as a split plot randomised complete block design. The main plot consisted of the different irrigation systems and the subplot the two rootstocks (M793 and M7). There were eight replicates with a replicate consisting of five-tree-plots. Analysis of variance was done using the General Linear Model (GLM) procedure in the Statistical Analysis System (SAS) programme (SAS Institute Inc, 2004, Cary, NC).

Results and discussion

2004/05:

Fruit and leaf samples:

Significant differences were only found between treatments for the N, Ca and Mg concentrations and Ca content (Table 5). No significant differences were found in concentration of any element, Ca content or fruit mass between the trees on different rootstocks. The micro jet and fertigation drip treatments resulted in significantly lower N concentrations in fruit at harvest (46.69 and 45.75 mg.100g^{-1} FW respectively) compared to the pulsating drip treatment (56.47 mg.100g^{-1} FW). Ca concentrations of fruit at harvest for the micro jet and fertigation drip treatments (5.57 and 5.27 mg.100g^{-1} FW respectively) were significantly higher than that of the pulsating drip treatment (4.34 mg.100g^{-1} FW) (Table 5). The Ca content followed the same tendency as described for the Ca concentration (Table 5).

Trees were young and vigorously growing during the 2004/05 season in order to fill their allocated space. The trees received a nutrient solution that was high in N up to the end of that season and the nutrient solution was thereafter changed to a mixture for bearing trees. Results found in the first season will not be discussed in detail, as the yield was very low and variation probably occurred because of different bearing positions. According to Ferguson et

al. (1993) the position of fruit on the tree is one of the major sources of variation in mineral content and storage potential in fruit.

Ca concentration of fruit at harvest varied between 4 to 6 mg.100g^{-1} FW (data not shown). The higher N value found in the fruit at harvest of the pulsating drip treatment might be the reason for the lower Ca concentration in that fruit. According to Korcak (1980) high levels of N can negatively influence the uptake of Ca. Also vigorous extension growth near an apple fruit may lower its Ca concentration (Perring, 1979).

Significant interactions were found for Ca percentage and Na and Zn concentrations in the leaves between the treatments (Table 6). Significant differences were found for P percentage and Fe concentration between treatments and P and K percentages and B concentrations in the leaves of trees on different rootstocks.

Norms established by Kotzé (2001) for mineral analyses of leaf samples for the apple industry of South Africa recommends that N percentage in leaves should generally be between 2.09 and 2.60%. As expected, N percentage in the leaves was high, because of the higher level of N in the nutrient solution given to the trees to stimulate vegetative growth of the young trees. K percentage in the leaves was also higher than the 1.2-1.6% interval suggested by Kotzé (2001).

2005/06:

Fruit and leaf samples:

At 40 dafb, the interaction between the treatments and the rootstocks were significant for N concentration and Ca concentration of fruit (Pr > F = 0.0165 and 0.0002 respectively) (Table

7). The Ca concentration of fruit with micro jets and on M7 was significantly higher than both the fertigation and pulsating drip treatments on M793 and M7, but not significantly higher than the Ca concentration of fruit with micro jets and on M793 (Data not shown). There were significant differences found for K concentrations of fruit between the treatments and for P concentrations between the rootstocks (Table 7).

No significant differences were found in Ca concentration of fruit at harvest between the treatments or rootstocks (Table 8). There were significant interactions found in N and P concentrations between treatments and rootstocks.

Ca concentration of fruit at harvest for the 2005/06 season varied between 7 and 8 mg.100g^{-1} FW (data not shown). Terblanche et al. (1980) listed threshold values for apple fruit N, K, Mg and Ca concentrations of fruit at harvest to ensure that fruit are free of bitter pit. Their values were mainly based on 'Golden Delicious' apple fruit and our study was done on 'Brookfield Gala' apple fruit which is not as prone to bitter pit. Under South African conditions Terblanche et al. (1980) recommended that the minimum Ca concentration for 'Golden Delicious' apples to prevent bitter pit is 5.4 and 6.6 mg.100g^{-1} FW for unsprayed and Ca-sprayed fruit, respectively. Levels found in our trial for 'Brookfield Gala' apples were higher than the values recommended by Terblanche et al. (1980) for Ca-sprayed 'Golden Delicious' apple fruit. Therefore, high fruit quality could be expected, even when no additional Ca sprays were applied.

The Ca content of the fruit at harvest followed the same tendency as described for the Ca concentration between treatments, but that tendency was not the same for rootstocks (Table 8). Ca content of fruit at harvest for the micro jet treatment was 10.17 mg.fruit^{-1}, for

fertigation drip 9.22 mg.fruit^{-1} and for pulsating drip 8.92 mg.fruit^{-1}. It was expected that no differences would be found in Ca content of fruit, because there were no significant differences found in the fruit mass or the Ca concentrations between the treatments (Table 8) as it is known that there is a strong relationship between fruit size and Ca concentration, with large fruit having less Ca concentration and a much higher risk of bitter pit (Ferguson et al., 1993).

There was a significant interaction found in K percentage between treatment and rootstock. Significant differences for P, Ca and Mg percentages and Mn, Zn and B concentrations were found between the treatments (Table 9). Comparing the Ca percentage in the leaves between the treatments, fertigation drip was significantly higher (1.92%) than the micro jets (1.72%), but that did not differ significantly from the pulsating drip (1.82%). Leaves of trees on M7 had significantly higher Ca percentage (1.89%) than on M793 (1.75%).

Leaf analysis of the 2004/05 season indicated that the nutrient solution had to be changed to a mixture that was lower in N and K, but higher in Ca for the 2005/06 season. Leave analyses for the 2005/06 season showed that N, P and K percentages in leaves were in line with the norms set by Kotzé (2001). Kotzé (2001) recommended that Ca percentage in leaves should be between 1.2 and 1.6% to ensure that enough Ca is provided to the tree. Levin et al. (1980) found that the leaf Ca percentage, in an orchard where drip irrigation was used, varied between 1.3 and 1.9% (dry weight basis). In our results the leaf Ca percentage of the fertigation drip and pulsating drip treatments were 1.92% and 1.82%, respectively which agreed with the results of Levin et al. (1980), but even that of the micro jet treatment was 1.72% which was still in the range of Levin et al. (1980) for drip irrigation. Faust (1980) stated that when leaf Ca concentration is above 1.8% of the dry weight, Ca influx to the tree

had to be continuous throughout the entire season. In such a case (such as for the 2005/06 season) it is likely that the fruit also received sufficient levels of Ca and that fruit Ca concentrations are also high as we found. These results suggested that the nutrient solution applied to the trees was well balanced. The higher percentage Ca in leaves of trees on M7 than on M793 could be due to better uptake of Ca through the larger volume of fine roots developed with rootstock M7 (results later). It is known that most Ca uptake generally occurs through young, white, nonsuberized roots (Faust, 1989; Jackson, 2003) and Hebbar et al. (2004) found that optimum root growth and distribution is needed for water uptake, nutrient uptake and crop yield.

Yield:

The number of fruit per tree was counted in the second season (2005/06). The fertigation drip and the pulsating drip treatments had significantly more fruit per tree than the micro jet treatment (Table 10). The systems however, determine the number of fruits on the trees indirectly. The reason for the more fruit is that the trees where fertigation drip and pulsating drip were used had more branches in the first year. Therefore more spurs as well as short shoots developed during the second season, which resulted in trees with more bearing positions than in the case where micro jets were used. After thinning, there were fewer fruit on the trees that received the micro jet treatment than in the case of the trees under fertigation and pulsating drip treatments.

For the same reason yield of the fertigation drip treatment (29.93 $t.ha^{-1}$) and pulsating drip treatment (33.63 $t.ha^{-1}$) were significantly higher than that of the micro jets (19.64 $t.ha^{-1}$) during the 2005/06 season. Yield efficiency of the fertigation drip and the pulsating drip treatments were also significantly higher than that of the micro jets. No significant difference

was found between the yields of trees on the two rootstocks, but yield efficiency of trees on M7 was significantly higher than trees on M793. Yields were directly related to the number of fruits per tree. Perring (1979) stated that heavier crop loads of small apples usually have high Ca concentrations. As mentioned earlier in our results we found no significant differences in Ca concentrations of fruit at harvest. There were also no significant differences in the fruit mass of trees between any of the application systems, but fruit of trees on M793 were significantly larger than fruit on M7 (Table 10). It seems that the rootstock has a direct effect on fruit size, with the dwarfing rootstock M7 having smaller fruit in our study.

Root distribution:

Photographs of the different root profiles are illustrated in Fig. 2a-f. Trees where the micro jet system was used had a lower root volume through the soil profile than the drip systems. Furthermore, the roots of trees where micro jets were used were distributed throughout the whole soil profile, compared with the more prolific root growth under the drippers where both drip systems were used. It was observed that roots between the soil surface and 20 cm depth were thinner than roots at 40–60 cm depth. This was observed for all three treatments. Trees that were subjected to micro jets had lower total fine root concentration than trees that were subjected to drip systems.

The difference in root distribution for the two rootstocks is worth mentioning. For trees where micro jets were used the fine roots were concentrated between 0 and 60 cm depth on M7, but were visible in smaller quantities between 0–30 cm depth for the trees on M793 (Fig. 2a-b). Trees that were planted on M7 and that were subjected to either the fertigation or the pulsating drip system, had roots (especially fine roots) concentrated at 0–50 cm depth. Trees that were planted on rootstock M793 and that were subjected to the same conditions as mentioned above had the highest concentration of fine roots from 0-30 cm depth and thicker

roots between 30 and 60 cm (Fig. 2c-f). Levin et al. (1972) found that over 80% of the root system was restricted to the upper 60 cm soil layer. In general, trees on M7 had a more prolific concentration of feeder roots compared to M793.

After soil preparation a pH of 5.2 (KCl), K level in the region of 3-4 percent, Ca in the region of 70-80 percent and Mg in the region of 10-15 percent of the cation exchange capacity (C.E.C.) provides optimum conditions for tree growth, fruit production and fruit quality (Terblanche et al., 1980). An ideal pH and correct percentages of Na, K, Ca and Mg were present in the C.E.C. of the orchard soil after preplant soil preparation (Table 2). The overall chemical and physical soil conditions for root development were excellent in this well aerated loamy sand soil after preplant preparation. The organic fraction of the soil was approximately 1.5% in the case where pulsating drip and fertigation drip were used, but only approximately 0.2% where micro jets were used (Table 2). The sawdust and straw that were applied to the soil during preplant soil preparation rot quicker under the micro jets, possibly due to the larger surface area wetted with the treatment compared with the drip systems. However, soil samples were taken near to a dripper. This tendency might explain the lower root volume observed in the case where micro jets were used. It is known that most Ca uptake occurs through young, white, nonsuberized, actively growing roots (Faust, 1980; Faust, 1989; Jackson, 2003). With detail management of irrigation a correct soil water status can be maintained which is not only effective in improving root growth, but also increases Ca uptake by the roots and transport of Ca to the fruits (Faust, 1989). Our results suggests that the management of orchard conditions (e.g. soil preparation, irrigation, fertilization) and practices (e.g. pruning, thinning, pest control) were done successfully for all three water and nutrient application systems, even for trees grown under micro jet irrigation. Adequate Ca fruit levels were attained even without any additional foliar Ca applied to the trees.

Conclusions

In conclusion, it seems as if the following overriding factors played a role: The soil medium was ideal for stimulation of fine roots, because of good aeration and drainage. The C.E.C. was optimal as recommended and the pH was ideal and the P content in the soil correct. Pruning and tree training was done correctly and that resulted in optimal light management inside the trees. Thinning was done according to tree potential. Furthermore, trees were not under stress e.g. waterlogging conditions.

The study was done on young trees and results may change once competition between fruit is high, as in full bearing trees. The results of this trial indicate that if trees are grown under well managed conditions, uptake of Ca into the fruit will be adequate, specifically with the cultivar 'Royal Gala'.

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Table 1. The average percentages stone, clay, silt and sand in the composition of the loamy sand Tukulu soil (0-60 cm depth) of the trial orchard, as well as the mean water holding capacity of the soil.

Classification	Stone	Clay	Silt	Fine Sand	Medium Sand	Coarse Sand	Stone Volume	Water holding capacity
				%			(v/v)	mm/m
Loam sand	0.75	5.21	9.61	57.4	21.8	5.91	0.34	130.3

Table 2. The pH, carbon (C) percentage and the percentages of elements that contribute to the cation exchangeable capacity in the top soil and sub soil of the loamy sand Tukulu soil used for the trial. The samples were collected in March 2005.

Treatment	Depth cm	pH (KCl)	C %	Na %	K %	Ca %	Mg %	T-value $cmol.kg^{-1}$
Micro	0-30	5.7	0.32	2.15	3.97	77.03	11.26	5.72
Micro	30-60	5.9	0.11	3.32	2.88	78.76	10.81	7.58
Fertigation drip	0-30	6.1	1.37	5.61	3.20	79.22	11.98	8.47
Fertigation drip	30-60	4.8	1.49	5.55	2.38	70.09	11.54	5.26
Pulsating drip	0-30	5.9	2.14	2.03	4.09	76.48	13.51	8.24
Pulsating drip	30-60	4.7	1.73	5.25	1.71	64.42	16.36	5.22

Table 3. Predetermined annual amount of water for full bearing trees, with 100% leaf coverage, as well as the duration and the frequency per month at which water and nutrients were applied to 'Brookfield Gala' apple trees through the three application systems during the 2005/06 season for a predicted fruit production of 25 ton.ha^{-1} (Drippers received 25% less).

Month	Annual	Micro		Fertigation Drip		Pulsating Drip	
	Water $M^3.ha^{-1}$	Duration (minutes)	Times per week	Duration (minutes)	Times per week	Duration (minutes)	Times per week
Jan	1044	83	2	120	7	20	42
Feb	977	78	2	112	7	19	42
Mar	806	129	1	92	7	18	35
Apr	644	103	1	74	7	18	28
May	548	88	1	63	7	21	21
Jun	50	8	1	6	7	6	7
Jul	50	8	1	6	7	6	7
Aug	50	8	1	6	7	6	7
Sept	50	8	1	6	7	6	7
Oct	411	66	1	47	7	16	21
Nov	660	106	1	76	7	19	28
Des	874	70	2	100	7	20	35

Table 4. The predetermined annual nutrient requirement for 1 ton fruit.ha^{-1}, as well as the computed annual amount of each element given to third leaf 'Brookfield Gala' trees for a predicted production of 25 tons.ha^{-1} during the 2005/06 season.

Ton fruit	N	K	Ca	Mg	P	Cu	Zn	B	Mo	Mn	Fe
	kg.ha^{-1}.year^{-1}										
1	2.3	1.8	1.8	0.5	0.5	0.007	0.02	0.01	0.001	0.02	0.13
25	57.5	45.0	45.0	12.5	12.5	0.18	0.50	0.25	0.00	0.50	3.25

Table 5. Macro element concentrations and Ca content of fruit, as well as fruit mass at harvest for the 2004/05 season of second leaf 'Brookfield Gala' trees planted on two rootstocks and subjected to three water and nutrient application systems. Mean separation in columns using LSD (5%).

Treatment	N	P	K	Ca	Mg	Ca content	Fruit mass
	mg.100g^{-1} fresh weight					mg.fruit^{-1}	g.fruit^{-1}
Irrigation system:							
Micro jets	46.69 b	14.43 ns[z]	165.93 ns	5.57 a	7.99 a	8.48 a	153.64 ns
Fertigation drip	45.75 b	14.83	157.00	5.27 a	7.49 ab	8.45 a	160.71
Pulsating drip	56.47 a	14.49	150.20	4.34 b	7.25 b	6.43 b	147.26
Rootstock:							
M793	47.73 ns	15.01 ns	157.44 ns	4.84 ns	7.44 ns	7.20 ns	149.84 ns
M7	51.17	14.17	158.29	5.30	7.72	8.36	157.90
Sign. Level Pr > F:							
Irrigation system (I)	0.0293	0.8111	0.1627	0.0164	0.0420	0.0047	0.1640
Rootstock (R)	0.4105	0.3181	0.7336	0.2153	0.3465	0.0828	0.1863
I * R	0.5522	0.9976	0.7728	0.4827	0.6825	0.8203	0.5824

[z] Means with the same letters are not significantly different ($P \leq 0.05$; ns = non significant)

Table 6. Leaf mineral analyses results of second leaf 'Brookfield Gala' apple trees that were subjected to three different water and nutrient application systems and two rootstocks. Samples were collected at the end of January for the 2004/05 season. Means in columns are separated by using LSD (5%).

Treatment	N	P	K	Ca	Mg	Na	Mn	Fe	Cu	Zn	B
	%					mg.kg^{-1}					
Irrigation system:											
Micro	3.35 ns[z]	0.28 a	2.34 ns	1.09 b	0.36 ns	207.56 a	72.88 ns	200.94 b	7.31 ns	59.63 ns	40.56 ns
Fertigation drip	3.32	0.24 b	2.32	1.25 a	0.36	133.53 b	79.07	231.47 a	6.27	77.93	37.07
Pulsating drip	3.24	0.26 ab	2.23	1.22 a	0.36	114.44 b	75.75	196.44 b	6.50	79.19	39.25
Rootstocks:											
M793	3.31 ns	0.28 a	2.40 a	1.20 ns	0.37 ns	172.91 a	78.70 ns	215.83 ns	6.61 ns	76.65 a	43.39 a
M7	3.30	0.24 b	2.20 b	1.17	0.35	132.42 b	73.08	202.75	6.79	67.79 b	34.79 b
Sign. level Pr > F											
Irrigation system (I)	0.5614	0.0458	0.4256	0.0333	0.8430	0.0009	0.7605	0.0180	0.6631	0.0668	0.1925
Rootstock (R)	0.8924	0.0136	0.0230	0.5995	0.0913	0.0397	0.4200	0.2057	0.9114	0.0424	0.0002
I * R	0.4353	0.1654	0.3736	0.0280	0.0782	0.0370	0.1022	0.3676	0.3190	0.0088	0.0708

[z] Means with the same letters are not significantly different ($P \leq 0.05$; ns = non significant)

Table 7. Macro element concentrations and Ca content in fruit, as well as fruit mass at 40 dafb during 2005/06 of third leaf 'Brookfield Gala' trees planted on two rootstocks and subjected to three water and nutrient application systems. Mean separation in columns using LSD (5%).

Treatment	N	P	K	Ca	Mg	Fruit mass
	mg.100g^{-1} fresh weight					g.fruit^{-1}
Irrigation system:						
Micro jets	153.63 ns[z]	22.52 ns	193.19 b	17.59 a	13.99 ns	21.29 ns
Fertigation drip	154.63	22.49	208.44 a	18.47 a	14.94	20.39
Pulsating drip	149.75	21.41	192.69 b	14.75 b	13.94	21.69
Rootstock:						
M793	154.29 ns	22.76 a	199.33 ns	15.95 b	14.03 ns	21.38 ns
M7	151.04	21.52 b	196.88	17.93 a	14.55	20.87
Sign. Level Pr > F:						
Irrigation system (I)	0.6192	0.2530	0.0488	0.0286	0.0767	0.0631
Rootstock (R)	0.3124	0.0390	0.5404	0.0031	0.1530	0.3195
I * R	0.0165	0.7800	0.4966	0.0002	0.0767	0.0993

[z] Means with the same letters are not significantly different (P ≤ 0.05; ns = non significant)

Table 8. Macro element concentrations and Ca content of fruit, as well as fruit mass at harvest for the 2005/06 season of third leaf 'Brookfield Gala' trees planted on two rootstocks and subjected to three water and nutrient application systems. Mean separation in columns using LSD (5%).

Treatment	N	P	K	Ca	Mg	Ca content	Fruit mass
	mg.100g^{-1} fresh weight					mg.fruit^{-1}	g.fruit^{-1}
Irrigation system:							
Micro jets	48.88 ns[z]	11.66 a	121.31 ns	7.42 ns	6.63 ns	10.17 ns	136.80 ns
Fertigation drip	47.50	9.66 b	118.69	7.41	6.43	9.22	125.04
Pulsating drip	47.06	10.24 ab	116.38	7.17	6.38	8.92	126.16
Rootstock:							
M793	47.50 ns	10.86 a	121.46 ns	7.17 ns	6.46 ns	9.64 ns	135.31 a
M7	48.13	10.16 b	116.13	7.49	6.49	9.26	123.31 b
Sign. Level Pr > F:							
Irrigation system (I)	0.8839	0.0478	0.6966	0.7792	0.3550	0.1430	0.0565
Rootstock (R)	0.7517	0.0294	0.1393	0.2674	0.8674	0.3415	0.0282
I * R	0.0322	0.0258	0.1042	0.2213	0.5442	0.2899	0.7652

[z] Means with the same letters are not significantly different ($P \leq 0.05$; ns = non significant)

Table 9. Leaf mineral analyses results of third leaf 'Brookfield Gala' apple trees that were subjected to three different water and nutrient application systems and two rootstocks. Samples were collected at the end of January for the 2005/06 season. Means in columns are separated by using LSD (5%).

Treatment	N	P	K	Ca	Mg	Na	Mn	Fe	Cu	Zn	B
	%					mg.kg^{-1}					
Irrigation system:											
Micro	2.55 ns[z]	0.22 a	1.64 a	1.72 b	0.34 b	265.92 ns	151.42 b	200.50 ns	5.67 ns	66.75 ab	37.00 a
Fertigation drip	2.49	0.18 b	1.37 b	1.92 a	0.41 a	261.33	183.75 a	211.17	5.50	67.83 a	33.75 b
Pulsating drip	2.54	0.19 b	1.38 b	1.82 ab	0.41 a	244.08	166.42 ab	189.5	5.75	64.00 b	34.58 b
Rootstocks:											
M793	2.50 ns	0.20 ns	1.56 a	1.75 b	0.37 b	249.67 ns	168.33 ns	205.89 ns	5.50 ns	66.94 ns	34.22 b
M7	2.58	0.19	1.37 b	1.89 a	0.41 a	264.56	166.06	194.89	5.78	65.44	36.00 a
Sign. Level Pr > F											
Irrigation system (I)	0.3137	0.0117	0.0011	0.0128	0.0019	0.2935	0.0173	0.4703	0.8481	0.0800	0.0053
Rootstock (R)	0.0554	0.2287	<0.0001	0.0072	0.0002	0.0706	0.6531	0.3040	0.2544	0.3670	0.0119
I * R	0.1445	0.7047	0.0039	0.5199	0.0597	0.5915	0.6492	0.2362	0.6816	0.6192	0.9458

[z] Means with the same letters are not significantly different (P ≤ 0.05; ns = non significant)

Table 10. Fruit parameters for third leaf 'Brookfield Gala' apple trees that were subjected to three different water and nutrient application systems and two rootstocks for the 2005/06 season.

Treatment	Average Mass (g)	Number	Yield in kg.tree^{-1} (Estimated yield t/ha)	Yield efficiency kg/cm^2
Irrigation systems:				
Micro jets	147.77 ns[z]	70.69 b	9.82 b (19.64)	0.565 b
Fertigation drip	139.15	111.75 a	14.97 a (29.93)	0.866 a
Pulsating drip	135.68	120.88 a	17.10 a (33.63)	1.028 a
Rootstock:				
M793	147.68 a	94.00 ns	12.94 ns (25.87)	0.666 b
M7	134.05 b	108.21	14.99 (29.60)	0.974 a
Sign. Level Pr > F:				
Treatment	0.0656	<0.0001	<0.0001	0.0005
Rootstock	0.0007	0.1947	0.1064	0.0013

[z] Means with the same letters are not significantly different ($P \leq 0.05$; ns = non significant)

Figure 1. The grid with dimensions of 80 cm wide, 80 cm long and 20 x 20 cm squares that was used to evaluate the concentration of roots vertically distributed through the soil profile as well as horizontally from the dripper.

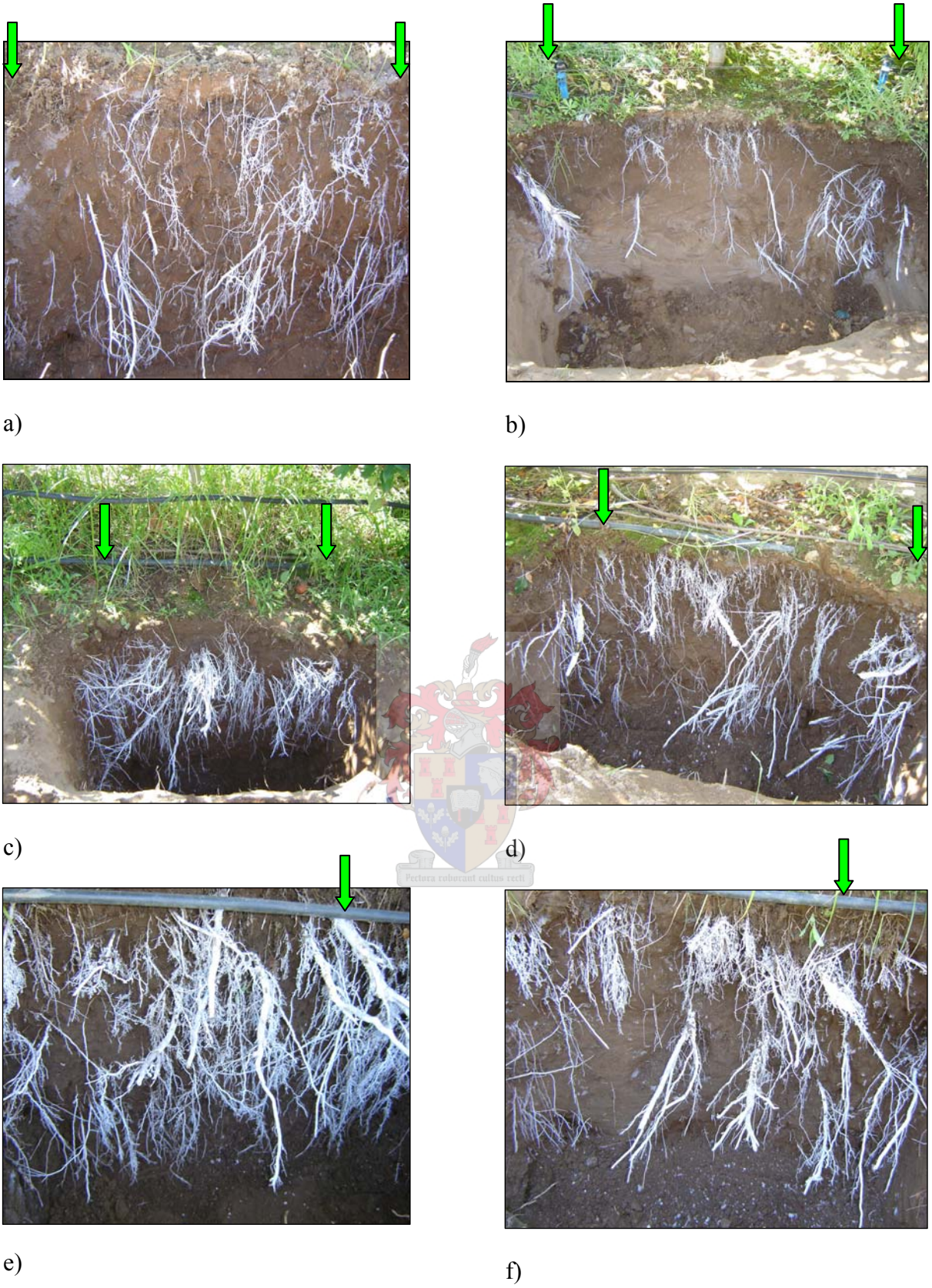

Figure 2. Root distribution and concentration of fine roots of 'Brookfield Gala' apple trees. The system and rootstock were: a) micro jets and M7 b) micro jets and M793 c) fertigation drip and M7 d) fertigation drip and M793 e) pulsating drip and M7 and f) pulsating drip and M793.

Pectora roborant cultus recti

CHAPTER 4

EFFECT OF CALCIUM APPLICATION LEVELS DURING THREE PHENOLOGICAL PERIODS ON CALCIUM UPTAKE INTO APPLE FRUIT

Abstract

Sufficient calcium (Ca) levels in fruit are needed at harvest to ensure optimum fruit quality and post harvest storage life. The major challenge associated with Ca nutrition is the transport from the roots upwards with the transport vessels into the fruit. To evaluate the effectiveness of soil Ca applications to increase the uptake of Ca into apple fruit, three different Ca application levels, viz., low, standard and high were applied during three phenological periods to 'Brookfield Gala' apple trees on two rootstocks, viz., M7 and M793, planted on loamy sand soil, over two consecutive seasons. In 2004/05 there were significant differences found at harvest between treatments for N, P, K, Ca and Mg concentrations and the Ca content in fruit. The high-high treatment had the highest Ca concentration in fruit at harvest for 2005/06 (8.85 mg.100g^{-1} FW), but it was not significantly higher than the low-std or the high-low treatments (8.13 and 7.83 mg.100g^{-1} FW). A significant difference was found in the Ca concentration and the Ca content between the two rootstocks when fruit mass was used as covariate and fruit of trees on M7 had higher values than on M793. There was no significant difference between the treatment means for yield (Pr > F = 0.2675), but there was a significant difference in the yield of trees on the two rootstocks (Pr > F = 0.0020). Trees on M7 gave a mean yield of 11.46 kg.tree^{-1} and trees on M793 gave a mean yield of 8.74 kg.tree^{-1}. At harvest there were only significant differences found in TSS and starch breakdown between treatments, and after nine weeks of storage only in TSS between treatments. Values found for flesh firmness showed that fruit had good quality.

Introduction

Calcium (Ca) is perhaps the most important mineral element determining fruit quality (Conway et al., 2002) and the post harvest storage life of fruit (Siddiqui & Bangerth, 1993; Casero et al., 2002). It is especially important in apples and pears, because these fruits are stored for extended periods (Faust, 1989). Furthermore, a number of physiological disorders of apples are associated with low fruit tissue Ca (Faust, 1989; Jackson, 2003) and bitter pit is considered as the most important disorder (Yuri et al., 2002; Jackson, 2003).

The major challenge associated with Ca nutrition is that Ca not only needs to be taken up by the tree, but also be transported into the fruit (Faust, 1989). Most nutrients are applied annually to the soil surface. However, some nutrients do not penetrate into the soil easily (i.e. Ca) and therefore must be applied before the tree is planted, in a quantity that may supply the tree for the duration of its life (Faust, 1989). Soil treatments with Ca to increase fruit Ca concentration have often achieved very little success (Conway et al., 2002). Ca redistribution within plants is limited, and the presence of adequate levels of Ca in the soil solution does not ensure sufficient uptake or translocation to the tree, and especially to the fruit (Shear, 1980). A high level of Ca in the cation exchange capacity (C.E.C.) of the soil is necessary. Ca should constitute 70-80% of the C.E.C. in the soil (Terblanche et al., 1980). Nevertheless, Ca deficiency is common phenomena in all major apple-growing areas, even on calcareous soil (Wojcik & Szwonek, 2002).

Ca occurs in soils predominantly as the divalent cation (Ca^{2+}) held on exchange sites and to a lesser extent in chelated forms, insoluble phosphates, sulphates or silicates, as ion pairs, or in microbes (Korcak, 1980). The first stage in Ca uptake involves movement of Ca in the soil towards the roots (Jackson, 2003). For this process to proceed with maximum efficiency, the

ion concentration in the soil water must be high enough to enable the nutrients needed by the plant to reach the root by mass flow (Jackson, 2003). Ca uptake and translocation is generally accepted to be an ion exchange phenomenon, but it is also dependent on optimum soil moisture (Shear, 1980; Ernani et al., 2002).

Ca is considered to move preferentially in the xylem sap (Vang-Peterson, 1980; Jackson, 2003), upward with the transpiration stream (Faust, 1989). Upward movement is dependant on the Ca exchange adsorption on the xylem walls, the transpirational flux (Bangerth, 1979; Jackson, 2003), the xylem functionality of the fruit (Dichio et al., 2003) and is not simply a matter of mass flow (Jackson, 2003). As the xylem provides the only route for major distribution of Ca in plants, any permanent loss of this element during movement in the xylem must be of significance in the overall Ca nutrition of the plant (Ferguson & Bollard, 1976).

The final stages of movement into organs and tissues, or the flux into the fruit are partly under metabolic control (Jackson, 2003). Accumulation of Ca and other elements occurs rapidly during the first period of fruit growth, which usually lasts 4-6 weeks after full bloom (Faust, 1989). Palmer et al. (2003) classified the first period as the initial 35-50 days of exponential growth following fertilisation. Ferguson & Watkins (1989) note in their review on bitter pit that intensive cell division that takes place during the first period provides a considerable sink for Ca, and the extent of this stage may be crucial to the final Ca status of the fruit. During the second phase, the period of cell enlargement, Ca uptake into fruit still continues, but at a slower rate than initial Ca uptake. As fruit growth continues the Ca concentration of fruit decreases with the rate of final expansion of the fruit (Faust, 1989). This decrease in Ca concentration of fruit plays an important role in determining the storage quality of fruit. Large fruit, with high Ca dilution rates and a low Ca concentration at harvest, usually have a

very poor storage quality in contrast with small fruit with a higher Ca concentration at harvest (Faust, 1989). Perring (1979) found that wet conditions in the soil enhanced Ca uptake, but dry conditions caused outflow of Ca from the fruit. This might have caused poor fruit quality as a result of lower fruit Ca content. Irrigation in dry areas would therefore be appropriate to ensure that fruit remains in good condition. However, excessive irrigation during the period just before harvest should be avoided as far as possible as it will result in further dilution of the Ca concentration in the fruit (Faust, 1989).

Under appropriate orchard soil management, the exchange complex of the soil is dominated by Ca (Korcak, 1980). Except for adequate amounts of Ca in the soil solution, Faust (1989) noted that active root growth is needed for maximum Ca uptake, more so than in uptake of K or P. Storey & Treeby (2002) agreed that the dependence on root uptake is likely to be greater for Ca as it is not very mobile in the phloem compared with K, which is phloem-mobile. Water application through a drip system is localised as it is applied to a portion of the plant's potential root zone (Elfving, 1982) which results in the development of a restricted root zone in which moisture stress can be prevented by frequent water application (Haynes, 1985). The advantage of this method is more accurate management over plant processes, because nutrients (i.e. Ca) and water can be applied during certain phenological periods as needed (Stassen et al., 1999). This raises the following questions: Can an increase in the amount of Ca in the nutrient solution applied to the soil through a pulsating drip system increase the Ca uptake of fruit? Can this increase raise the Ca uptake into apple fruit during the first 40 days after full bloom (dafb)? Can this increase raise the Ca uptake into apple fruit from 40 dafb until harvest? Therefore, this study was conducted to evaluate the effectiveness of a high Ca nutrient solution applied continuously to the root system to increase fruit Ca.

Materials and methods

The study was done at the same site as described in Chapter 3.

Water and nutrient management:

Only the pulsating drip system described in Chapter 3 was used in this trial. Trees were subjected to three Ca application levels (low, standard (std) and high) which were applied through the pulsating drip system.

The standard Ca application level was determined for each season as mentioned by the different predicted fruit yields. The low Ca application level contained 40% less Ca than the standard Ca application level and the high Ca application level contained 40% more Ca than the standard Ca application level (Table 1). The Ca treatments were given at three time periods: The periods used were i) from full bloom to 40 dafb, ii) from 40 dafb to harvest and iii) the period from full bloom to harvest. After harvest all the trees received the standard nutrient solution.

The three nutrient mixtures for the Ca application levels were mixed in three separate 2500 L tanks (Fig. 1 of appendix). The nutrient mixtures were made up by only changing the amount of Ca, but all three mixtures contained exactly the same amount of all other elements.

As a result of the three Ca application levels there were three dripper lines in each row, one for each Ca application level. Whenever water and nutrients were applied, drippers were opened at the replicates of three trees where a specific Ca application level was applied, but the drippers were blank at the replicates where that Ca application level was not applied. Dripper lines were laid according to the statistical design of the trial (Fig. 2 in Appendix).

Fruit, leaf and soil samples for mineral analyses:

In the first season (2004/05) fruit samples were only collected at harvest for mineral analyses. During the second season (2005/06), fruit samples were collected every 20 days (starting at 20 dafb until harvest) for mineral analyses to establish Ca uptake patterns. Three fruit were collected per sample, one from each tree of the three trees of a replicate. For uniformity, fruit samples were collected from spurs on two-year-old shoots. Leaf samples were collected for both seasons at the end of January (SH) for mineral analyses. In addition leaf samples were collected at 60 and 80 dafb for mineral analyses as described in Chapter 3. Soil samples were collected during March 2005 as described in Chapter 3. Mineral analyses on fruit, leaves and soil were performed by a commercial laboratory (Bemlab Pty Ltd, Strand) on each fruit sample just after it was collected. The whole fruit without the pips and core was analysed.

Yield:

During 2004/05 yield of all trees was low, an average of 7 fruit.tree^{-1}. These fruit were collected for mineral analyses only. Yield during the second season (2005/06) was higher and all fruit were harvested and weighed. Yield was calculated in kg.tree^{-1} per treatment.

Fruit samples for quality and maturity analyses:

At harvest in the second season (2005/06) 25 fruit per replicate per treatment were harvested at random for fresh quality and maturity analyses done by our laboratory, whereas a second sample of 25 fruit was analysed following nine weeks of cold storage at -0.5 °C. Fruit diameter was measured with an electronic calliper, fruit mass was determined with a one decimal balance and flesh firmness of fruit was determined on pared, opposite cheeks with a GÜSS fruit texture analyser (8 mm tip). The juice of slices cut from each side of the fruit was used to determine the total soluble solids (TSS) with a refractometer (PR32, ATAGO Co.

Ltd., Tokyo, Japan). The malic, tartaric and citric acid was determined by titrating NaOH (0.1 mol.L^{-1}) to the juiced fruit with a Metrohm 760 Sample Changer. Starch breakdown of fruit was determined by applying 1% iodine solution to one half of the cut fruit. The percentage breakdown could be read from a starch breakdown chart for apples.

Statistical layout and analysis:

The treatments were laid out in a 3x3x2 factorial design. There were three different Ca application levels, viz., low, standard and high applied at three phenological stages and two rootstocks viz., M793 and M7. There were nine treatments in total. Three of these treatments were the high, standard and low Ca application levels applied from full bloom to harvest. The other six treatments consisted of combinations of the three Ca application levels. The 18 treatments were laid out as a randomised complete block design and three trees represented one replicate.

Analysis of variance was done using the General Linear Model (GLM) procedure in the Statistical Analysis System (SAS) programme (SAS Institute Inc, 2004, Cary, NC). In addition, single degree of freedom, orthogonal, polynomial contrasts were fitted to some of the variables, comparing treatments with another (Clewer & Scarisbrick, 2001). Low Ca application level were compared to high Ca application level for early (full bloom to 40 dafb) and late (40 dafb to harvest) application periods.

Results and discussion

2004/05:

Fruit samples:

Due to the young trees used and the low yield (average of 7 fruit.tree^{-1}), trees bore only a few fruit that could be collected in the first season and much variation in element concentrations of fruit were expected due to fruit position. A conclusion could not be drawn from the results after only the first season of treatments, and therefore the results will not be discussed in detail.

Significant differences for N, P, K, Ca and Mg concentrations in fruit and for Ca content in fruit at harvest were found between treatments (Table 2). The low-low treatment had the highest concentration of all these elements in fruit, but that was not significantly higher than one or more of the other treatments for all cases. In this season (2004/05) there were no significant differences found in the fruit mass between treatments. The K/Ca ratio in fruit was calculated and no significant differences were found between the treatments or rootstocks, but the ratios were higher than the 22:1 that Tomala (1997) recommended (Table 3). There were no significant differences found for Ca concentrations or Ca content in fruit between trees on the different rootstocks. Contrasts showed that trees that received the low Ca application level between 40 dafb and harvest had significantly higher fruit Ca as opposed to trees that received the high Ca application level. That was in contrast with what was expected and cannot be explained.

Leaf samples:

As expected the N percentage in leaves of the 2004/05 season was high, because of the higher amount of N in the nutrient solution given to the trees to stimulate vegetative growth of the

young trees (Table 4). K percentage in the leaves was also higher than the 1.2-1.6% range set by Kotzé (2001). These results indicated that for the 2005/06 season the nutrient solution had to be changed to a mixture that was lower in N and K and higher in Ca for a higher predicted yield.

2005/06:

Fruit samples:

No significant differences were found between treatments or rootstocks for N, K and Mg concentrations in the fruit (Table 5). A significant interaction between treatment and rootstock was found for fruit mass. The mean fruit mass was a significant covariate for P and Ca concentration and Ca content of fruit at harvest. Fruit from all trees used for this season were relatively small, due to a high fruit load. Adjusted means of the element concentrations and Ca content for fruit mass used as covariate showed that treatment and rootstock affects these elements. The high-high treatment had the highest Ca concentration of fruit at harvest (8.85 mg.100g^{-1} FW), but it was not significantly higher than that of the low-std or the high-low treatments (8.13 and 7.83 mg.100g^{-1} FW respectively). Trees on M7 had significantly higher values for Ca concentration (8.05 mg.100g^{-1} FW) in fruit compared with M793 (7.01 mg.100g^{-1} FW). Ca content of fruit had the same differences between treatments and rootstock as described for Ca concentration of fruit. The K/Ca ratio of fruit at harvest was calculated and no significant difference was found between treatments, but there was a significant difference between rootstocks. Comparing the K/Ca ratio in fruit between rootstocks, trees on M793 had a significant higher ratio (18:1) than trees on M7 (15:1) (Table 3). However, both rootstocks were lower than the 22:1 ratio that Tomala (1997) recommended. Contrasts of low versus high early (full bloom until 40 dafb) were only

significant for K concentration in fruit ($p = 0.0386$), but low versus high late (40 dafb until harvest) was not significant for any of the element concentrations in fruit.

Terblanche et al. (1980) listed threshold values for apple fruit N, K, Mg and Ca concentrations of fruit at harvest to ensure that fruit are free of bitter pit. Their values were mainly based on 'Golden Delicious' apple fruit and our study was done on 'Brookfield Gala' apple fruit which is not as prone to bitter. Since these values are also applicable to fruit quality, we used the values of Terblanche et al. (1980), and compared it to those of other researchers. Terblanche et al. (1980) suggested that N concentration in fruit at harvest should not be less than 24 $mg.100^{-1}$ FW and that P concentration of 'Golden Delicious' apple generally varies between 6.0 and 12.0 $mg.100g^{-1}$ FW. The threshold value for P concentration in 'Cox' fruit is 11 $mg.100g^{-1}$ FW according to Waller (1980). We found the N concentrations in fruit above 24 $mg.100^{-1}$ FW and P concentration of fruit at harvest varied between 9.5 and 10.5 $mg.100g^{-1}$ FW which was within the norms of both researchers. Furthermore, Faust (1980) stated that an N/Ca ratio of approximately 10:1 in fruit would ensure high fruit quality. Such a ratio was found in our data. Terblanche (1985) and Terblanche et al. (1980) stated that K concentration of fruit must at least be 95 $mg.100g^{-1}$ FW to ensure maximum Ca uptake by fruit. K concentration of fruit in our data was such that Ca uptake should took place adequately. Also, the K/Ca ratio for fruit at harvest in the 2005/06 season was less than the 22:1 ratio suggested by Tomala (1997) as the threshold value for the prevention of commercial losses due to bitter pit. Terblanche et al. (1980) recommends a fruit Ca concentration at harvest of above 5.4 and 6.6 $mg.100g^{-1}$ FW for unsprayed and Ca-sprayed fruit respectively to control bitter pit effectively. In 2005/06 trees had fruit Ca concentrations at harvest which were much higher than the Ca-sprayed value of Terblanche et al. (1980). For the 2005/06 season the nutrient mixture was changed to a nutrient solution that was lower in

both N and K and higher in Ca for a higher expected fruit yield. Neilsen et al. (2005) found values between 3.2 and 3.5 mg.100g^{-1} FW in 'Braeburn' apple fruit at harvest when they applied Ca thiosulphate once a year to the soil. We found Ca concentrations at harvest were higher than their values.

The changes in macro element concentrations that took place during fruit development from 20 dafb until harvest are shown in Table 6-9. The values in these tables indicate that all element concentrations decreased over time. In contrast with that the values for Ca content and fruit mass increased over time. Since the study focused on Ca uptake into fruit only the decrease in Ca concentration, and the increase in Ca content were illustrated (Fig. 1-2).

Fig. 1a illustrates the decrease in Ca concentration of fruit that took place over time for the three main Ca application levels (low-low, std-std and high-high) and Fig. 1b that for the two rootstocks. No significant differences were found in the Ca concentration of fruit between treatments for all sampling dates (although clear differences were visible at 20 dafb), except at harvest. Unlike the treatments, Ca concentration in fruit of trees on M7 was significantly higher than on M793 at each sampling date.

Both figures illustrate that Ca concentration of fruit decreased as the fruit expanded, but it is unsure where the peak in Ca concentration occurred, as a rapid decrease was already observed from 20 to 40 dafb. Thereafter, the decrease continued at a much slower rate until harvest, as fruit increased in size. According to Ferguson & Watkins (1989) the reason for the decrease in Ca concentration is that the expansion of fruit is greater than the rate of mineral uptake, resulting in a dilution of the mineral. The patterns found for Ca concentration in our trial is in contrast to i) the trends of Ca concentration for avocado fruit when peak concentrations were

found at six weeks after fruit set (Witney et al., 1986) and ii) 'Golden Delicious' apple fruit discussed in Chapter 6 when Ca concentrations peaked at 40 dafb. Unfortunately no fruit samples were collected at 20 dafb in the latter study. The shorter growing season of 'Royal Gala' fruit (approximately 125 days) compared with 'Golden Delicious' fruit (approximately 140 days) possibly explains the trends observed.

Fig. 2a illustrates the increase in Ca content of fruit that took place over time for the three main Ca application levels (low-low, std-std and high-high) and Fig. 2b that for the two rootstocks. Ca accumulation into fruit for all treatments between 20 and 40 dafb and 60 and 80 dafb continued at the same rate, but the increase seemed to be slower between 40 and 60 dafb except for the low-low treatment. Between 80 dafb and harvest there was actually a decrease in the Ca content of fruit from the low-low and the std-std treatments, but the high-high treatment continued to accumulate more Ca into the fruit. The trend observed for fruit of the high-high treatment, was also observed for fruit of trees on M7, but fruit of trees on M793, however, continued to accumulate Ca rapidly up to 60 dafb and only thereafter accumulation slowed down. The Ca content at 60 dafb was significantly higher for fruit of trees on M793 than on M7, but at harvest the opposite was found. Fruit of trees on M7 had significantly higher Ca content than on M793. According to Lang (1990), the xylem flow reverses at times, in other words it flows from the fruit to the tree, particularly during periods of high evaporative demand. Xylem sap flows into the fruit during the night, but frequently flows out to the tree during the day. As mineral elements are carried with the water in the xylem, this reversal may have an important effect on the final mineral composition of the fruit. High temperatures during the day were observed during that stage of fruit growth and a high evaporative demand might explain the decrease in the Ca content of the low-low and std-std treatments, but it does not explain the accumulation of Ca during that period for the high-high

treatment. The slower increase in Ca content of fruit between 40 and 60 dafb could be due to the change-over from a predominantly xylem supply of Ca to one from the phloem. This change-over has been associated with a levelling off of total fruit Ca content between the cell division and the cell expansion phases of fruit growth (Ferguson & Watkins, 1989).

Leaf samples:

At 60 and 80 dafb there were no significant differences found for the Ca percentage in the leaves between treatments (Table 10-11). At harvest, however, there was a significant interaction found for the Ca percentage in the leaves between treatments and rootstocks (Table 12).

Table 10-12 indicate the decrease in N, P and K percentages and the increase in Ca and Mg percentages that took place in the leaves over time. Faust (1989) agreed with this finding. The Ca percentage in the leaves at harvest was higher than the norms recommended by Kotzé (2001), for apples of the South African apple industry (between 1.2 and 1.6%). It was also slightly higher than the norms suggested by Basso & Wilms (1988) for apple orchards in Southern Brazil and the norms set by Faust (1989) for Ca percentage in leaves (1.10-1.70% and 1.5-1.8% respectively). As 'Royal Gala' fruit is not prone to bitter pit the slightly higher Ca levels in the leaves are acceptable. Kotzé (2001) recommends that the N percentage from a perfect nutrient solution should be between 2.09 and 2.60% in the leaves and the K percentage between 1.2 and 1.6% in leaves. The N percentages in the leaves of our data were within that interval, while K percentages in leaves were slightly lower. As mentioned earlier the fruit in this study had adequate Ca at harvest to be free from any Ca deficiency symptoms. It is speculated from the N percentages in the leaves that a good leaf/fruit ratio was maintained, since excessive vegetative growth was not stimulated. From both the fruit and

leaf mineral analyses, it seems that competition in the tree (between sinks) for metabolites did not influence the transport of Ca to the fruits negatively. The leaf mineral analyses further proved that a balanced nutrient solution was applied to the trees and maximum Ca uptake could occur.

Soil samples:

From the literature it is stressed that except for adequate amounts of Ca in the soil solution, active root growth is needed for maximum Ca uptake, more so than in uptake of K or P (Faust, 1980). Besides that, physical and chemical soil conditions also influence Ca uptake. Terblanche (1985) recommended that after soil preparation a pH of 5.2 (KCl), K level in the region of 3-4 percent, Ca in the region of 70-80 percent and Mg in the region of 10-15 percent of the C.E.C. provides optimum conditions for tree growth and fruit production. Soil analysis was done and an ideal pH as well as correct percentages of Na, K, Ca and Mg was present in the C.E.C. of the soil after preparation (Table 2, Chapter 3). The organic fraction of the soil was increased from 0% to approximately 1.5% (Table 2, Chapter 3). These results suggested that conditions were optimum for active root development. To prove that active root development took place, feeder roots was observed in the soil profile during root studies to ensure that Ca could be taken up and transported to the fruit at times when it was needed most.

Yield:

There was no significant difference between the treatment means for yield ($Pr > F = 0.2675$), but there was a significant difference for the yield of trees on the two rootstocks ($Pr > F = 0.0020$) (Fig. 3a-b). Trees on M7 gave a mean yield of 11.46 $kg.tree^{-1}$ and trees on M793 gave a mean yield of 8.74 $kg.tree^{-1}$ (Fig. 3b). As mentioned earlier fruit of trees on M7 were

smaller and had higher Ca concentrations and Ca contents at harvest than that of fruit of trees on M793. Furthermore, we know that M7 is a more dwarfing rootstock than M793 and therefore a tree on M7 will be less vigorous and smaller than a tree on M793. The heavier crop load of trees on M7 resulted in smaller fruit. This agrees with Perring (1979) that heavier crops of small apples usually have high Ca concentrations. This also agrees with the concluding remark of Witney et al. (1986) that less vigorous trees are more likely to have high-Ca fruits at maturity, than vigorously growing trees.

Fruit samples for quality and maturity analyses:

Fruit at harvest from the high-low treatment had the highest º Brix value (14.86) for TSS, but it was not significantly higher than for the high-high treatment (14.53) (Table 13). The starch break down of fruit of the high-high treatment was the highest (61.95%), but it was not significantly higher than that of the high-low or the high-std treatments (57.21 and 52.02% respectively). Fruit of trees on M793 had significantly higher values for TSS, firmness, malic, citric and tartaric acid compared to M7, but fruit of trees on M7 had significantly higher starch percentage breakdown on M793 (Table 13). After nine weeks of storage there were no significant differences found for fruit diameter, starch, firmness, malic, citric or tartaric acid between treatments (Table 14). The only significant difference found was for TSS content between treatments. However, significant differences for all the parameters were found between rootstocks. Fruit of trees on M793 had significantly higher values for diameter, TSS, pressure, malic, citric and tartaric acid as opposed to M7, but again the starch percentage breakdown was significantly higher in fruit on M7 than on M793 (Table 14).

Terblanche et al. (1980) stated that a maximum fruit diameter threshold of 61 mm for 'Golden Delicious' apples will ensure freedom from bitter pit. In this trial fruit diameter was between

61 and 64 mm in the 2005/06 season, which mean that quality of fruit was expected to be good. The results of firmness proved that even after nine weeks of storage, the fruit were still firm. From the starch content of the fruit in this study it showed that the fruit of trees on M7 was more matured than that of M793, but it was expected from the slightly smaller fruit and higher yield of trees on M7.

Conclusions

Trees were still young and received a nutrient solution that was high in N to stimulate growth in trees to fill the space allocated to them during the first season (2004/2005). Therefore, a conclusion could not really be drawn from the results after only one season of treatments.

The results of 2005/06 showed that during fruit growth, Ca concentration decreased as cell expansion took place and the Ca in the fruit diluted. However, Ca content increased as the fruit size increased and it continued until harvest.

The fruit yield found for fruit of trees on M7 explained the higher Ca concentrations in fruit of M7. M7 is a more dwarfing rootstock than M793 and therefore a tree on M7 will be less vigorous and smaller than a tree on M793. The heavier crop load of trees on M7 resulted in smaller fruit, which caused the higher Ca concentrations, since the dilution effect in small fruit is less than that in large fruit. These higher values were found during the whole fruit growth period. Therefore, it will be recommended that trees on M7 receive different water and nutrition management than trees on M793.

To conclude, soil conditions after preplant soil preparation was correct to ensure active root development. The pH and the Na, K, Ca and Mg percentages in the C.E.C. of the soil were

adequate. Furthermore the leaf mineral analyses of the 2005/06 season showed that a well balanced nutrient solution was applied to the trees. From all these results it seems that the orchard conditions (e.g. soil preparation, irrigation, fertilization) and practices (e.g. pruning, thinning, pest control) were managed successfully that even trees that received the low Ca application level had no problem in producing fruit of good quality and with associated high Ca levels, when no additional foliar Ca was applied to the trees. It might be possible that optimum levels of Ca were reached in the fruit, and further increase under the conditions was not possible.

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Table 1. The amount of each element given to third leaf 'Brookfield Gala' trees that were subjected to three Ca application levels (low, standard and high) and two rootstocks (M793 and M7) calculated for a production of 25 tons.ha^{-1} for the 2005/06 season.

Treatment	N	P	Ca	Mg	P	Cu	Zn	B	Mo	Mn	Fe
					kg.ha^{-1}year^{-1}						
Std	57.5	45.0	45.0	12.5	12.5	0.18	0.50	0.25	0.00	0.50	3.25
Low	57.5	45.0	27.0	12.5	12.5	0.18	0.50	0.25	0.00	0.50	3.25
High	57.5	45.0	63.0	12.5	12.5	0.18	0.50	0.25	0.00	0.50	3.25

Table 2. The macro element concentrations, Ca content of fruit as well as the fruit mass at harvest during the 2004/05 season of second leaf 'Brookfield Gala' apple trees on two rootstocks and subjected to three Ca application levels. Mean separation in columns using LSD (5%).

Treatment	N	P	K	Ca	Mg	Ca content	Fruit mass
	mg.100g^{-1} fresh weight					mg.fruit^{-1}	gram
Ca levels:							
Low-Low	59.00 a	16.64 a	189.83 a	6.03 a	9.35 a	9.07 a	150.37 ns^{z}
Std-Std	51.33 abc	16.29 a	178.50 ab	5.42 abc	8.63 ab	8.61 ab	159.07
High-High	50.00 abcd	15.07 ab	175.17 abc	5.17 bcd	7.83 cd	7.33 bc	142.02
Low-Std	50.67 abcd	14.25 bc	157.00 c	5.25 abcd	7.93 bcd	8.34 ab	158.50
Std-Low	41.17 d	14.16 bc	165.50 bc	4.80 cd	7.63 cd	6.50 c	137.65
Low-High	45.67 bcd	12.94 c	156.67 c	4.42 d	7.30 d	6.51 c	147.57
High-Low	47.50 bcd	13.39 c	179.17 ab	5.98 ab	8.35 bc	8.94 a	149.90
Std-High	44.67 cd	13.24 c	165.83 bc	4.82 cd	7.45 d	6.88 c	142.23
High-Std	54.50 ab	14.05 bc	171.50 abc	5.02 cd	8.32 bc	7.83 abc	157.13
Rootstock:							
M793	46.74 b	13.95 b	165.96 b	5.10 ns	7.95 ns	7.77 ns	152.86 ns
M7	52.04 a	14.94 a	176.07 a	5.32	8.23	7.79	145.91
Sign.Level Pr > F:							
Ca levels (Ca)	0.0250	0.0001	0.0205	0.0055	0.0002	0.0015	0.2584
Rootstock (R)	0.0232	0.0114	0.0288	0.2755	0.1466	0.9517	0.1305
Ca * R	0.8487	0.5945	0.1915	0.2712	0.3666	0.0684	0.4480
Contrasts:							
Low vs High early (0 – 40 dafb)	0.6864	0.3338	0.1790	0.5167	0.9029	0.8885	0.6569
Low vs High late (40dafb – harvest)	0.3766	0.0371	0.0301	0.0018	0.0003	0.0039	0.7135

^{z} Means with the same letters are not significantly different ($P \leq 0.05$; ns = non significant)

Table 3. The K/Ca ratio at harvest of 'Brookfield Gala' apple trees on two rootstocks subjected to three Ca application levels during both the 2004/05 and 2005/06 season. Mean separation in columns using LSD (5%).

Treatment	K/Ca ratio 2005	K/Ca ratio 2006
Ca levels:		
Low-Low	31.63 ns[z]	17.15 ns
Std-Std	33.03	17.75
High-High	34.40	14.44
Low-Std	30.69	14.18
Std-Low	35.12	16.02
Low-High	35.94	16.69
High-Low	30.05	17.87
Std-High	34.81	17.91
High-Std	34.36	17.86
Rootstock:		
M793	33.06	18.00 a
M7	33.61	15.29 b
Sign. Level Pr > F:		
Ca levels (Ca)	0.2362	0.0972
Rootstock (R)	0.6415	0.0006

[z] Means with the same letters are not significantly different ($P \leq 0.05$; ns = non significant)

Table 4. Mineral analyses results of leaf samples at harvest for the 2004/05 season of second leaf 'Brookfield Gala' apple trees that were subjected to three Ca application levels and two rootstocks. Means in columns are separated using LSD (5%).

Treatment	N	P	K	Ca	Mg	Na	Mn	Fe	Cu	Zn	B
	%					mg.kg^{-1}					
Ca levels:											
Low-Low	3.22 ns[z]	0.26 ns	2.14 c	1.33 ns	0.43 c	207.17 ns	124.50 ns	281.17 de	6.17 ns	68.83 bc	42.67 ns
Std-Std	3.19	0.29	2.15 c	1.42	0.43 c	204.33	110.17	254.33 e	8.83	82.50 bc	44.83
High-High	3.17	0.26	2.12 c	1.33	0.48 abc	191.00	125.50	294.00 cde	9.50	65.50 c	43.33
Low-Std	3.23	0.26	2.30 bc	1.43	0.46 bc	200.50	114.67	279.67 de	6.83	83.83 bc	45.33
Std-Low	3.13	0.27	2.56 ab	1.46	0.44 c	224.33	124.00	359.33 b	6.33	97.00 bc	49.50
Low-High	3.16	0.24	2.49 ab	1.53	0.55 a	238.67	119.83	345.17 bc	8.50	87.17 bc	47.67
High-Low	3.11	0.23	2.71 a	1.53	0.55 a	238.83	141.17	431.50 a	8.50	182.67 a	52.67
Std-High	3.31	0.26	2.53 ab	1.51	0.52 ab	230.83	108.67	323.83 bcd	8.50	101.33 b	47.67
High-Std	3.32	0.25	2.49 ab	1.53	0.53 ab	235.67	103.67	267.50 e	7.50	83.00 bc	44.00
Rootstock:											
M793	3.19 ns	0.27 a	2.38 ns	1.39 b	0.49 ns	215.41 ns	118.52 ns	317.44 ns	8.78 ns	99.53 ns	46.78 ns
M7	3.22	0.24 b	2.39	1.52 a	0.49	222.67	119.74	312.89	6.93	89.70	46.04
Sign.Level Pr > F:											
Ca levels (Ca)	0.5425	0.4262	0.0014	0.4601	0.0013	0.5517	0.6525	<0.0001	0.8949	<0.0001	0.0980
Rootstock (R)	0.5253	0.0070	0.8094	0.0210	0.8931	0.5848	0.8899	0.7102	0.1339	0.2113	0.6438
Ca * R	0.3123	0.2386	0.2792	0.1721	0.6898	0.6204	0.2339	0.2651	0.1791	0.0122	0.6556
Contrasts:											
Low vs High early	0.9723	0.7437	0.1311	0.6022	0.0486	0.6943	0.7269	0.0597	0.3730	0.0030	0.4627
Low vs High late	0.3478	0.9420	0.3106	0.8229	0.0338	0.8400	0.2756	0.0200	0.2230	0.0022	0.2979

[z] Means with the same letters are not significantly different ($P \leq 0.05$; ns = non significant)

Table 5. The macro element concentrations, Ca content and mass of fruit at harvest during the 2005/06 season of third leaf 'Brookfield Gala' apple trees on two rootstocks that were subjected to three Ca application levels. Mean separation in columns using LSD (5%). Adjusted means were used for P and Ca concentrations and Ca contents of fruit where fruit mass was a significant covariate (cov).

Treatment	N	P	K	Ca	Mg	Ca content	Fruit mass
	mg.100g^{-1} fresh weight					mg.fruit^{-1}	gram
Ca levels:							
Low-Low	26.33ns[z]	10.53 a	120.33 ns	7.14 c	6.22 ns	7.14 c	94.67 cd
Std-Std	60.83	9.95 a	121.67	6.99 c	6.15	6.93 c	110.43 a
High-High	46.33	10.10 a	122.17	8.85 a	6.75	8.81 a	87.00 d
Low-Std	58.67	9.49 a	115.00	8.13 ab	6.45	8.22 ab	106.12 ab
Std-Low	35.17	10.04 a	120.00	7.54 bc	6.35	7.59 bc	97.27 bcd
Low-High	43.50	10.21 a	120.67	7.32 bc	6.82	7.33 bc	103.42 abc
High-Low	49.33	10.44 a	138.50	7.83 abc	6.73	7.81 abc	103.92 abc
Std-High	51.67	9.81 a	125.33	7.03 c	6.53	7.08 c	104.82 abc
High-Std	59.67	9.80 a	121.50	6.96 c	6.32	7.00 c	99.57 bc
Rootstock:							
M793	53.41 ns	9.28 b	124.78 ns	7.01 b	6.47 ns	7.04 b	107.73 a
M7	42.48	10.80 a	120.82	8.05 a	6.49	8.05 a	93.87 b
Sign.Level Pr > F:							
Ca at 20 dafb cov	0.8013	0.0004	0.2963	<0.0001	0.3127	0.8383	<0.0001
Mass covariate	0.8713	0.0168	0.7669	0.0067	0.3448	<0.0001	-
Ca without cov	0.7912	0.9358	0.1338	0.0084	0.0573	0.1361	0.0045
Ca with Ca 20 cov	0.8061	0.8438	0.1758	0.0155	0.0820	0.1558	0.0039
Ca with mass cov	0.7907	0.6513	0.1446	0.0275	0.0189	0.0285	-
Rootstock Without cov	0.2940	<0.0001	0.2395	0.0002	0.8164	0.7855	<0.0001
Rootstock With Ca 20 cov	0.2441	0.0187	0.1660	0.1249	0.8900	0.6008	0.0070
Rootstock With mass cov	0.1293	0.0010	0.1413	0.0009	0.1144	0.0017	-
Ca * R	0.6095	0.7043	0.5018	0.4595	0.6386	0.1923	0.0307
Contrasts:							
Low vs High early	0.4811	0.9674	0.0386	0.2630	0.4421	0.8956	0.1437
Low vs High late	0.4212	0.4846	0.3866	0.4266	0.0576	0.5791	0.9467

[z] Means with the same letters are not significantly different ($P \leq 0.05$; ns = non significant)

Table 6. The macro element concentrations, Ca content and mass of fruit at 20 dafb during the 2005/06 season of third leaf 'Brookfield Gala' apple trees on two rootstocks that were subjected to three Ca application levels. Mean separation in columns using LSD (5%).

Treatment	N	P	K	Ca	Mg	Ca content	Fruit mass
	mg.100g^{-1} fresh weight					mg.fruit^{-1}	gram
Ca levels:							
Low-Low	255.17 ns[z]	34.19 abc	237.33 ns	45.73 ns	28.67 b	1.52 ns	3.40 b
Std-Std	243.33	32.12 d	230.33	42.48	26.92 b	1.67	3.98 a
High-High	249.00	32.40 cd	240.50	49.35	28.25 b	1.74	3.62 ab
Low-Std	272.33	35.84 a	251.67	50.88	31.85 a	1.59	3.20 b
Std-Low	251.17	33.20 bcd	244.17	47.33	28.23 b	1.71	3.68 ab
Low-High	265.17	34.69 ab	248.33	53.32	32.28 a	1.66	3.18 b
High-Low	250.83	34.60 ab	250.50	43.38	27.08 b	1.71	4.03 a
Std-High	250.67	32.91 bcd	259.83	47.48	28.65 b	1.73	3.70 ab
High-Std	255.00	33.72 bcd	247.83	45.18	27.98 b	1.60	3.58 ab
Rootstock:							
M793	257.82 ns	33.68 ns	237.44 b	41.25 b	26.32 b	1.60 ns	3.92 a
M7	251.67	33.80	253.78 a	53.23 a	31.44 a	1.72	3.27 b
Sign.Level Pr > F:							
Ca levels (Ca)	0.3563	0.0061	0.1213	0.1087	0.0005	0.7629	0.0317
Rootstock (R)	0.2713	0.7736	0.0007	<0.0001	<0.0001	0.0620	<0.0001
Ca * R	0.6491	0.9732	0.4277	0.8111	0.5533	0.8131	0.6712

[z] Means with the same letters are not significantly different ($P \leq 0.05$; ns = non significant)

Table 7. The macro element concentrations, Ca content and mass of fruit at 40 dafb during the 2005/06 season of third leaf 'Brookfield Gala' apple trees on two rootstocks that were subjected to three Ca application levels. Mean separation in columns using LSD (5%).

Treatment	N	P	K	Ca	Mg	Ca content	Fruit mass
	mg.100g^{-1} fresh weight					mg.fruit^{-1}	gram
Ca levels:							
Low-Low	153.67 ns[z]	22.21 ab	195.33 bcd	19.58 ns	15.67 ns	3.67 ns	18.85 ns
Std-Std	159.83	22.29 ab	209.33 ab	20.83	14.68	4.46	22.30
High-High	152.50	22.87 a	202.67 abc	22.88	16.33	4.58	20.05
Low-Std	146.50	20.91abcd	182.00 d	19.40	15.38	4.08	21.03
Std-Low	142.00	19.74 d	188.50 cd	18.77	14.10	3.69	19.65
Low-High	152.67	20.14 cd	194.17 bcd	19.75	15.40	4.10	20.63
High-Low	164.00	21.92 abc	216.17 a	21.83	15.67	4.38	20.78
Std-High	150.83	20.62 bcd	195.00 bcd	17.63	14.67	3.45	19.68
High-Std	165.67	22.07 abc	197.50 bcd	16.92	14.65	3.52	20.78
Rootstock:							
M793	158.44 ns	21.91 a	200.63 ns	17.96 b	14.57 b	3.76 ns	21.05 a
M7	149.93	20.92 b	195.07	21.38 a	15.77 a	4.22	19.79 b
Sign.Level Pr > F:							
Ca levels (Ca)	0.1880	0.0399	0.0183	0.4250	0.1564	0.2618	0.0626
Rootstock (R)	0.0510	0.0442	0.1899	0.0073	0.0022	0.0680	0.0095
Ca * R	0.1900	0.7577	0.3778	0.4021	0.9296	0.6648	0.0317
Contrasts:							
Low vs High early	0.0665	0.0464	0.0059	0.5969	0.8819	0.4901	0.5178

[z] Means with the same letters are not significantly different ($P \leq 0.05$; ns = non significant)

Table 8. The macro element concentrations, Ca content and mass of fruit at 60 dafb during the 2005/06 season of third leaf 'Brookfield Gala' apple trees on two rootstocks that were subjected to three Ca application levels. Mean separation in columns using LSD (5%), since fruit mass was not a significant covariate for any of the parameters.

Treatment	N	P	K	Ca	Mg	Ca content	Fruit mass
	mg.100g^{-1} fresh weight					mg.fruit^{-1}	gram
Ca levels:							
Low-Low	114.67 a	17.32 ns[z]	165.50 ns	13.27 ns	11.32 ns	5.44 ns	41.50 a
Std-Std	120.33 a	17.02	174.50	14.03	11.20	5.60	40.27 abc
High-High	106.83 ab	17.32	168.50	13.58	11.33	4.87	36.33 d
Low-Std	110.67 a	16.77	165.17	13.37	11.68	5.23	39.23 abcd
Std-Low	115.33 a	16.83	177.17	14.83	11.65	5.55	37.80 bcd
Low-High	107.83 ab	16.17	156.83	14.30	11.30	5.36	37.38 cd
High-Low	120.83 a	18.28	178.67	17.38	12.18	7.03	40.77 ab
Std-High	112.67 a	16.79	170.17	12.97	11.40	5.39	41.40 a
High-Std	92.67 b	17.05	166.50	13.48	11.28	5.18	38.22 bcd
Rootstock:							
M793	110.63 ns	17.53 ns	167.63 ns	13.11 b	11.94 a	5.90 a	39.29 a
M7	112.00	16.60	170.82	15.16 a	11.03 b	5.13 b	39.13 a
Sign.Level Pr > F:							
Mass as covariate	0.1191	0.9159	0.9610	0.0754	0.3492	0.1968	-
Ca levels without cov.	0.0428	0.8603	0.3297	0.2518	0.9212	0.1285	0.0086
Ca levels with cov.	0.0424	0.8242	0.2944	0.1970	0.8166	0.1906	-
Rootstock without cov.	0.7165	0.1021	0.4490	0.0123	0.0096	0.0176	0.8179
Rootstock with cov.	0.7190	0.1004	0.4717	0.0148	0.0101	0.0159	-
Ca* R	0.0009	0.5864	0.7578	0.1586	0.3289	0.4562	0.0020
Contrasts:							
Low vs High early	0.3572	0.2419	0.0959	0.2235	0.6855	0.3557	0.2856
Low vs High late	0.0965	0.3033	0.1001	0.1115	0.3681	0.0417	0.0636

[z] Means with the same letters are not significantly different ($P \leq 0.05$; ns = non significant)

Table 9. The macro element concentrations, Ca content and mass of fruit at 80 dafb during the 2005/06 season of third leaf 'Brookfield Gala' apple trees on two rootstocks that were subjected to three Ca application levels. Mean separation in columns using LSD (5%).

Treatment	N	P	K	Ca	Mg	Ca content	Fruit mass
	mg.100g^{-1} fresh weight					mg.fruit^{-1}	gram
Ca levels:							
Low-Low	75.83 ns[z]	14.63 ns	156.50 ns	11.62 ns	9.67 ns	7.42 abc	65.48 ns
Std-Std	82.33	15.13	184.00	12.03	9.82	7.80 a	64.73
High-High	89.00	13.82	170.17	11.88	9.33	6.65 cd	56.72
Low-Std	76.33	13.27	151.33	11.77	9.50	7.72 ab	66.08
Std-Low	80.00	14.32	167.67	10.63	9.10	6.44 d	61.03
Low-High	78.17	12.95	157.33	10.40	9.03	6.56 cd	63.45
High-Low	79.17	15.09	182.33	10.62	9.68	6.80 bcd	65.82
Std-High	81.00	14.92	170.17	10.72	10.02	6.66 cd	63.30
High-Std	71.83	14.61	173.00	11.42	9.37	7.07 abcd	62.42
Rootstock:							
M793	80.26 ns	14.89 a	167.70 ns	10.53 b	9.13 b	6.89 ns	66.16 a
M7	78.33	13.72 b	168.41	11.93 a	9.87 a	7.14	60.29 b
Sign.Level Pr > F:							
Ca levels (Ca)	0.5238	0.4685	0.1142	0.5538	0.8606	0.0440	0.4391
Rootstock (R)	0.5710	0.0355	0.9013	0.0040	0.0234	0.2754	0.0049
Ca * R	0.3819	0.2713	0.8767	0.2328	0.3889	0.0038	0.0096
Contrasts:							
Low vs High early	0.4399	0.1803	0.0062	0.9369	0.8745	0.1644	0.1697
Low vs High late	0.2946	0.2384	0.6721	0.9369	0.9542	0.3480	0.2251

[z] Means with the same letters are not significantly different ($P \leq 0.05$; ns = non significant)

Table 10. Mineral analyses results of leaf samples at 60 dafb during the 2005/06 season of third leaf 'Brookfield Gala' apple trees that were subjected to three Ca application levels and two rootstocks. Means in columns are separated using LSD (5%).

Treatment	N	P	K	Ca	Mg	Na	Mn	Fe	Cu	Zn	B
	%					mg.kg^{-1}					
Ca levels:											
Low-Low	2.63 ns[z]	0.21 ns	1.50 ns	0.86 ns	0.36 ns	160.33 bc	184.67 a	153.33 ab	8.83	44.83	28.33 a
Std-Std	2.64	0.21	1.51	0.94	0.30	162.33 abc	169.33 ab	157.67 a	7.17	41.83	27.50 ab
High-High	2.63	0.20	1.57	0.92	0.36	166.83 abc	187.00 a	148.67 abc	6.67	46.17	26.50 b
Low-Std	2.70	0.21	1.43	0.92	0.39	146.83 c	190.00 a	136.67 bc	7.00	49.17	28.33 a
Std-Low	2.66	0.20	1.60	0.98	0.35	170.50 abc	174.83 ab	133.00 c	6.83	42.00	26.17 b
Low-High	2.61	0.19	1.44	0.87	0.37	150.67 c	171.33 ab	136.50 bc	7.33	44.17	26.50 b
High-Low	2.63	0.19	1.53	0.94	0.33	189.83 a	168.83 ab	137.50 bc	7.00	42.33	26.33 b
Std-High	2.64	0.19	1.46	0.90	0.39	182.33 ab	153.67 b	131.83 c	7.50	41.33	26.67 b
High-Std	2.64	0.19	1.50	0.90	0.37	186.00 ab	155.00 b	131.67 c	7.00	42.83	26.17 b
Rootstock:											
M793	2.60 b	0.20 a	1.54 ns	0.85 b	0.35 b	165.30 ns	170.59 ns	139.04 ns	7.15 ns	43.33 ns	26.93 ns
M7	2.68 a	0.19 b	1.47	0.98 a	0.38 a	171.52	174.89	142.48	7.37	44.37	26.96
Sign.Level Pr > F:											
Ca levels (Ca)	0.8906	0.0651	0.5642	0.6108	0.2190	0.0300	0.0564	0.0267	0.0682	0.1102	0.0070
Rootstock (R)	0.0018	0.0445	0.0877	<0.0001	0.0094	0.3394	0.4716	0.4069	0.4602	0.4173	0.9098
Ca * R	0.8251	0.5476	0.7511	0.6618	0.4833	0.2922	0.2009	0.2564	0.6913	0.6353	0.1461
Contrasts:											
Low vs High early	0.5853	0.0605	0.1376	0.3203	0.1422	0.0010	0.1141	0.5691	0.0285	0.1501	0.0013
Low vs High late	0.6786	0.2688	0.3041	0.3203	0.0829	0.3834	0.4565	0.6531	0.2934	0.5936	0.3347

[z] Means with the same letters are not significantly different ($P \leq 0.05$; ns = non significant)

Table 11. Mineral analyses results of leaf samples at 80 dafb during the 2005/06 season of third leaf 'Brookfield Gala' apple trees that were subjected to combinations of three Ca application levels and two rootstocks. Means in columns are separated using LSD (5%).

Treatment	N	P	K	Ca	Mg	Na	Mn	Fe	Cu	Zn	B
	%					mg.kg^{-1}					
Ca levels:											
Low-Low	2.50 ns[z]	0.17 ns	1.34 dc	1.31 ns	0.44 ns	274.33 ns	204.50 a	162.00 ns	7.17 abc	65.67 ns	30.50 a
Std-Std	2.49	0.15	1.34 dc	1.39	0.42	283.00	191.33 abc	162.83	7.83 ab	62.33	28.83 cde
High-High	2.50	0.16	1.36 abcd	1.34	0.41	298.50	199.17 ab	171.33	8.33 a	63.50	28.33 de
Low-Std	2.58	0.16	1.24 d	1.32	0.45	267.00	189.83 abc	159.33	7.83 ab	63.33	29.83 abc
Std-Low	2.47	0.17	1.37 abcd	1.36	0.39	285.83	185.00abcd	149.50	7.50 cd	61.83	27.83 e
Low-High	2.52	0.16	1.35 bcd	1.29	0.43	255.83	184.67abcd	160.50	6.67 bcd	64.00	30.17 ab
High-Low	2.50	0.16	1.48 ab	1.36	0.39	301.50	175.00 bcd	150.67	6.17 cd	61.17	28.50 de
Std-High	2.47	0.16	1.45 abc	1.34	0.42	280.67	168.67 cd	155.00	6.17 cd	64.00	29.17 bcd
High-Std	2.49	0.16	1.48 a	1.33	0.42	269.67	163.83 d	155.17	5.83 d	60.17	28.67 cde
Rootstock:											
M793	2.43 b	0.16 ns	1.44 a	1.23 b	0.41 ns	279.67 ns	177.78 b	158.56 ns	7.04 ns	60.26 b	28.74 b
M7	2.57 a	0.16	1.31 b	1.44 a	0.43	279.52	191.56 a	158.41	6.85	65.52 a	29.44 a
Sign.Level Pr > F:											
Ca levels (Ca)	0.5169	0.7430	0.0083	0.7767	0.2596	0.3438	0.0278	0.2529	0.0006	0.9026	0.0014
Rootstock (R)	<0.0001	0.7329	<0.0001	<0.0001	0.1195	0.9871	0.0201	0.9698	0.5002	0.0046	0.0218
Ca * R	0.8577	0.8698	0.8356	0.7397	0.8487	0.7835	0.2910	0.0591	0.4484	0.4050	0.3666
Contrasts:											
Low vs High early	0.1788	0.7539	0.0010	0.2874	0.0166	0.0368	0.0565	0.7454	0.1906	0.2084	<0.0001
Low vs High late	0.8648	0.1010	0.8543	0.5140	0.3349	0.4295	0.5671	0.0927	0.1906	0.6592	0.4436

[z] Means with the same letters are not significantly different ($P \leq 0.05$; ns = non significant)

Table 12. Mineral analyses results of leaf samples at harvest during the 2005/06 season of third leaf 'Brookfield Gala' apple trees that were subjected to three Ca application levels and two rootstocks. Means in columns are separated by using LSD (5%).

Treatment	N	P	K	Ca	Mg	Na	Mn	Fe	Cu	Zn	B
	%					mg.kg^{-1}					
Ca levels:											
Low-Low	2.60 ab	0.16 ns[z]	1.08 bc	1.82 bcd	0.49 ns	279.50 c	192.50 a	231.83 d	5.50 ns	51.67 ns	30.00 abc
Std-Std	2.55 abcd	0.16	1.11 bc	1.95 ab	0.47	320.17 abc	187.67 ab	230.67 d	5.33	53.83	29.67 abc
High-High	2.44 d	0.15	1.13 abc	1.79 d	0.47	351.17 ab	186.83 ab	272.33 ab	5.17	54.67	28.50 c
Low-Std	2.57 abc	0.16	1.02 c	1.81 cd	0.50	294.83 c	181.33 ab	246.00 cd	5.50	53.00	30.17 ab
Std-Low	2.57 abc	0.18	1.19 ab	1.99 a	0.47	356.00 a	188.17 a	242.67 d	5.17	53.83	30.33 ab
Low-High	2.57 abc	0.16	1.11 bc	1.91 abc	0.53	312.67 bc	182.17 ab	253.83 bcd	5.83	54.67	31.00 a
High-Low	2.48 cd	0.15	1.20 ab	1.90 abc	0.47	354.50 ab	176.00 ab	283.33 a	5.00	47.67	28.83 bc
Std-High	2.51 bcd	0.16	1.16 ab	1.92 ab	0.48	302.83 c	167.50 b	271.67 abc	6.00	52.83	31.00 a
High-Std	2.65 a	0.15	1.23 a	1.85 bcd	0.47	305.17 c	167.50 b	281.83 a	5.67	53.00	30.00 abc
Rootstock:											
M793	2.51 b	0.16 ns	1.24 a	1.74 b	0.47 b	309.44 b	176.44 ns	245.82 b	5.30 ns	51.26 b	30.26 ns
M7	2.58 a	0.15	1.03 b	2.02 a	0.50 a	329.85 a	185.70	268.44 a	5.63	54.33 a	29.63
Sign.Level Pr > F:											
Ca levels (Ca)	0.0282	0.3612	0.0382	0.0030	0.5753	0.0043	0.1471	0.0003	0.1613	0.3764	0.0294
Rootstock (R)	0.0105	0.1625	<0.0001	<0.0001	0.0269	0.0474	0.0564	0.0007	0.0644	0.0295	0.0889
Ca * R	0.0665	0.9603	0.3526	0.0160	0.9507	0.5541	0.0719	0.0618	0.5973	0.5603	0.3671
Contrasts:											
Low vs High early	0.0998	0.1670	0.0018	0.9688	0.0636	0.0017	0.1454	<0.0001	0.1279	0.4266	0.0065
Low vs High late	0.1542	0.4253	0.5478	0.2530	0.2348	0.5263	0.2498	0.0822	0.0451	0.0791	0.3200

[z] Means with the same letters are not significantly different ($P \leq 0.05$; ns = non significant)

Table 13. The quality parameters for fruit at harvest during the 2005/06 season of third leaf 'Brookfield Gala' apple trees on two rootstocks that were subjected to combination of three Ca application levels. Mean separation in columns using LSD (5%). Adjusted means were used for fruit diameter, TSS and starch where fruit mass was a significant covariate.

Treatment	Diameter mm	TSS ° Brix	Starch %	Firmness kg	Malic acid	Citric acid	Tartaric acid
Ca levels:							
Low-Low	63.30ns[z]	14.06 bc	42.75 b	9.08 ns	0.42 ns	0.40 ns	0.45 ns
Std-Std	63.19	13.97 c	35.82 bc	9.25	0.42	0.40	0.45
High-High	62.76	14.53 ab	61.95 a	9.02	0.43	0.41	0.45
Low-Std	63.02	13.82 c	23.21 c	9.36	0.44	0.41	0.46
Std-Low	63.06	13.86 c	41.93 b	9.30	0.44	0.42	0.46
Low-High	62.59	14.22 bc	38.35 bc	9.10	0.43	0.41	0.46
High-Low	62.85	14.86 a	57.21 ab	9.06	0.43	0.42	0.46
Std-High	62.65	14.29 bc	42.35 b	9.21	0.44	0.42	0.47
High-Std	62.54	14.32 bc	52.02 ab	9.00	0.42	0.39	0.44
Rootstock:							
M793	62.55 ns	14.71 a	27.91 b	9.37 a	0.44 a	0.42 a	0.47 a
M7	63.22	13.72 b	59.99 a	8.93 b	0.41 b	0.40 b	0.44 b
Sign. Level Pr > F:							
Mass covariate	<0.0001	<0.0001	0.0002	0.6541	0.4083	0.3905	0.4196
Ca levels without cov.	0.4469	0.0021	0.0111	0.9216	0.9753	0.9872	0.9833
Ca levels with cov.	0.9750	0.0001	0.0630	0.9010	0.9774	0.9866	0.9868
Rootstock without cov	<0.0001	0.6988	<0.0001	0.0030	0.0079	0.0092	0.0114
Rootstock with cov	0.7055	<0.0001	<0.0001	<0.0001	0.0045	0.0093	0.0094
Ca * R	0.2485	0.4554	0.8919	0.3064	0.8604	0.8202	0.8120

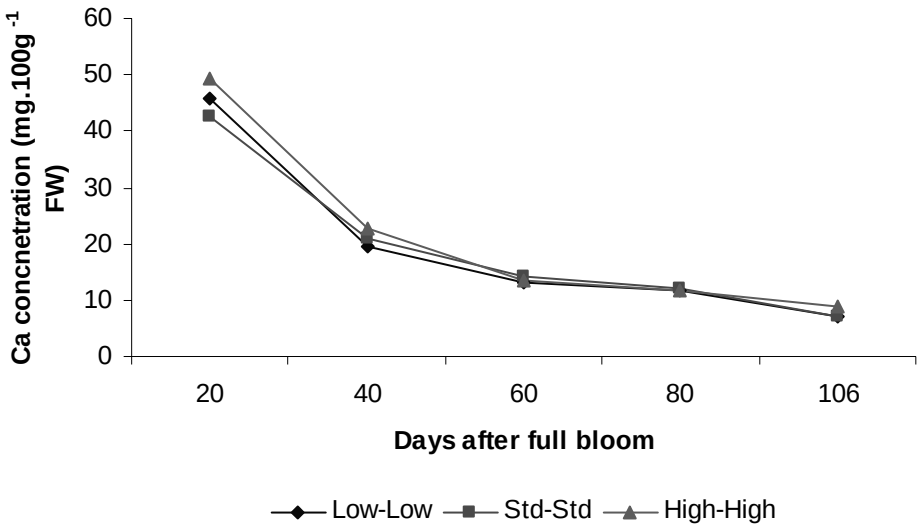
[z] Means with the same letters are not significantly different (P ≤ 0.05; ns = non significant)

Table 14. Quality parameters determined for fruit after nine weeks of storage during the 2005/06 season of third leaf 'Brookfield Gala' apple trees on two rootstocks that were subjected to three Ca application levels. Mean separation in columns using LSD (5%).

Treatment	Diameter mm	TSS ° Brix	Starch %	Firmness kg	Malic acid	Citric acid	Tartaric acid
Ca levels:							
Low-Low	61.63 ns[z]	14.83 bc	85.35 ns	7.76 ns	0.42 ns	0.40 ns	0.46 ns
Std-Std	64.10	14.55 c	85.53	7.33	0.42	0.41	0.46
High-High	61.76	15.13 abc	92.73	7.39	0.42	0.40	0.46
Low-Std	63.92	14.63 c	81.27	7.45	0.42	0.40	0.46
Std-Low	63.93	14.78 bc	87.00	7.39	0.45	0.43	0.48
Low-High	64.00	14.63 c	83.20	7.55	0.43	0.41	0.47
High-Low	63.27	15.43 ab	93.13	7.41	0.43	0.41	0.47
Std-High	64.86	14.90 abc	87.93	7.27	0.44	0.42	0.48
High-Std	62.22	15.57 a	91.60	7.52	0.42	0.40	0.46
Rootstock:							
M793	64.81 a	15.26 a	80.29 b	7.68 a	0.45 a	0.43 a	0.49 a
M7	61.79 b	14.62 b	94.77 a	7.22 b	0.41 b	0.39 b	0.44 b
Sign. Level Pr > F:							
Ca levels (Ca)	0.0899	0.0353	0.2323	0.8461	0.9417	0.9410	0.9487
Rootstock (R)	<0.0001	0.0002	<0.0001	0.0022	0.0017	0.0009	0.0010
Ca * R	0.2896	0.7645	0.8446	0.3406	0.8560	0.8226	0.8112

[z] Means with the same letters are not significantly different ($P \leq 0.05$; ns = non significant)

a)



b)

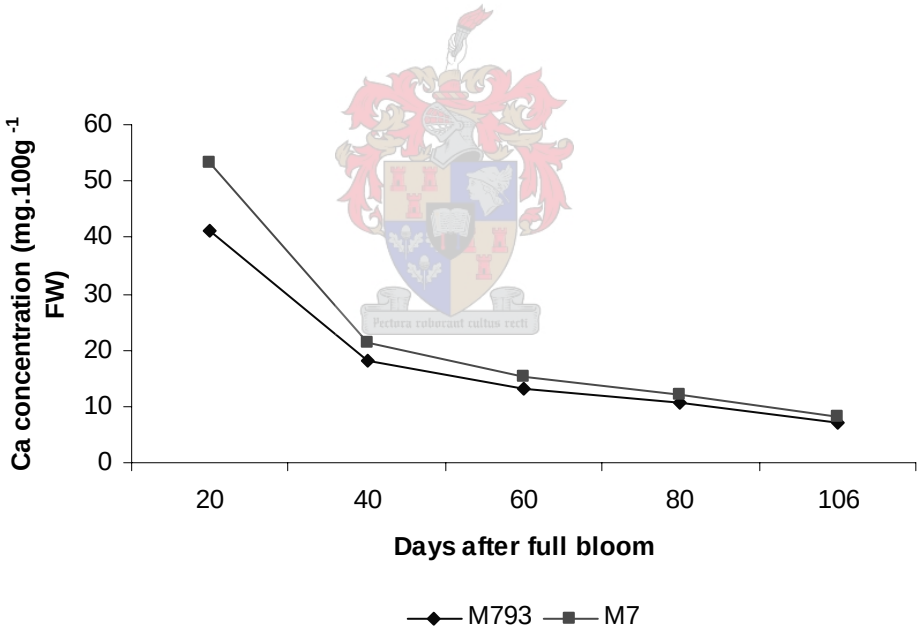
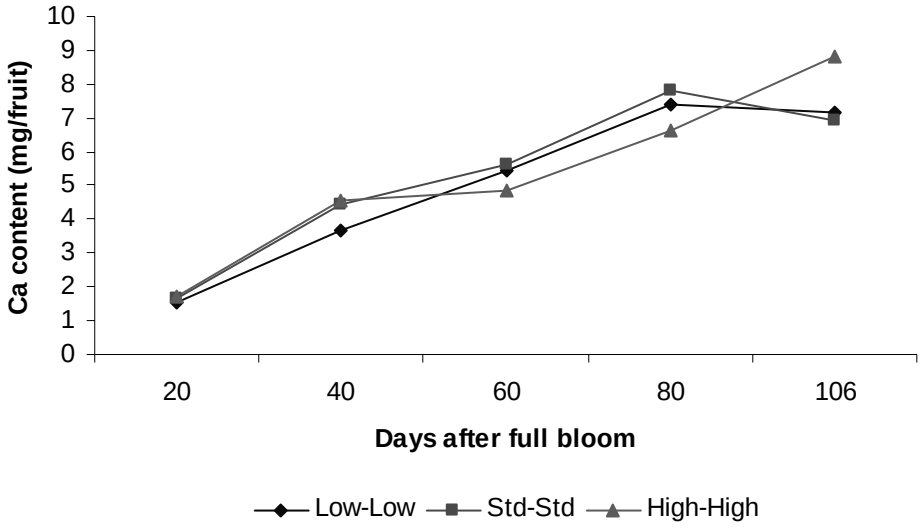


Figure 1. The decrease in mean Ca concentration of third leaf 'Brookfield Gala apple trees that were subjected to a) the three main Ca application levels (high, standard and low) and b) the two rootstocks from full bloom until harvest during the 2005/06 season. There was only a significant difference found in the Ca concentration of fruit between the treatments at harvest ($Pr > F = 0.0084$). There was a significant difference found in the Ca concentration of fruit between the rootstocks at every sampling date. (Significance level was $P \leq 0.05$).

a)



b)

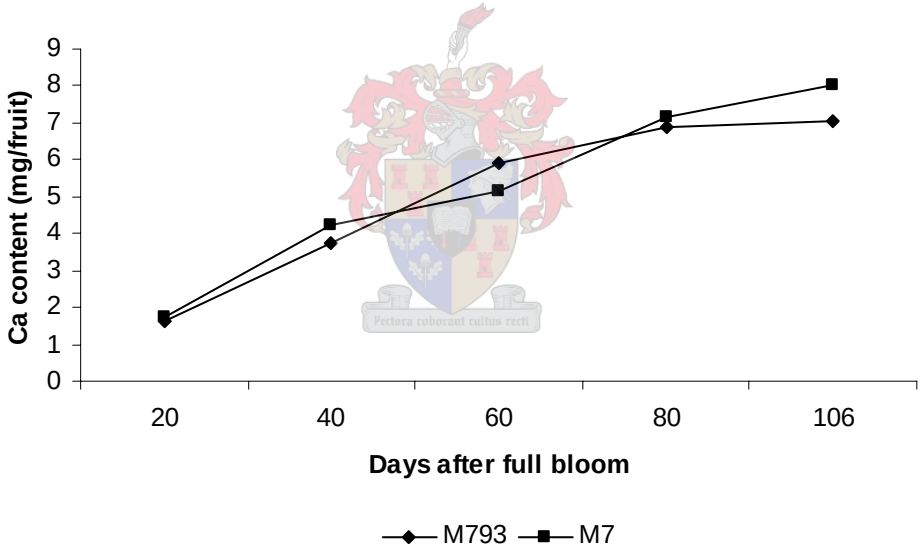
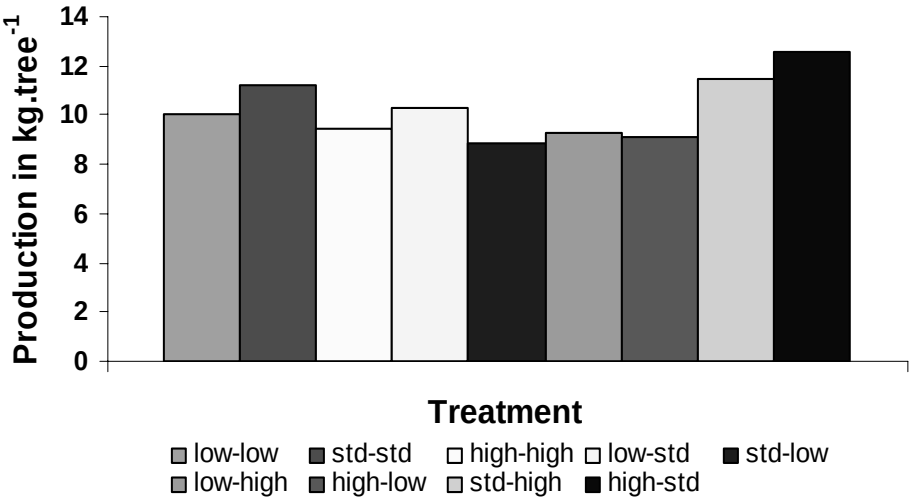


Figure 2. The decrease in mean Ca content of third leaf 'Brookfield Gala' apple trees that were subjected to a) the three main Ca application levels (high, standard and low) and b) the two rootstocks from full bloom until harvest during the 2005/06 season. There was a significant difference found in the Ca content of fruit between the treatments at harvest when fruit mass were used as covariate (Pr > F = <0.0001). There were significant differences at 60 dafb when M793 had the highest Ca content and at harvest when M7 had the highest Ca content for fruit mass used as covariate (Pr > F = 0.0176 and <0.0001 respectively).

a)



b)

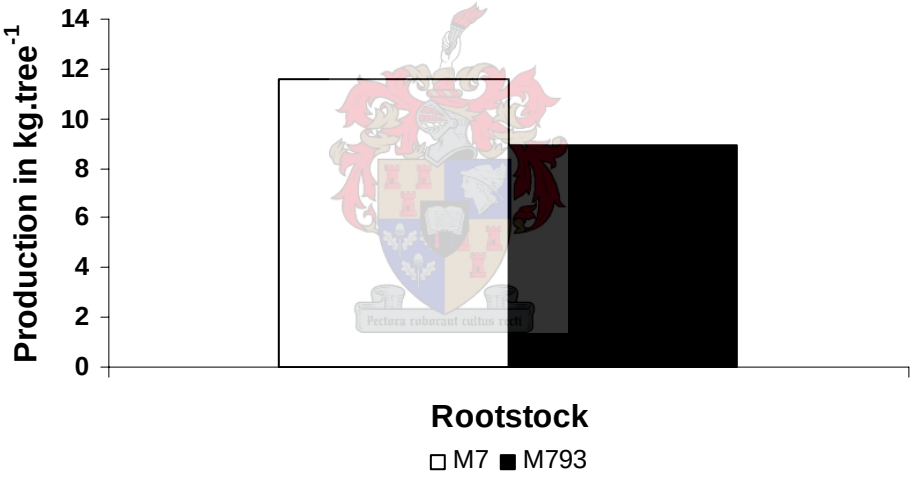


Figure 3. The mean yield values of third leaf 'Brookfield Gala' apple trees for the 2005/06 season that was subjected to three Ca application levels and two rootstocks. a) No significant difference was found on the production of the treatments (Pr > F = 0.2675), but b) a significant difference was found on the production of trees planted on the two rootstocks (Pr > F = 0.0020).

Pectora roborant cultus recti

Pectora roborant cultus recti

CHAPTER 5

EFFECT OF ROOTSTOCKS ON INTAKE OF CALCIUM INTO 'REINDERS GOLDEN DELICIOUS' APPLE FRUIT UNDER SOUTH AFRICAN CONDITIONS

Abstract

The effect of six rootstocks (M9 (Cepiland), CG222, M7A, CG707, M793 and CG239) on the uptake of calcium (Ca) into 'Reinders Golden Delicious' apple fruit was studied during the 2004-2006 seasons. Mineral analyses were done on fruit and leaf samples collected at harvest in both seasons, as well as at 40 days after full bloom (dafb) in the 2005/06 season. In the 2004/05 season fruit of trees on rootstocks M7 and M793 had higher Ca concentrations at harvest than all other rootstocks. At 40 dafb during the 2005/06 season Ca concentration of the fruit of trees on rootstock CG707 were significantly higher than that for M9 and M793, although that was not significantly higher than for the fruit of trees on rootstocks M7, CG239 and CG222. At harvest (2005/06), there were no significant differences found for the Ca concentration and the Ca content of fruit between the treatments, when fruit mass was used as a covariate. The fruit mass of trees on rootstock CG239 was significantly higher compared with that for M7, M9 and CG707. No significant differences were found between treatments for leaf mineral values in 2004/05, but in 2005/06 CG707 had significantly higher leaf Ca percentage than all other rootstocks.

Introduction

Genetic factors, such as rootstocks, play an important role in apple tree nutrition (Jadczuk et al. 2001). Effects of rootstock on scion tree yield, growth, and uptake of minerals has been reported (Lockard & Schneider, 1981; Sharma & Schaunan, 1991). Rootstocks have a greater

influence on the uptake of micronutrients than macronutrients (Heinz, 1989). However, Slowinski & Sadowski (2001) found the variation of leaf nutrient concentrations due to season and sampling date (Jadczuk et al. 2001) was often larger than rootstock effects. According to Ershadi & Talaie (2001) the variation of leaf nutrient content in relation to rootstock was small, and the total effect of rootstock on composition of scion apple leaves was not big. The effect of rootstock on the absorption and transport of mineral elements is so complex that an interaction between rootstock and cultivar seems to exist for accumulation, utilisation and redistribution of nutrients (Oukabli & Lahlou, 2005). Therefore it can influence Ca uptake and transport to the fruits.

One of the most important objectives of growers is to control fruit quality using rootstocks (Slowinska et al., 2004). Rootstock effects on internal fruit quality parameters such as firmness, soluble solids content, and acidity (Fallahi et al., 1985; Drake et al., 1991) and apple fruit mineral composition (Ben, 1995) have been found. Slowinska et al. (2004) found that rootstock affects fruit firmness, however, there was no persistent tendency for higher firmness associated with any particular rootstock. They found trends for higher firmness values, for fruit from trees on rootstocks where higher values for Ca content of fruit were obtained. Ca is perhaps the most important mineral element determining fruit quality (Conway et al., 2002) and the post harvest storage life of fruit (Siddiqui & Bangerth, 1993). The rootstock effect on physiological disorders, storage diseases and percentage of sound fruits was significant and the low percentage of physiological disorders of fruits for some rootstocks was associated with a high Ca and low K/Ca ratio (Slowinska et al., 2004). Under South African conditions Terblanche et al. (1980) recommended that the minimum Ca concentration for 'Golden Delicious' apples for bitter pit control is 5.4 and 6.6 $mg.100g^{-1}$ fresh weight (FW) for

unsprayed and Ca-sprayed fruit, respectively. Tomala (1997) stated that a K/Ca ratio above 22:1 is likely to be associated with commercial losses due to bitter pit.

The objective of this study was to examine the effect of six rootstocks in different vigour classes on Ca uptake into fruit during fruit growth of 'Reinders Golden Delicious' trees.

Materials and methods

This study was done on 'Reinders Golden Delicious' apple trees, planted in a commercial orchard on the farm Bo-Radyn that is situated in the Villiersdorp fruit production area (33º59´S; 19º18´E). Trees on 12 different rootstocks were planted, but for the purpose of this study, only six rootstocks were selected. These were (in sequence of increasing vigour): M9 (Cepiland) (dwarfing), CG222, M7A, CG707, M793 (standard rootstock currently used in South Africa) and CG239 (Costa & Stassen, 2006). Tree spacing was 4.5 x 2 m with 'Royal Gala' and 'Granny Smith' apple trees planted as cross-pollinators. Trees were planted on a well-drained, well-aerated sandy loam soil and soil preparation was done to achieve an ideal pH (KCl) of 5.5 and a correct balance of Na, K, Ca and Mg express as percentage of the cation exchange capacity (C.E.C.) of the soil to an effective depth of > 700 mm (Table 1). In order to achieve these conditions, calcitic lime was mixed into the soil horizons during preparation. All agricultural practices were as for a commercial orchard, including eight $Ca(NO_3)_2$ foliar applications for the control of bitter pit.

Fruit and leaf samples for mineral analysis:

In the first season (2004/05) fruit samples were collected only at harvest. Generally, leaf samples are annually collected at 31 January, but for this trial leaf samples were collected the same day as the fruit samples (14 February). In the second season (2005/06), fruit samples

were collected at 40 days after full bloom (dafb) as well as at harvest. Leaf samples were again collected on the same day as the fruit samples for harvest. For uniformity, fruit samples were collected from spurs on two-year-old shoots. Three fruit picked per tree represented one sample. For the leaf samples, one sample per three trees was picked. Three fruit per replicate were pooled and mineral analyses on fruit and leaves were performed by a commercial laboratory (Bemlab Pty Ltd, Strand). The whole fruit without the pips and core was analysed.

Yield:

The yield data was obtained from Costa & Stassen (2006).

Statistical analysis:

The layout of this trial was a randomised complete block design. There were 6 treatments with three replicates each. Three trees represented one replicate. Analysis of variance was done using the General Linear Model (GLM) procedure in the Statistical Analysis System (SAS) programme (SAS Institute Inc, 2004, Cary, NC). The data were analysed by performing a two-way ANOVA. Analysis of covariance was used for both fruit sample data sets of the 2005/06 season. Fruit mass was used as covariate, but not for the previous season (2004/05) as there was not a significant difference between treatments.

Results and discussion

2004/05:

Fruit and leaf sample results:

The mineral analyses of fruit at harvest showed that there were significant differences found in all mineral element concentrations between treatments (Table 2). Trees on CG239 had significantly the highest nitrogen (N) concentration in the fruit (88.75 mg.100g^{-1} FW). Trees

on CG707 had the highest phosphorus (P) concentration in fruit (12.55 mg.100g^{-1} FW), but that was not significantly higher than CG239 and M793 (12.18 and 11.71 mg.100g^{-1} FW, respectively). Fruit potassium (K) concentration of trees on CG707 was also the highest (158.56 mg.100g^{-1} FW), but again it was not higher than found for CG239 and M793 (149.37 and 149.22 mg.100g^{-1} FW). However, fruit of trees on M7A and M793 had significantly higher Ca concentrations (5.54 and 5.12 mg.100g^{-1} FW, respectively) than fruit of trees on all other rootstocks, but their Ca concentrations did not differ significantly from each other. The differences found for the Mg concentrations of fruit were significant, but for fruit mass no significant differences were found between treatments during this season. The actual Ca content in the fruit at harvest was calculated and fruit of trees on M7A had significantly the highest Ca content (9.39 mg.fruit^{-1}). There were significant differences found between the treatments for the K/Ca ratio of fruit at harvest. Fruit of trees on CG239 had the highest ratio, but it was not significantly higher compared to the values for CG707 and M9 (Table 3). Nevertheless, the values for the K/Ca ratio of all the treatments were extremely high.

Terblanche et al. (1980) stated that P concentrations normally lie between 6.0 and 12.0 mg.100g^{-1} FW for 'Golden Delicious' apple fruit at harvest. P concentrations of the fruit at harvest in this season were within this range. According to Terblanche (1985), K concentration in fruit must lie between 95 and 105 mg.100g^{-1} FW at harvest to assure that ion antagonism between K and Ca is avoided. All fruit at harvest in our study had higher K concentrations than that suggested by Terblanche (1985). From the literature K reduces Ca uptake by ion antagonism (Failla et al., 1990) and the balance between these two elements is important in the susceptibility of fruit to bitter pit (Ferguson & Watkins, 1983). Terblanche et al. (1980) also recommended that the minimum Ca concentration for 'Golden Delicious' apples for bitter pit control is 5.4 and 6.6 mg.100g^{-1} FW for unsprayed and Ca-sprayed fruit,

respectively. Most fruit from this season showed a lower Ca concentration than the recommended unsprayed value (5.4 mg.100g^{-1} FW). Furthermore, the K/Ca ratio of fruit from all treatments was higher than the 22:1 threshold value suggested by Tomala (1997) for the prevention of commercial losses due to bitter pit. This was the consequence of high K concentrations and low Ca concentrations in fruit of this season.

There were no significant differences found for leaf mineral concentrations between treatments in the 2004/05 season, except for Mg percentage (Table 4). Apart from P and K percentages and Na and B concentrations in leaves, all other minerals had higher concentrations than the standard norms used by the industry for apple leaves (Kotzé, 2001). Since K percentage is correct in the leaves and Ca percentage in the C.E.C. of the soil was corrected with soil preparation, the only possible explanation for the lower Ca concentration in fruit is that K and N in the nutrient solution applied to the trees was higher than needed by the trees and might have lowered the Ca uptake into the apple fruit.

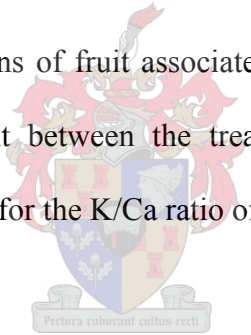
2005/06:

Fruit and leaf sample results:

Mineral analyses of fruit at 40 dafb were significant between treatments for N, P, K and Ca concentrations of fruit, but not for Mg concentration of fruit. The mean fruit mass was a significant covariate for P, K and Ca concentrations and Ca content of fruit at 40 dafb. Adjusted means for K concentration of fruit of trees on rootstock M9 (Cepiland) were significantly higher than that of all other rootstocks (196.74 mg.100g^{-1} FW) (Table 5). Adjusted means for Ca concentration of fruit of trees on CG707 were significantly higher (14.33 mg.100g^{1} FW) as opposed to M9 (Cepiland) and M793 (12.78 and 11.60 mg.100g^{-1} FW, respectively), but that was not significantly higher than Ca concentrations for fruit of

trees on M7A, CG239 and CG222. The fruit mass of fruit of trees on M793 was significantly higher (20.06 g.fruit^{-1}) than fruit of trees on all other rootstocks, except on M9 (Cepiland). The mean fruit mass was also a significant covariate for actual Ca content in the fruit and it followed the same pattern as described for Ca concentration (Table 5).

At harvest there were significant differences found for N, P, K and Mg concentrations between treatments (Table 6). Fruit mass of trees on CG239 was significantly higher (164.13 g.fruit^{-1}) compared with that for M7A, M9 (Cepiland) and CG707 (146.18, 146.68 and 150.68 g.fruit^{-1}, respectively), but not significantly higher than for M793 and CG222 (158.08 and 154.55 g.fruit^{-1}, respectively). The mean fruit mass was a significant covariate for Ca concentration of fruit only. Adjusted means of Ca concentration showed no persistent tendency for higher Ca concentrations of fruit associated with any particular rootstock. Ca content in fruit was not significant between the treatments. There was no significant difference found between treatments for the K/Ca ratio of the fruit at harvest (Table 3).



P concentrations of all fruit at harvest were within the range suggested by Terblanche et al. (1980). K concentration of fruit at harvest was mostly within the range set by Terblanche (1985). The fruit in this trial was subjected to eight foliar Ca sprays and none of the treatments had a higher Ca concentration than the recommended 6.6 mg.100g^{-1} FW value of Terblanche et al. (1980). The K/Ca ratio of fruit of most treatments except for CG707 and CG222 did not exceed the 22:1 threshold value suggested by Tomala (1997), which should assure good fruit quality. Slowinska et al. (2004) found that mean fruit mass was affected by rootstock and they observed a trend towards larger fruit on more vigorous rootstocks and smaller fruits were noted for dwarfing rootstocks. From our results a similar trend was

observed, since CG239 is a very vigorous rootstock and fruit were larger than on the more dwarfing rootstocks M9 and M7, but fruit mass of CG222 was in-between.

There were significant differences found for N and Ca percentages in the leaves between treatments in the 2005/06 season (Table 7). Leaves from trees on M9 had significantly higher N percentage (2.91%) than for CG707 and CG222 (2.62 and 2.73%, respectively), but that was not significantly higher than for CG239, M7A and M793. However, N percentage was higher for all rootstocks than the standard norms set by Kotzé (2001) for N percentage of apple leaves (2.04 and 2.52%). In the case of Ca percentage, leaves from trees on CG707 had significantly higher leaf Ca (2.92 %). The leaf Ca percentage of the other treatments did not differ significantly from each other. The mean leaf Ca percentage for the 2005/06 season was higher than the standard norm used for apple leaves by the industry, (between 1.36 and 1.82%) set by Kotzé (2001). Furthermore, the leaf mineral analyses showed that the K percentage of leaves was lower than that of the standard norm (1.14 and 1.52%) in apple leaves. Mg percentage in leaves was higher as opposed to that of the standard norm for Mg percentage.

Zocchi & Mignani (1995) stated that Ca deficiencies may be attributable to the low mobility of the ion in the plant transport systems. Ca shows a very low mobility in the phloem (Zocchi & Mignani, 1995). This indicates the importance of the early xylem transport for the supply of Ca to the developing fruit (Casero et al., 2002) at the stage when cell division in fruit is the strongest sink for carbohydrates and the leaf/fruit ratio is still low. The higher leaf Ca levels found in our trees showed that Ca was present within the trees. Furthermore, Faust (1989) stated that active root growth is needed for maximum Ca uptake and an adequate amount of Ca (70-80%) in the C.E.C. of the soil solution is needed (Terblanche et al., 1980). Ca was

adequately available in the soil since it constituted 79% of the C.E.C. in the soil and Mg percentage and K percentage were also appropriate in the C.E.C. of the soil (14% and 5% respectively). Conditions in the soil were appropriate for root development since the C percentage in the soil was 0.86% and the soil was well aerated. The pH of 6.3 (KCl) was higher than the ideal pH of 5.5 (KCl), but not harmful for activities in the soil (Table 1). Overall physical and chemical conditions in the soil were thus ideal for Ca uptake. However, the high N levels that were found in the leaves of trees in this trial could have lead to more vigorous growth in all trees, even the more dwarfing trees. This could have resulted in shoot growth that was a stronger sink than fruit growth development at the time when Ca uptake into fruit was critical (Ferguson & Watkins, 1989). According to Korcak (1980), interactions between Ca and N occur and therefore the nutritional balance of the tree must always be considered when fruit quality is low.

Yield for both seasons:

In 2005 trees on CG239 had higher yields (22.51 kg.tree^{-1}) than for M9 (Cepiland) (14.51 kg.tree^{-1}), but it was not significantly higher. In 2006 the same trend was observed. Trees on CG239 had higher yields (67.6 kg.tree^{-1}) than both M9 (Cepiland) and M7A (40.6 and 47.2 kg.tree^{-1}, respectively), but again not significantly higher. CG222 is a very dwarfing rootstock, but it seems to have higher yield than other dwarfing rootstocks.

From the literature it is known that a strong relationship between fruit size and Ca concentration exists, with large fruit having less Ca and a much higher risk of bitter pit (Ferguson et al., 1993). Furthermore, at the whole tree level, crop load is an important determinant of fruit size, with heavy crop loads associated with smaller average fruit size (Broom et al., 1998). In this trial, all fruit of trees were thinned according to standard farm

norms, which include chemical thinning followed by hand thinning. In both seasons results points towards fruit yield being higher on more vigorous rootstocks CG239 and CG707 as opposed to the dwarfing rootstocks M9 (Cepiland) and M7A, with the dwarfing rootstock CG222 in-between. No significant difference was found in fruit mass in the 2004/05 season between the rootstocks although CG239 had slightly higher fruit mass than both M9 (Cepiland) and M7A. The more vigorous rootstocks experienced strong vegetative growth which might have resulted in higher leaf/fruit ratio and probably larger fruit depending on the crop load. In contrast to that the more dwarfing rootstocks might have had more favourable leaf/fruit ratios and smaller fruit size according to the crop load. Comparing the fruit mass at harvest of the 2005/06 season between the rootstocks, the dwarfing M9 (Cepiland) and M7A had smaller fruit than the standard rootstock M793, but those of the dwarfing rootstock CG222 were not smaller. Furthermore, CG707 (almost the same vigour as M793), and the more vigorous rootstock CG239 had larger fruit. There were already significant differences at 40 dafb in 2005/06. When fruit mass was used as covariate in that season, no differences in Ca concentration of fruit were found. Therefore it seems that the vigour of the rootstock could play an indirect role on fruit size and because of that a difference in fruit Ca may be observed.

Conclusions

From these results it can be concluded that rootstocks do not play a direct role on the Ca concentration in apple fruit in this specific trial. The results of Ca levels in leaves of the trees on the different rootstocks for both seasons showed that Ca was taken up in adequate amounts from the roots, but it seems that the transport to the fruit was not sufficient due to a number of factors. Firstly, in the 2004/05 season the results of fruit at harvest showed that K concentration in fruit was high and as a result the K/Ca ratio was also higher than the

threshold value described by researchers. That means that K could have been the preferred element to be taken into the fruit in stead of Ca, since K is mobile in the phloem. Soil analyses showed that the conditions in the soil were ideal for good root development and Ca was present in adequate amounts to be available for uptake. This indicates the importance of the application of a well balanced nutrient solution.

To summarise, there was a tendency that more vigorous rootstocks that experienced strong vegetative growth and probably higher leaf/fruit ratios had larger fruit and more dwarfing rootstocks that had more favourable leaf/fruit ratios had smaller fruit size. Therefore it seemed that the vigour of the rootstocks could have played an direct role on fruit size and because of that a difference in fruit Ca was observed in this trial.

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Table 1. The pH, carbon (C) percentage and the percentages of elements that contribute to the cation exchangeable capacity in the sandy loam soil used for the trial. Sample was collected at the beginning of 2006.

Soil	Depth cm	pH (KCl)	C %	Na %	K %	Ca %	Mg %
Sandy loam	0-60	6.3	0.86	1.5	5	79	14

Table 2. Macro and micro element concentrations, fruit mass as well as Ca content of fruit at harvest for fifth leaf 'Reinders Golden Delicious' apple trees for the 2004/05 season. Mean separation in columns using LSD (5%).

Rootstock	N	P	K	Ca	Mg	Ca content	Fruit mass
	mg.100g^{-1} fresh weight					g.fruit^{-1}	mg.fruit^{-1}
M793	74.44 b	11.71 ab	149.22ab	5.12 a	6.94 a	8.21 b	159.62 ns[z]
CG707	69.00 bc	12.55 a	158.56 a	4.15 b	6.67 ab	6.95 c	166.87
M9Cepiland	59.44 d	9.93 cd	142.11 bc	4.35 b	6.21 bc	7.14 bc	164.63
CG222	61.33 d	11.06 bc	142.00 bc	4.50 b	6.08 c	7.30 bc	163.17
M7A	64.44 dc	9.50 d	134.78 c	5.54 a	7.12 a	9.39 a	169.18
CG239	88.75 a	12.18 ab	149.37 ab	3.93 b	7.00 a	6.77 c	171.92
Sign. Level Pr > F:							
Rootstock	*<0.0001*	*<0.0001*	*0.0040*	*<0.0001*	*0.0004*	<0.0001	*0.2426*
LSD (5%)	7.44	1.22	11.65	0.62	0.52	1.09	10.38

[z] Means with the same letters are not significantly different ($P \leq 0.05$; ns = non significant)

Table 3. K/Ca ratio of fruit at harvest for sixth leaf 'Reinders Golden Delicious' apple trees during the 2004/05 and the 2005/06 season. Mean separation in columns using LSD (5%).

Rootstock	K/Ca ratio 2005	K/Ca ratio 2006
M793	29.38 bc	17.73 ns[z]
CG707	38.54 a	23.03
M9Cepiland	33.43 ab	22.11
CG222	32.10 b	22.99
M7A	24.92 c	20.12
CG239	38.88 a	20.03
Sign. Level Pr > F:		
Rootstock	< 0.0001	0.2061

[z] Means with the same letters are not significantly different (P ≤ 0.05; ns = non significant)

Table 4. Leaf mineral analyses results at harvest for fifth leaf 'Reinders Golden Delicious' apple trees during the 2004/05 season.

Rootstock	N	P	K	Ca	Mg	Na	Mn	Fe	Cu	Zn	B
	%					mg.kg^{-1}					
M793	2.89 ns[z]	0.167 ns	1.61 ns	1.74 ns	0.49 a	325.3 ns	271.3 ns	800.7 ns	8.00 ns	72.7 ns	36.3 ns
M7A	2.99	0.163	1.34	1.97	0.50 a	353.0	234.0	151.0	12.00	83.0	32.7
M9 Cepiland	2.86	0.160	1.32	2.14	0.50 a	317.7	316.3	532.7	12.67	114	32.7
CG 239	2.98	0.163	1.52	1.87	0.52 a	355.7	234.7	183.7	11.67	78.3	37.0
CG 707	2.78	0.180	1.58	2.34	0.54 a	357.3	281.3	181.3	8.30	87.0	38.7
CG 222	2.97	0.167	1.39	2.12	0.39 b	358.7	307.0	223.0	10.00	133	36.7
Sign.level Pr > F:											
Rootstock	*0.2332*	*0.1790*	*0.4884*	*0.2044*	*0.0374*	*0.4380*	*0.0913*	*0.5509*	*0.0725*	*0.0725*	*0.3427*
LSD (5%)	0.21	0.02	0.40	0.50	0.087	55.69	67.90	907.38	3.66	43.83	6.83

[z] Means with the same letters are not significantly different ($P \leq 0.05$; ns = non significant)

Standard norms set for leaf minerals for 'Golden Delicious' apple trees (Kotzé, 2001)

Standard	Apple	N	P	K	Ca	Mg	Na	Mn	Fe	Cu	Zn	B
Low	Norms	2.04	0.14	1.14	1.36	0.30	0	21	102	4	30	24
High		2.52	0.17	1.52	1.82	0.40	500	95	191	9	50	39

Table 5. Macro and micro element concentrations, fruit mass as well as Ca content of fruit at 40 dafb for sixth leaf 'Reinders Golden Delicious' apple trees during the 2005/06 season. Means were adjusted in columns for P, K, Ca concentrations and Ca content for fruit mass used as covariate (cov). Mean separation in columns for N and Mg concentrations of fruit and fruit mass using LSD (5%).

Rootstock	N	P	K	Ca	Mg	Ca content	Fruit mass
	mg.100g^{-1} fresh weight					g.fruit^{-1}	mg.fruit^{-1}
M793	194.75 a	21.91 a	158.33 c	11.60 c	11.25 ns[z]	2.13 c	20.06 a
CG707	178.33 b	22.30 a	174.60 bc	14.33 a	11.84	2.67 a	18.58 bc
M9Cepiland	186.11 ab	22.48 a	196.74 a	12.78 bc	11.50	2.38 bc	18.96 ab
CG222	177.56 b	19.39 b	173.03 bc	13.01 abc	11.60	2.41 abc	18.02 bc
M7A	195.00 a	21.01 ab	164.42 c	13.73 ab	11.62	2.54 ab	18.73 b
CG239	175.44 b	19.97 b	179.23 b	13.26 abc	11.73	2.47 ab	17.41 c
Sign. Level Pr > F :							
Fruit mass c	0.2066	0.0071	0.0299	0.0151	0.3342	0.0179	-
Rootstock without cov	0.0028	0.0002	<0.0001	0.0113	0.8137	0.1033	0.0033
Rootstock with cov	0.0090	0.0033	<0.0001	0.0246	0.8495	0.0184	-
LSD (5%)	12.00	-	-	-	0.85	-	1.24

[z] Means with the same letters are not significantly different ($P \leq 0.05$; ns = non significant)

Table 6. Macro and micro element concentrations, fruit mass as well as Ca content of fruit at harvest for sixth leaf 'Reinders Golden Delicious' apple trees during the 2005/06 season. LS means were only used for Ca as fruit mass was the covariate (cov). Mean separation in the rest of the columns using LSD (5%).

Rootstock	N	P	K	Ca	Mg	Ca content	Fruit mass
	mg.100g^{-1} fresh weight					g.fruit^{-1}	mg.fruit^{-1}
M793	40.67 b	7.88 abc	109.11 ab	5.49 a	5.84 a	7.97 ns[z]	158.08 ab
CG707	42.56 b	8.48 a	115.67 a	5.14 a	5.98 a	8.13	150.68 bc
M9Cepiland	43.56 b	7.22 c	107.00 ab	5.43 a	5.59 a	7.96	146.68 c
CG222	39.38 b	7.52 bc	95.50 c	5.33 a	4.86 b	8.52	154.55 abc
M7A	41.44 b	7.38 bc	105.22 abc	4.72 a	5.63 a	7.68	146.18 c
CG239	49.67 a	8.23 ab	104.44 bc	5.18 a	5.78 a	7.52	164.13 a
Sign. Level Pr > F:							
Fruit mass	0.3060	0.2451	0.1132	0.0372	0.5791	0.1318	-
Rootstock without cov	0.0010	0.0301	0.0450	0.1631	0.0016	0.6130	0.0102
Rootstock with cov	0.0014	0.0529	0.0239	0.4576	0.0018	0.4335	-
LSD (5%)	4.59	0.86	11.14	0.74	0.49	8.82	10.83

[z] Means with the same letters are not significantly different ($P \leq 0.05$; ns = non significant)

Table 7. Leaf mineral analyses results at harvest for sixth leaf 'Reinders Golden Delicious' apple trees for the 2005/06 season.

Rootstock	N	P	K	Ca	Mg	Na	Mn	Fe	Cu	Zn	B
	%					mg.kg^{-1}					
M793	2.83 ab	0.19 ns[z]	1.28 ns	2.33 b	0.43 ns	271.33 ns	302.33 ns	169.33 ns	13.67 ns	96.33 ns	39.33 ns
M7A	2.84 ab	0.15	1.07	2.28 b	0.49	277.00	267.00	153.33	12.67	95.00	37.00
M9 Cepiland	2.91 a	0.16	1.12	2.42 b	0.49	278.33	307.00	185.33	10.00	103.67	35.67
CG 239	2.87 ab	0.17	1.14	2.43 b	0.50	202.33	261.67	175.00	8.67	104.00	38.33
CG 707	2.62 c	0.16	1.16	2.92 a	0.53	271.33	293.67	138.33	8.00	98.00	36.67
CG 222	2.73 bc	0.15	1.11	2.55 b	0.42	304.67	295.33	153.00	11.00	109.67	35.33
Sign.level Pr > F:											
Rootstock	*0.0128*	*0.1930*	*0.5937*	*0.0293*	*0.2426*	*0.2459*	*0.1467*	*0.2265*	*0.1173*	*0.0684*	*0.7687*
LSD (5%)	0.146	0.035	0.2562	0.3637	0.1103	85.281	40.953	41.851	4.5663	10.311	6.8528

[z] Means with the same letters are not significantly different ($P \leq 0.05$; ns = non significant)

Standard norms set for leaf minerals for 'Golden Delicious' apple trees (Kotzé, 2001)

Standard	Apple	N	P	K	Ca	Mg	Na	Mn	Fe	Cu	Zn	B
Low	Norms	2.04	0.14	1.14	1.36	0.30	0	21	102	4	30	24
High		2.52	0.17	1.52	1.82	0.40	500	95	191	9	50	39

Table 8. Production of fifth and sixth leaf 'Reinders Golden Delicious' apple trees for both the 2004/05 and 2005/06 seasons (Costa & Stassen, 2006).

Rootstock	kg.tree^{-1} in 2005	kg.tree^{-1} in 2006
M9 – Cepiland	14.51 ns[z]	40.6 ns
CG222	18.02	52.4
M7A	18.23	47.2
CG707	20.47	64.4
M793	17.93	52.1
CG239	22.51	67.6
Significance level: Pr >F	0.0972	0.1826

[z] Means with the same letters are not significantly different ($P \leq 0.05$; ns = non significant)

CHAPTER 6

EVALUATING PRE-HARVEST FOLIAR CALCIUM APPLICATIONS FOR INCREASING FRUIT CALCIUM CONTENT AND REDUCING BITTER PIT INCIDENCE OF 'GOLDEN DELICIOUS' APPLES

Abstract

Applications of foliar calcium (Ca) products to increase fruit Ca content and to reduce the incidence of bitter pit in apples are used worldwide. In Experiment 1, we evaluated the effectiveness of four pre-harvest foliar Ca products on mature, bearing 'Golden Delicious' trees to increase fruit Ca content and reduce the incidence of bitter pit. CalNitro ($Ca(NO_3)_2$), Calcimax® (organic chelated complex), Terra CalAc (Ca acetate) and Ca fulvate were applied at the concentrations and time recommended by the suppliers. $Ca(NO_3)_2$ resulted in the highest Ca content of fruit at harvest, but not significantly higher than Ca acetate. In Experiment 2, $Ca(NO_3)_2$, Calcimax® and Ca acetate were applied, commencing at three (early, mid and late) different phenological stages of fruit growth. Late $Ca(NO_3)_2$ (80 days after full bloom (dafb)) applications increased the Ca content of fruit at harvest more than early (six dafb) and mid (40 dafb) applications. Although there was a trend towards better bitter pit control with early vs late applications, the incidence of bitter pit during the 2005/06 season was too low (less than seven percent) for significant differences between the treatments in both experiments.

Introduction

Foliar applications of nutrients to improve quality and decrease deficiencies are common in fruit production (Ernani et al., 2002; Faust, 1989). Neilsen & Neilsen (2003) define foliar nutrition as the direct supply of nutrients to trees via a spray application of dilute concentrations of minerals to foliage, buds and even bark. Pre-harvest foliar Ca applications to increase the Ca content of fruit and reduce bitter pit incidence, are widely used (Hewett &

Watkins, 1991; Yuri et al., 2002). According to Saure (2002), Ca absorption from foliar applications occurs directly via the fruit skin and little, if any Ca, is transported from the leaves to the fruit.

Foliar Ca applications are reported to be highly successful in some cases, but in a substantial number of cases, results showed little effect on Ca content of fruit or bitter pit control (Carbo et al., 1988; Hewett & Watkins, 1991; Le Grange et al., 1998a). Van Goor (1971) reported on the effect of frequent sprays with $Ca(NO_3)_2$ solutions on the incidence of bitter pit of 'Cox's Orange Pippen' apple. In most cases, bitter pit was reduced by Ca sprays and adequate commercial control of the disorder was obtained (reduction 74-94%). According to Terblanche et al. (1980), a series of three to five sprays with either $Ca(NO_3)_2$ or $CaCl_2$ can control bitter pit potential of about 16 percent in the orchard effectively. Rease & Drake (2000) applied foliar Ca at high rates either early (early June, late June and mid-July) or late (mid-July, early August, late August) in the season (Wenatchee, WA, Northern Hemisphere (NH)) and improved fruit quality of 'Red Delicious' and 'Golden Delicious' apples. Trees sprayed with $CaSO_4$ had healthy leaves and large fruits, but the Ca concentration in the fruit peel and cortex was equal to that of the control (no Ca sprays). The incidence of bitter pit was also very high, especially in the 'Golden Delicious' fruit.

Rease & Drake (2002) found that foliar sprays with $CaCl_2$, had the lowest bitter pit incidence and the highest Ca concentration in the fruit cortex when compared to non $CaCl_2$-based materials. In South-Africa, $Ca(NO_3)_2$ is preferred above $CaCl_2$, as it is less likely to cause leaf scorch, especially in sensitive cultivars such as 'Granny Smith' (Wooldridge et al., 1998).

The K/Ca and (K + Mg)/Ca ratios may play a role in the susceptibility of fruit to bitter pit (Tomala, 1997). Sharples (1980) found that a high K/Ca ratio was associated with increased

wastage due to rotting and bitter pit. Tomala (1997) stated that a K/Ca ratio above 22:1 is likely to be associated with commercial losses due to bitter pit.

Numerous spray rate application experiments have been confounded by application at different times (Le Grange et al., 1998b). A study by Casero et al. (2002) showed that early (starting at 10 dafb) foliar Ca applications did not increase Ca accumulation in 'Golden Delicious Smoothee' apples, while late (starting at 70 dafb) Ca sprays increased the Ca absorption rate and accumulation in fruit. Their reasoning was, that during the first period of fruit growth, Ca is provided mainly by root absorption, but in the latter part of the season, when fruit Ca absorption is limited, foliar applications are more effective and results in an increase in fruit Ca content. Rapid penetration into young fruits can take place through trichomes and stomata, but after 45 dafb fruit lose the trichomes and then penetration of Ca mainly occur via lenticels (Schlegel & Schönherr 2002). Lötze & Theron (2006) reported on the effectiveness of pre-harvest Ca application for bitter pit control in 'Golden Delicious apples' under South African conditions, indicated that foliar applications during the first 40-70 dafb effectively increased fruit Ca content early in the season and also reduced the incidence of bitter pit. Also under South African conditions, Calcimax® (organically complexed Ca carrier) controlled bitter pit more effectively than conventional treatments ($Ca(NO_3)_2$ or $CaCl_2$) if applied as a series of sprays, either from mid November (Southern Hemisphere) to harvest (4.56 $ml.litre^{-1}$), or from beginning of December until harvest (9.12 $ml.litre^{-1}$) (Wooldridge et al., 1994). That is in contrast with present recommendations to start Ca applications later in the season (after 70 dafb) for a high fruit Ca at harvest, and associated low incidence of bitter pit. However, the aim of Lötze & Theron (2006) was primarily to reduce bitter pit and not necessarily to increase the final fruit Ca content. Work by Neilsen et al. (2005) on 'Braeburn' apples agrees with their findings. Five weekly sprays of $CaCl_2$

commencing the first week of June (N.H.) were as effective as a similar treatment applied later (end of August to end of September (N.H.)) in the season, to reduce bitter pit incidence. This was despite a minimal impact on whole fruit Ca concentration at harvest (Neilsen et al., 2005).

$Ca(NO_3)_2$ is the standard Ca source for controlling bitter pit in commercial apple orchards in South Africa (Terblanche et al.1969; 1971). The aims of this study are first to evaluate the effectiveness of other Ca products with organic components as opposed to $Ca(NO_3)_2$ regarding the fruit Ca content and bitter pit control. Secondly, to determine the most effective phenological stage for Ca foliar applications to reduce the incidence of bitter pit by varying the time of application during the season.

Materials & Methods

The study was done in the 2005/06 season and consisted of two experiments. Trees were selected in a bitter pit prone orchard in the Vyeboom region (33º59´S; 19º18´E), in the Western Cape. The trial was conducted on mature, uniform, full bearing 'Golden Delicious' trees on rootstock M793, planted in 1986 at a planting distance of 4 m x 1.5 m.

Experiment 1:

Four different Ca formulations/products were applied at the concentration and time recommended by the suppliers (Table 1 & 2). Five treatments were applied in total, including the control with no Ca application (Table 1). In Experiment 1, two tree plots were used, with eight replicates for each of the five treatments. On both sides of each replicate, a buffer tree was included to prevent possible drift contamination from other treatments. The trial layout was a randomised complete block design. All the treatments were applied with a handgun until runoff.

For this experiment, fruit samples were collected only at harvest (five fruit per replication). For uniformity, fruit samples were collected from spurs on two-year-old shoots. The mineral analyses were performed by a commercial laboratory (Bemlab Pty Ltd, Strand). The whole fruit without the pips and core was analysed.

The financial implications of the different foliar Ca products applied in Experiment 1 were also compared (Table 3).

Experiment 2:

The trial lay out for Experiment 2 was also a randomised complete block design, but with 10 treatments and five replicates, including the control that received no foliar Ca applications. Each replicate consisted of two trees, with buffer trees on both sides. Three different Ca formulations/products were applied, commencing at three different phenological stages of fruit growth (Table 4 & 5). The first stage started at six dafb; the second stage started approximately at 40 dafb; and third stage, approximately at 80 dafb. Each treatment was applied weekly over a period of six weeks (Table 4). All treatments were applied with a handgun until runoff. Under local conditions, the growing season for 'Golden Delicious' fruit is approximately 140 days.

Fruit samples (five fruit per replicate) were collected for mineral analyses at six, 41 and 83 dafb, as well as at harvest. The procedure for sampling and mineral analysis of the fruit was the same as for Experiment 1.

Pre-optimum fruit samples (approximately 100 fruit per replicate with a weight of approximately 17 kg, depending on fruit size) were picked prior to commercial harvest to

increase the probability of bitter pit development. Fruit were stored for nine weeks at -0.5 ºC until external bitter pit symptoms were visible. Thereafter each apple was examined individually for signs of bitter pit and the percentage bitter pit for each treatment determined. This was done for both experiments.

Statistical analysis:

Analyses of variance of the data were performed using the general linear model (GLM) procedure in the Statistical Analysis System (SAS) programme (SAS Institute Inc, NC, CARY, 2004). In addition, single degree of freedom, orthogonal, polynomial contrasts were used for Experiment 2. Logit transformation was performed on the percentage bitter pit data for both experiments (Snedecor & Cockran, 1997).

To illustrate the absorption rates of Ca per product after each application the slopes for each treatment were determined for i) six – 41 dafb ii) 41 – 83 dafb and iii) 83 – 125 dafb. Slopes were calculated with the formula $s = [Ca]_2 - [Ca]_1 / Time_2 - Time_1$. The turning points (1-4) represent the Ca concentrations of each treatment at six, 41, 83 and 125 dafb.

Results and discussion

Experiment 1:

Only Ca and magnesium (Mg) concentrations of fruit differed significantly at harvest between the treatments (Table 6). All treatments, except Ca fulvate, had significantly higher fruit Ca concentration than the control. $Ca(NO_3)_2$ had a significantly higher fruit Ca concentration than all the other treatments. Ca acetate followed with the second highest Ca concentration. The Ca concentration of the treatments Calcimax® and Ca fulvate did not differ significantly from each other (Table 6).

Fruit from the Calcimax® and Ca acetate treatments had significantly higher Mg concentrations at harvest than fruit from treatments $Ca(NO_3)_2$ and Ca fulvate. The Mg of none of the Ca treatments differed significantly from that of the control treatment. It is possible that the Ca products with the organic formulations (CHO chelated complexes) contain Mg in their compositions and this could explain the significant differences between treatments in Mg, but the exact composition of these products is unknown. The concentrations of nitrogen (N), phosphate (P) and potassium (K) in the fruit were not significantly different at harvest between the treatments (Table 6). This was surprising as in the case of N it is an ingredient of the $Ca(NO_3)_2$ treatment.

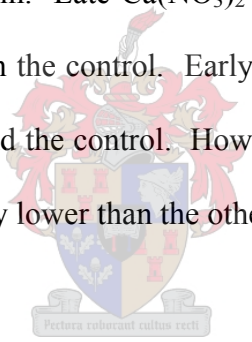
Fruit mass did not differ significantly between the treatments (Table 7). The K/Ca ratio for the control treatment was significantly higher than that of all other treatments, except for that of the Ca fulvate treated trees. $Ca(NO_3)_2$ and Ca acetate treated trees had K/Ca ratios less than 22:1 in their fruit, which is within the norm for bitter pit control according to Tomala (1997) (Table 7). The same trend was observed between treatments for the (K + Mg)/Ca ratio. The Ca content of fruit from the $Ca(NO_3)_2$ treatment was the highest, although not significantly higher than the Ca acetate treatment (Table 8). The fruit of the control treatment had the lowest Ca content, but that was not significantly lower than the Ca content of the Ca fulvate or Calcimax® treatments. $Ca(NO_3)_2$ had the lowest bitter pit percentage in their fruit, but did not differ significantly from the other treatments (Table 8).

Bitter pit percentage did not differ significantly between the treatments (Table 8). $Ca(NO_3)_2$ showed a tendency towards less actual bitter pit (1.84%) than the other treatments, especially the control (4.30%).

As mentioned, products were applied as recommended by their suppliers. Therefore, $Ca(NO_3)_2$ and Calcimax® were applied eight times and Ca acetate and Ca fulvate only three times during the season. When the total cost per treatment was calculated for Experiment 1 (Table 3), $Ca(NO_3)_2$ was the most affordable treatment, in spite of the high (eight) number of applications (R2.55/ L vs R15.71/ L). Calcimax®, also applied eight times, was the most expensive treatment.

Experiment 2:

Significant differences were found in Ca content of fruit at harvest between the different treatments ($Pr > F = 0.0252$) (Table 9). The Ca content of the control fruit was very high (5.48 mg) which is difficult to explain. Late $Ca(NO_3)_2$ had the highest fruit Ca content, but that was not significantly higher than the control. Early Ca acetate had a significantly lower Ca content than the late $Ca(NO_3)_2$ and the control. However, the Ca content of fruit from the early Ca acetate, was not significantly lower than the other treatments.



Contrasts were performed on the Ca content of fruit at harvest. No significant interaction between product and time was found ($Pr > F = 0.0653$). There were significant differences between early vs late application ($Pr > F = 0.0206$) and between the products $Ca(NO_3)_2$ and Ca acetate ($Pr > F = 0.0057$).

The logit bitter pit percentage did not differ significantly between treatments ($Pr > F = 0.2363$). The actual bitter pit incidence for this season (2005/06) was very low, with less than seven percent per treatment. The early $Ca(NO_3)_2$ treatment had the lowest bitter pit percentage (1.69%), although it was not the treatment with the highest Ca content of fruit at harvest (Table 9). The contrast for bitter pit percentage between $Ca(NO_3)_2$ and Ca acetate

was significant (Pr > F = 0.0393). In this experiment, there were also no significant differences in fruit mass at harvest between the treatments (Pr > F = 0.9301) (Data not shown).

These results show a tendency towards early and middle treatments being as effective or even better in bitter pit control than the late treatments, especially where $Ca(NO_3)_2$ and Calcimax® were used. Work by Lötze & Theron (2006) as well as Neilsen et al. (2005) agrees with this finding. In both cases, bitter pit incidence was reduced more with mid season than late foliar applications. Wooldridge et al. (1994) found that a series of Calcimax® applications, either from 17 November (SH) (at 4.56 $ml.litre^{-1}$), or from 6 December, to harvest at 21 February (at 9.12 $ml.litre^{-1}$), was more effective than the conventional treatments ($Ca(NO_3)_2$ and/or $CaCl_2$). In contrast with Wooldridge et al. (1994) the actual bitter pit percentage of the Calcimax® treatments was not lower than with the $Ca(NO_3)_2$ treatments.

The efficiency of the different products to increase the Ca concentration of fruit and reduce bitter pit incidence at different phenological stages may differ. The early treatments were mainly applied during the cell division phase (up to 40 dafb), when various researchers describe uptake and penetration of Ca into fruit to be rapid (Faust, 1989; Casero et al., 2002). The middle treatments were applied at the end of cell division and beginning of cell expansion phase (between 40 and 80 dafb). This is the period when uptake of Ca into fruit is normally low, because of strong sink competition for carbohydrates in the tree e.g. vigorously growing shoots. The late applications were applied at cell expansion and end of shoot growth phase (starting at 80 dafb). Fig. 1a-c illustrates the change in Ca concentration in fruit per treatment between two phenological stages. The slopes and turning points for the graphs are presented in Tables 10a-b. The turning points represent the Ca concentration of fruit at the sampling

date (dafb), before the treatments were performed. Peak Ca concentration was at 41 dafb for all treatments.

The Ca concentration of the fruitlets at six dafb was not significantly different between the $Ca(NO_3)_2$ treatments and control (Fig. 1a). Early $Ca(NO_3)_2$ had the highest turning point at 41 dafb, although it was not significantly higher than for the middle, late and control treatments. The first slope of early $Ca(NO_3)_2$ is steeper than that of the control (not significant). As expected, middle $Ca(NO_3)_2$ had the highest turning point at 83 and late $Ca(NO_3)_2$, at 125 dafb, confirming an increase in fruit Ca following the application of Ca. In the case of the middle treatment, the turning point was not significantly higher than for the rest of the treatments, but it was significantly higher for the late treatment. In both cases, the decline in the slope was the smallest during the time of application (not significant). Table 9 showed that fruit from early $Ca(NO_3)_2$ had the lowest bitter pit percentage although not significant, followed by middle $Ca(NO_3)_2$, and this was not associated with the highest Ca content at harvest. This confirms the results discussed earlier.

The slopes of the Calcimax® treatments did not follow the same trend as the $Ca(NO_3)_2$ treatments. Fig. 1b illustrates the small differences between the values of the Ca concentration of fruit of the Calcimax® treatments at each turning point (0.20, 1.06, 0.36, and 0.18, respectively). Although not significant, early Calcimax® had the lowest Ca concentration in fruit at six dafb, but the highest Ca concentration in fruit at 125 dafb, as well as the highest Ca content of all Calcimax® treatments at harvest. The early Calcimax® had the lowest bitter pit percentage of all the Calcimax® treatments (not significant).

Early Ca acetate treatment had a significantly lower Ca concentration in fruit at six dafb than the other Ca acetate treatments. Although not significant, the first slope for the early Ca acetate treatment was as expected steeper (0.42) between six and 41 dafb than the other Ca acetate treatments and the control. Early Ca acetate had a significantly lower Ca concentration in fruit at 125 dafb than the control. Middle Ca acetate had the least negative slope (-0.17) between 41 and 83 dafb and late Ca acetate the least negative slope between 83 dafb and 125 dafb (-0.07).

Late $Ca(NO_3)_2$ and late Ca acetate showed that foliar applications during this phase increased the final Ca concentration of fruit at harvest most successfully. Ca content of fruit at harvest from the late $Ca(NO_3)_2$ treatment was the highest of all treatments. These results confirmed that, in order to achieve fruit with maximum Ca concentration at harvest, Ca should be applied closer to harvest (Neilsen et al., 2005).

Ca concentrations of fruit from Experiments 1 & 2 were compared to the average industry Ca concentrations of commercial 'Golden Delicious' apple orchards in the Western Cape (2005/06) (W.A.G. Kotzé, 2006, personal communication, Bemlab (Somerset Wes)). The Ca concentration for these orchards varied between 3.5–4.5 $mg.100g^{-1}$ FW, similar to our data. In Experiment 1, the $Ca(NO_3)_2$ and the Ca acetate treatments had higher Ca concentrations (5.95 and 4.98 $mg.100g^{-1}$ FW) than that generally found for the region. In Experiment 2, the late $Ca(NO_3)_2$ application was the only treatment to show a higher Ca concentration than orchards in the region. Analysis of the industry Ca concentrations for 2005/06 indicated a low (1.4-2 $mg.100g^{-1}$ FW) and high threshold (6-8 $mg.100g^{-1}$ FW). Our results were within these boundaries.

Conclusions

Experiment 1 showed that, if the different Ca products are applied according to supplier specifications, the highest fruit Ca concentration at harvest was achieved with $Ca(NO_3)_2$, followed by Ca acetate. Eight applications of $Ca(NO_3)_2$ and Calcimax® applied between 40 dafb and 80 dafb were as effective in controlling bitter pit as three applications of Ca acetate and Ca fulvate, once a month, from six dafb and the control. In this trial, $Ca(NO_3)_2$ was the most affordable product to increase fruit Ca concentration.

In Experiment 2, differences between product and timing of Ca sprays were found if one ignores the fact that the control treatment where no Ca sprays were applied, had unexplainable high Ca content in fruit. Indications are that late applications (starting at 80 dafb) of $Ca(NO_3)_2$ and Ca acetate are more effective in increasing the Ca content of fruit at harvest than mid season and early season applications (40-80 dafb and shortly after full bloom respectively). These results agreed with the results of other researchers (Neilsen et al., 2005; Casero et al., 2002). That was not the case with Calcimax® applications. When products were considered, late $Ca(NO_3)_2$ (80dafb) gave the highest Ca content of fruit at harvest.

Finally, for effective control of bitter pit incidence, results seem to point towards early (shortly after full bloom) and middle (40 dafb to 80 dafb) applications with foliar Ca. However, results seem to point towards the stage between 41 and 83 dafb to be the most effective time to apply Ca acetate to reduce the incidence of bitter pit. Nevertheless, this did not co-inside with the highest Ca content of fruit at harvest.

No satisfactory conclusions on the effectiveness of the treatments in both experiments regarding bitter pit control could be drawn, due to the low incidence of bitter pit this season.

Even in the control treatments of both experiments bitter pit percentages were less than six percent.

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Table 1. Application schedule of four Ca products sprayed to 'Golden Delicious' trees during the 2005/06 season.

Treatment	Concentration (ml per 100 L water)	Days after full bloom												
		6	14	20	27	34	41	48	55	62	69	76	83	90
Control														
$Ca(NO_3)_2$	675						x	x	x	x	x	x	x	x
Calcimax®	450						x	x	x	x	x	x	x	x
Ca acetate	500	x				x				x				
Ca fulvate	500	x				x				x				

Table 2. Product information and volume of Ca applied per treatment for the 2005/06 season.

Treatment	Number of Sprays	Recommended concentration ml per 100 L water	Water Applied (L)	Volume product applied (L)	% Ca	% Other elements	Volume applied per tree (L)	Total Ca per tree for season
$Ca(NO_3)_2$	8	675	50	2.7	12	N = 15	1.5-2	1.50 g
Calcimax®	8	450	50	1.8	10	B = 0.65	1.5-2	1.25 g
Ca acetate	3	500	50	1.5	6	Fulvin acid = 4	1.5-2	0.31 g
Ca fulvate	3	500	50	1.5	6		1.5-2	0.29 g

Table 3. Costs involved in applying the different Ca products for the 2005/06 season.

Product	Number of sprays	Volume of product applied	Product cost per L	Cost per tree for one season
$Ca(NO_3)_2$	8	2.7 L	R 2.55	R6.90
Calcimax®	8	1.8 L	R15.71	R28.28
Ca acetate	3	1.5 L	R7.10	R10.65
Ca fulvate	3	1.5 L	R10.50	R15.75

Table 4. Ca products sprayed to 'Golden Delicious' trees on three stages of fruit development during 2005/06. Each treatment was applied weekly for six consecutive weeks.

Treatment	Time of application	Concentration (ml per 100 L water)	Days after full bloom															
			6	14	20	27	34	41	48	55	62	69	76	83	90	97	104	111	118
Control																			
$Ca(NO_3)_2$	Early	675	x	x	-*	x	x	x	x										
$Ca(NO_3)_2$	Middle	675						x	x	x	x	x	x						
$Ca(NO_3)_2$	Late	675												x	x	x	x	x	x
Calcimax®	Early	450	x	x	-*	x	x	x	x										
Calcimax®	Middle	450						x	x	x	x	x	x						
Calcimax®	Late	450												x	x	x	x	x	x
Ca acetate	Early	500	x	x	-*	x	x	x	x										
Ca acetate	Middle	500						x	x	x	x	x	x						
Ca acetate	Late	500												x	x	x	x	x	x

*Ca sprays were not applied due to unfavourable weather.
x Time of application

Table 5. Product information and total Ca applied to 'Golden Delicious' trees for the 2005/06 season.

Treatment	Number of Sprays	Recommended concentration ml per 100 L water	Volume water applied (L)	Volume product applied (L)	% Ca	% Other elements	L of treatment per tree per application	Total Ca per tree for season
$Ca(NO_3)_2$					12	N = 15		
Early	6	675	50	2.025			1-1.5	0.96g
Middle	6	675	50	2.025			1.5-2	1.26g
Late	6	675	50	2.025			2	1.44g
Calcimax®					10	B = 0.65		
Early	6	450	50	1.350			1-1.5	0.8g
Middle	6	450	50	1.350			1.5-2	1.05g
Late	6	450	50	1.350			2	1.2g
Ca acetate					6	Fulvin acid = 4		
Early	6	500	50	3.000			1-1.5	0.48g
Middle	6	500	50	3.000			1.5-2	0.63g
Later	6	500	50	3.000			2	0.72g

Table 6. Mineral nutrient concentrations at harvest for 'Golden Delicious' apples sprayed with four Ca products during the 2005/06 season.

Treatment	N	P	K	Ca	Mg
	mg.100g $^{-1}$fresh weight				
Control	39.00 ns	7.87 ns	96.25 ns	2.71 d	4.34 ab
$Ca(NO_3)_2$	37.75	7.27	89.25	5.98 a	4.09 b
Calcimax®	36.75	7.49	90.25	3.71 c	4.54 a
Ca fulvate	37.25	7.87	91.50	2.88 cd	4.13 b
Ca acetate	36.13	7.98	93.50	4.94 b	4.45 a
Sign.level Pr > F	0.6811	0.2277	0.3753	<0.0001	0.0049
LSD (5%)	4.15	0.71	7.69	0.96	0.26

Table 7. Fruit mass, K/Ca ratio and (K+Mg)/Ca ratio of 'Golden Delicious' apples sprayed with four Ca products during the 2005/06 season.

Treatment	Fruit mass (g)	K/Ca	(K+Mg)/Ca
Control	132.06 ns	36.07 a	37.69 a
$Ca(NO_3)_2$	134.04	16.54 c	17.29 c
Calcimax®	131.71	25.54 b	26.80 b
Ca fulvate	137.53	33.65 a	35.14 a
Ca acetate	137.88	19.33 bc	20.24 c
Sign.level Pr > F	0.3844	<0.0001	<0.0001
LSD (5%)	8.16	6.31	6.51

Table 8. Ca content and bitter pit ratios at harvest of 'Golden Delicious' fruit sprayed with four Ca products during the 2005/06 season. Mean separation in columns using LSD (5%).

Treatment	Mean Ca/fruit at harvest (mg)	*% Bitter pit	% Bitter pit
Control	3.56 b	-3.14	4.30
$Ca(NO_3)_2$	8.04 a	-3.92	1.84
Calcimax®	4.88 b	-3.91	2.64
Ca fulvate	3.95 b	-3.40	3.25
Ca acetate	6.80 a	-3.54	3.07
Sign.level Pr > F	<0.0001	0.1952	

*Logit transformation = LOG ((Bitter pit fruit + 0.5) / (Total no. of fruit – Bitter pit fruit + 0.5))

Table 9. Ca content and bitter pit ratios at harvest for 'Golden Delicious' apple trees sprayed with Ca products commencing at three different times during the 2005/06 season. Mean separation in columns using LSD (5%).

Treatment	Mean Ca/fruit at harvest (mg)	*% Bitter pit	% Bitter pit
Control	5.48 ab	-2.85 ns	5.68
$Ca(NO_3)_2$ Early	4.17 bc	-4.07	1.69
$Ca(NO_3)_2$ Middle	5.08 bc	-4.06	2.54
$Ca(NO_3)_2$ Late	6.81 a	-3.15	5.04
Calcimax® Early	4.96 bc	-3.73	2.34
Calcimax® Middle	4.77 bc	-3.06	5.40
Calcimax® Late	4.75 bc	-3.62	5.88
Ca acetate Early	3.82 c	-2.85	6.47
Ca acetate Middle	4.42 bc	-3.26	3.61
Ca acetate Late	4.80 bc	-3.09	6.92
Sign.level Pr > F:			
Treatments	0.0252	0.2363	
Contrasts:			
Early vs Late	0.0057	0.4188	
Middle vs Late	0.0982	0.6019	
Early vs Middle	0.2223	0.7722	
$Ca(NO_3)_2$ vs Ca acetate	0.0206	0.0393	
$Ca(NO_3)_2$ vs Calcimax®	0.1869	0.3765	
Calcimax® vs Ca acetate	0.2889	0.2216	

* Logit transformation = LOG ((Bitter pit fruit + 0.5) / (Total no. of fruit – Bitter pit fruit + 0.5))

Table 10a. Slopes for the data of Fig. 1a-c. Slopes represent the changes in Ca concentration for 1) 6 – 41 dafb 2) 41 – 83 dafb and 3) 83 – 125 dafb.

Treatment	Slope 1	Slope 2	Slope 3
Control	0.36 ns[z]	-0.23 ns	-0.09 ns
$Ca(NO_3)_2$ Early	0.48	-0.33	-0.12
$Ca(NO_3)_2$ Middle	0.37	-0.21	-0.13
$Ca(NO_3)_2$ Late	0.38	-0.24	-0.06
Calcimax® Early	0.38	-0.23	-0.09
Calcimax® Middle	0.37	-0.23	-0.10
Calcimax® Late	0.40	-0.27	-0.09
Ca acetate Early	0.42	-0.25	-0.11
Ca acetate Middle	0.28	-0.17	-0.10
Ca acetate Late	0.38	-0.28	-0.07
Sign Level. Pr>F	0.1772	0.2550	0.1421

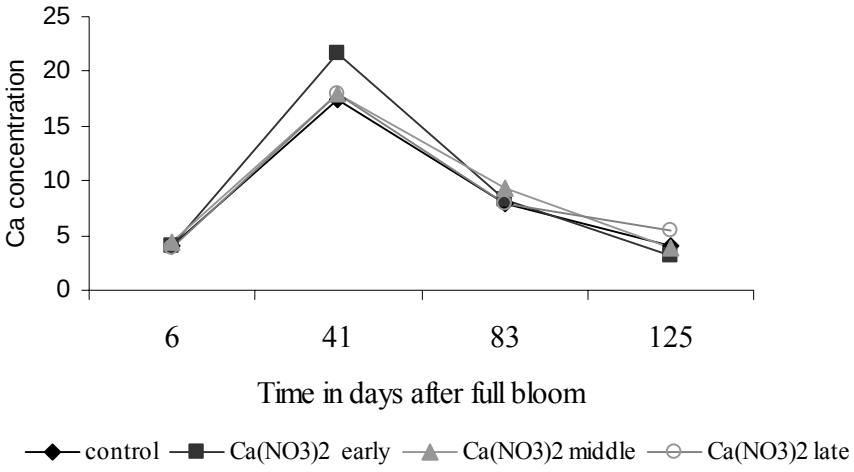
[z] Means with the same letters are not significantly different ($P \leq 0.05$; ns = non significant)

Table 10b. Turning points (TP) for the data of Fig. 1a-c. Turning point present the Ca concentration in fruit at 1) 6 dafb 2) 41 dafb 3) 83 dafb and 4) 125 dafb. Mean separation in columns using LSD (5%).

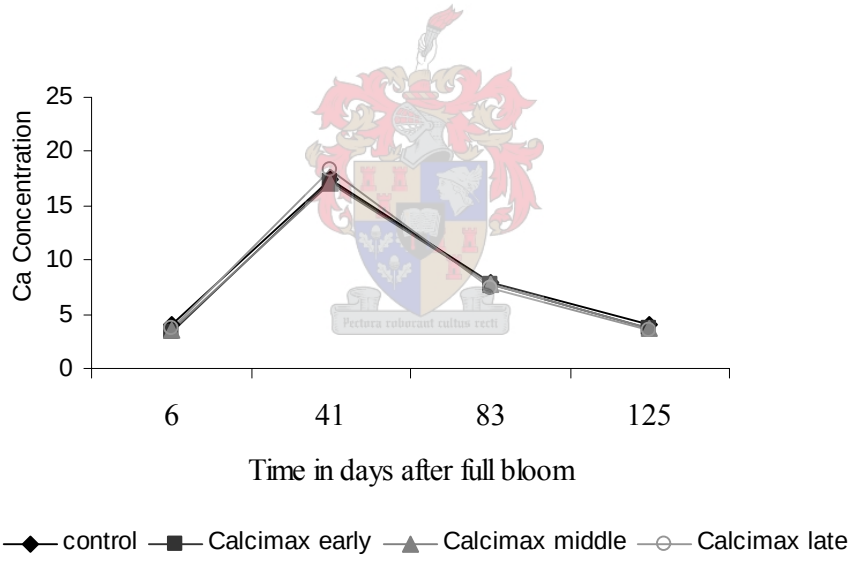
Treatment	TP1 - Ca concentration at 6 dafb	TP2 - Ca concentration at 41 dafb	TP3 - Ca concentration at 83 dafb	TP4 - Ca concentration at 125 dafb
Control	4.09 ab	17.44 ns[z]	7.90 ns	4.12 b
$Ca(NO_3)_2$ Early	3.99 ab	21.68	8.20	3.12 bc
$Ca(NO_3)_2$ Middle	4.40 a	17.96	9.36	3.84 bc
$Ca(NO_3)_2$ Late	3.94 ab	17.94	7.90	5.38 a
Calcimax® Early	3.42 b	17.32	7.74	3.76 bc
Calcimax® Middle	3.53 ab	17.10	7.42	3.58 bc
Calcimax® Late	3.62 ab	18.38	7.78	3.70 bc
Ca acetate Early	2.28 c	17.90	7.52	2.90 c
Ca acetate Middle	3.81 ab	14.30	7.22	3.44 bc
Ca acetate Late	4.28 ab	18.26	6.68	3.66 bc
Sign Level. Pr>F	0.0036	0.1693	0.2717	0.0110

[z] Means with the same letters are not significantly different ($P \leq 0.05$; ns = non significant)

a)


25
20
15
10
5
0
Ca concentration
6
41
83
125
Time in days after full bloom
control
Ca(NO3)2 early
Ca(NO3)2 middle
Ca(NO3)2 late

b)


25
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Ca Concentration
6
41
83
125
Time in days after full bloom
control
Calcimax early
Calcimax middle
Calcimax late

c)

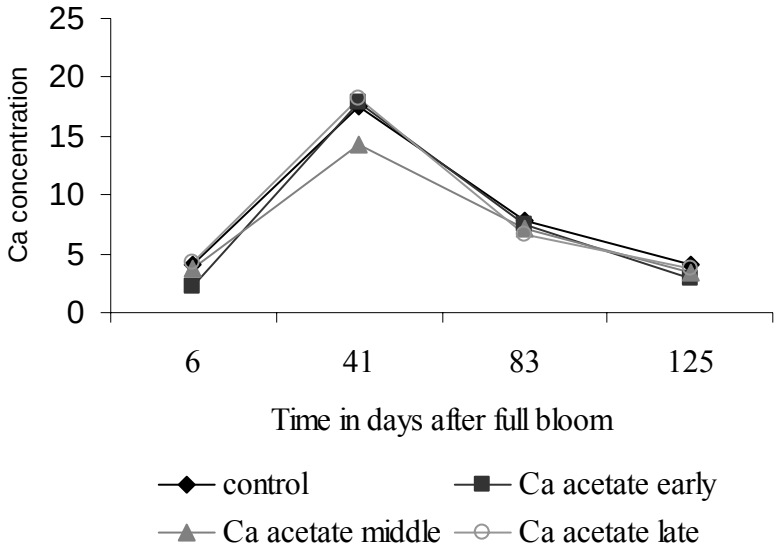


Figure 1. Ca concentrations of fruit in mg.100g^{-1} fresh weight of 'Golden Delicious' apple trees sprayed with a) $Ca(NO_3)_2$ b) Calcimax® and c) Ca acetate applications commencing at 6 (early), 41 (middle) and 83 (late) dafb against the Ca concentration of control fruit at the sampling dates.

CHAPTER 7

EVALUATING PRE-HARVEST ETHYLENE FORCING AND MAGNESIUM INFILTRATION TO PREDICT BITTER PIT INCIDENCE FOR 'GOLDEN DELICIOUS' APPLES

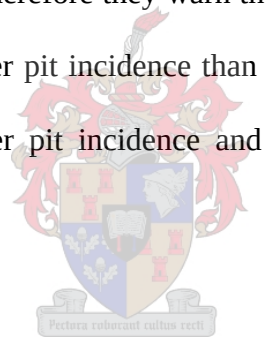
Abstract

An accurate pre-harvest prediction method for bitter pit incidence is becoming a necessity. The effectiveness of ethylene forcing and magnesium (Mg) infiltration as such methods were evaluated for 'Golden Delicious' apples. The Pearson correlation coefficient for Mg infiltration and actual bitter pit ($r = 0.45$) was higher compared to the coefficient found for ethylene treatment and actual bitter pit ($r = 0.13$). The R^2 value for the linear relationship between percentage bitter pit-like symptoms found with Mg infiltration and bitter pit-like symptoms found with ethylene treatment was 0.43. The low bitter pit incidence in the fruit of the past season (2005/06) could have influenced the accuracy of the results.

Introduction

The earlier in the season that the bitter pit potential of an orchard can be identified and quantified, the higher the possibility to reduce bitter pit incidence with pre-harvest treatments (Lötze & Theron, 2006). In South-Africa, some cultivars, e.g. 'Golden Delicious' and 'Braeburn', and orchards exist that are more susceptible than others to bitter pit. Therefore it is difficult to predict the potential for export of fruit after storage from such orchards. Although a number of physiological disorders in apples are associated with low calcium (Ca) levels in fruit tissue (Faust, 1989; Jackson, 2003), bitter pit is the most important disorder (Yuri et al., 2002; Jackson, 2003). Wills et al. (1976) found that fruit Ca concentration gave the best correlation with bitter pit from all individual minerals tested and fruit with less than

2 mg.100 g^{-1} fresh weight was always very susceptible to bitter pit. Various researchers (Martin et al., 1975; Sharples, 1980; Terblanche et al., 1980; Faust, 1989; Jackson, 2003) agreed that a negative relationship between the incidence of bitter pit and fruit Ca concentration exists. Under South African conditions Terblanche et al. (1980) recommended that the minimum Ca concentration for 'Golden Delicious' apples for bitter pit control is 5.4 and 6.6 mg.100g^{-1} fresh weight for unsprayed and Ca-sprayed fruit, respectively. Nevertheless, using mineral analysis of fruit, to predict bitter pit incidence has potential problems. Ferguson et al. (1979) warns that sample size and position of fruit within a tree complicate the accuracy of the results. Time of sampling, interpretation of results, as well as extrapolation of the results to orchard level, can potentially reduce the accuracy of this method (Lötze & Theron, 2006). Therefore they warn that although Ca concentration of fruit is more closely associated with bitter pit incidence than other elements, it should be applied with caution for prediction of bitter pit incidence and hence the need for more accurate prediction models exists.



To enhance the maturity and bitter pit-like symptoms of apples two types of ethylene treatment were introduced in South Africa. Ethylene forcing can be applied just before harvest to accelerate maturity and enhance the expression of bitter pit-like symptoms in apples (Eksteen et al., 1977). This treatment can be applied as i) the *Ginsburg method* where fruit is treated with one percent acetylene gas or ii) the *Bangerth method*, where fruit is immersed in a water solution containing 0.2 percent Ethephon (Eksteen et al., 1977; Lötze & Theron, 2006). These methods should be re-evaluated scientifically for various regions and different cultivars for consecutive seasons, due to a lack of local information regarding the accuracy of these methods under South African conditions.

A more recent destructive method for prediction of bitter pit pre-harvest, is the infiltration of apple fruit with magnesium (Mg) salts under vacuum. With this method, evidence of Ca deficiency (bitter pit-like symptoms) appears seven to 15 days after infiltration with a solution of $MgCl_2$, sorbitol and a surfactant (Burmeister & Dilley, 1993; Retamales et al., 2000, 2001; Retamales & Lepe, 2000; Retamales & Valdes, 2001). These bitter pit-like symptoms are inversely related to the Ca concentration of the fruit (Retamales et al., 2001).

The aims of this study were i) to evaluate the effectiveness and accuracy of ethylene forcing (Bangerth method) and Mg infiltration treatments pre-harvest to predict the incidence of bitter pit of 'Golden Delicious' apples by correlating it with the actual bitter pit incidence after storage, and ii) to compare the accuracy of these two methods with one another to determine the preferred prediction method for local commercial conditions.

Materials and methods

Plant material:

'Golden Delicious' apples were harvested from a bitter pit prone orchard in the Vyeboom area during the 2005/06 season. The experimental trees received six or less of the required minimum of eight foliar Ca applications for export fruit to reduce bitter pit.

Fruit sampling:

Fruit from two sources where foliar Ca products were evaluated for different purposes in the same block were sampled. Fruit samples from these trees were picked pre-optimum (approximately two weeks prior to optimum harvest) to enhance bitter pit incidence even further. These samples were further sub divided into a treatment sample (20-40 fruit) and a storage sample of about 100 fruit. The storage samples were stored at -0.5 °C in regular

atmosphere for nine weeks to develop bitter pit to determine the actual bitter pit potential of each replicate.

In Experiment 1, 50 sub samples of 20 of the 140 fruit were selected randomly for Mg infiltration. In Experiment 2, 50 sub samples of 40 fruit were selected, 20 for Mg infiltration and 20 for Ethylene forcing.

In Experiment 1, a 2000 ppm Ethephon solution was prepared using 42 ml Ethrel (48%) in 10 L distilled water. The 20 fruit per replicate were dipped in the Ethephon solution for one minute and thereafter placed in a pulp tray (Fig. 1a). Fruit were then left for seven days, at room temperature (approximately 25°C) and relative humidity at 52-63% in normal day light, to develop bitter pit–like symptoms.

In Experiment 2, a dessicator was used for the Mg infiltration. As the vacuum requirements for 'Golden Delicious' were not known, it was determined by first calculating the density of a 'Golden Delicious' apple (1.27 $g.cm^{-3}$) and comparing it to that for 'Granny Smith' (1.33 $g.cm^{-3}$) for which the vacuum was known (660 mm Hg), and then adjusting it accordingly for the 'Golden Delicious'.

The Mg solution of five litre contained 101.7 g $MgCl_2$ and 371.62 g sorbitol that was dissolved in deionised water. Once the pressure of 660 mm Hg was reached, the pump was switched off and the fruit kept under vacuum for two minutes. Thereafter the fruit were placed in pulp trays (Fig. 1a) and left under the same conditions as for the Experiment 1 to develop bitter pit–like symptoms, but for duration of seven and 10 days. The Mg solution

was used for five to seven repetitions before being refreshed. As the Mg concentration might have changed, the replicates were exposed to the solution, randomised.

After seven days, the ethylene forcing and Mg infiltrated fruit were investigated individually for any signs of superficial bitter pit. The number of fruit with bitter pit was counted for each treatment, regardless of the number of lesions per fruit. After 10 days fruit were evaluated again to determine if there was more fruit with signs of superficial bitter pit compared to the earlier evaluation at seven days (Fig. 1b, c). After nine weeks, the corresponding stored sub samples were removed from the cold store to determine the actual bitter pit.

Statistical analysis:

Data were analysed using the Statistical Analysis System (SAS) programme (SAS Institute Inc, NC, CARY, 2004). To determine if the ethylene forced and Mg infiltrated fruit respectively were linearly related to the actual bitter pit fruit after storage, data were analysed using the correlation procedure (PROC CORR) in SAS. A correlation was also performed to determine the extent to which these two methods are linearly related (Clewer & Scarisbrick, 2001). To determine the correlation between only Mg infiltration fruit and actual bitter pit after storage, data for Mg infiltration from both experiments were used to increase the data set, and analysed using the correlation procedure.

Results and discussion

The fruit that were ethylene forced had a yellower ground colour after seven days compared to the fruit that were infiltrated with the Mg solution (data not shown). Ethylene is known to enhance the ripening process of fruit and this acceleration of maturity also enhances the expression of bitter pit-like symptoms in apples (Eksteen et al., 1977). After 10 days at room

temperature, fruit from the ethylene treatment appeared over mature and the fruit from the Mg treatment still had a green ground colour and seemed firmer (observation). After seven days at room temperature, fruit from the Mg treatment showed more pronounced bitter pit-like symptoms than fruit from the ethylene treatment (Fig. 1b, c).

The Mg treatment showed a higher percentage (between 5 and 24%) of bitter pit-like symptoms than the actual bitter pit percentage (between 2 and 7%) observed in the stored replicates of source 1 (Table 1). The Mg treatment also showed a higher percentage (between 0 and 6) of bitter pit-like symptoms than the actual bitter pit percentage (between 0 and 1%) observed in the stored replicates from source 2 as well as the bitter pit-like symptoms of the ethylene treatment (mostly 0%, but in one case 6% was found) (Table 2).

The Pearson correlation coefficient for the Mg treatment and actual bitter pit ($r = 0.45$) was higher compared with the correlation coefficient found for ethylene treatment and actual bitter pit ($r = 0.13$) (Table 3). The value found for the Mg treatment and actual bitter pit was highly significant ($p = <0.0001$), but the value found for ethylene treatment and actual bitter pit was not significant ($p = 0.3519$). The R^2 value for the linear relation between percentage of bitter pit-like symptoms found with Mg treatment and percentage of bitter pit-like symptoms found with ethylene treatment was 0.43. This correlation was also significant ($p = 0.0020$).

The results showed that the percentage of bitter pit-like symptoms with Mg treatment related better linearly with the actual bitter pit percentage than was the case with the ethylene treatment. The R^2 value for our results (0.45) was lower than that of Retamales & Lepe (2000) for the incidence of Mg-induced pits and actual bitter pit after storage for 'Northern Spy' ($R^2 = 0.73$), and 40 days before harvest, for 'Braeburn' ($R^2 = 0.93$). The linear relation found between percentage of bitter pit-like symptoms with ethylene treatment and actual

bitter pit percentage after storage showed that only 13% of the actual bitter pit found could potentially be predicted correctly with ethylene treatment, because of the significance of the value.

Different factors may have influenced the lack of acceptable correlations in these experiments. Firstly, the incidence of bitter pit for the past season (2005/06) was extremely low (less than 7%) and the low values might have influenced the accuracy of the final results. Secondly, the experiment was only done for one season. More appropriate results might be found over a few seasons as bitter pit percentage may vary over seasons. Extra fruit per replication as well as more replicates can increase the data set for a more reliable correlation.

Conclusions

Although the number of bitter pit-like symptoms found with ethylene forcing was very low, the method did enhance the ripening process to such an extent that bitter pit-like symptoms developed in some fruit. From these results, 45% of the actual bitter pit found could be correctly predicted with Mg infiltration of fruit, which was better than the potential 13% correct prediction found with ethylene treatment. Based on these results it follows that Mg infiltration is a more acceptable method to predict actual bitter pit after storage than ethylene forcing.

The low bitter pit incidence in the fruit of this past season (2005/06) could have influenced the accuracy of the results. To increase the correlation coefficients, it is recommended to increase the number of fruit per replicate and the number of replicates. That should lead to a larger data set, which could result in better correlations. It could also be that it does not work for ‘Golden Delicious’.

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Table 1. Percentage ‘Golden Delicious’ fruit with Mg induced bitter pit-like symptoms and percentage actual bitter pit after nine weeks storage (source 1).

Treatments	Actual bitter pit %	Bitter pit % with Mg infiltration
Replication 1	5.68	14
Replication 2	1.69	14
Replication 3	2.54	8
Replication 4	5.04	10
Replication 5	2.34	5
Replication 6	5.40	16
Replication 7	5.88	16
Replication 8	6.47	24
Replication 9	3.61	19
Replication 10	6.92	7

Table 2. Percentage ‘Golden Delicious’ fruit with Mg- or ethylene induced bitter pit-like symptoms and percentage bitter pit after about nine weeks storage (source 2).

Replications *	Actual bitter pit %	Bitter pit % (Mg)	Bitter pit % (Eth)
Replication 1	0.99	6	6
Replication 2	0.51	3	2
Replication 3	0.47	0	0
Replication 4	0.93	2	1
Replication 5	0.65	2	0
Replication 6	0.79	3	1
Replication 7	0.93	3	0
Replication 8	0.00	1	0
Replication 9	0.36	4	0
Replication 10	0.00	5	0

Table 3. The correlation coefficient (r) calculated for the linear relation between Mg infiltration and actual bitter pit, ethylene forcing and actual bitter pit and the two methods with each other.

Treatments correlated	r-value	p-value
Mg infiltration and actual bitter pit	0.45	<0.0001
Ethylene forcing and actual bitter pit	0.13	0.3519
Mg infiltration and Ethylene forcing	0.43	0.0020

a)

b)

c)

Figure 1. ‘Golden Delicious’ fruit a) immediately after the Mg or ethylene treatments, b) with bitter pit-like symptoms at seven days after the Mg treatment and c) with bitter pit-like symptoms at seven days after the ethylene treatment.

CHAPTER 8

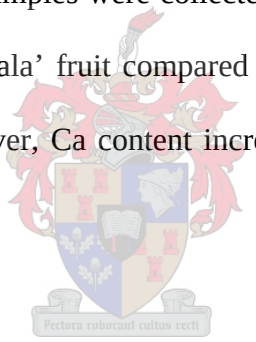
OVERALL DISCUSSION AND CONCLUSIONS

The first two trials of this study focused on increasing the Ca concentration/content in 'Brookfield Gala' apple fruit by subjection of the trees to different water and nutrient application strategies. Mineral analyses of fruit, leaves and soil as well as yield of second and third leaf trees on two rootstocks (M793 and M7) on a loamy sand soil were evaluated in these trials. Trees of both trials received a nutrient solution high in N during the 2004/05 season to stimulate growth in the young trees to fill the allocated space. According to Korcak (1980) the supply of high N levels can have a negative effect on the uptake of Ca. Therefore it was expected that fruit Ca concentration during the first season would be lower. Furthermore, for both trials, variation in mineral element concentrations was expected during the first season of fruit sampling, due to a low yield (average of 7 fruit.tree^{-1}) and different bearing positions. Ferguson et al. (1993) agrees that the position of fruit on the tree is one of the major sources of variation in mineral contents and storage potential in fruit.

There were no significant differences found between Ca concentration/content in fruit at harvest for the three treatments (micro jets, fertigation drip and pulsating drip) during the 2005/06 season or the rootstocks (M793 and M7). In the second trial 'Brookfield Gala' apple trees on the same rootstocks were subjected to three Ca application levels (high, standard and low) during three phenological periods to increase the Ca intake into apple fruit. High-high treatment had a significantly higher Ca concentration in fruit at harvest (2005/06) compared with the low-low and the std-std treatments, but not significantly higher than some of the combination treatments. Ca concentrations in both these trials were high. It is speculated that orchard and soil conditions were well managed and close to ideal and it could therefore be

possible that optimum levels of Ca were reached in fruit and resulted in treatments that did not differ as significantly from each other as expected.

Results of Chapter 4 and Chapter 6 showed that during fruit growth, Ca concentration decreased as cell expansion took place due to Ca dilution. According to Ferguson and Watkins (1989) the reason for the decrease in Ca concentration is that fruit expand at a greater rate than mineral input, resulting in dilution of the mineral. The patterns of Ca concentration in Chapter 4 are in contrast to the trends of Ca concentration for i) avocado fruit when peak Ca concentrations were found at six weeks after fruit set (Witney et al., 1986), and ii) for the control and Ca-sprayed 'Golden Delicious' fruit in Chapter 6 when Ca concentrations peaked at 40 dafb. Unfortunately no fruit samples were collected at 20 dafb in the latter study. The shorter growing season of 'Royal Gala' fruit compared with 'Golden Delicious' fruit might explain the trends observed. However, Ca content increased as the fruit size increased and continued until harvest.



In the third trial the effect of rootstocks on the intake of Ca into apple fruit was evaluated. It seems that rootstocks may affect the mean fruit mass of trees. Slowinska et al. (2004) found that mean fruit mass was affected by rootstock and they observed a trend towards larger fruit on more vigorous rootstocks and smaller fruit were noted for very dwarfing rootstocks. From our results a similar trend was observed. In Chapter 5 mean fruit mass was a significant covariate for Ca concentration of fruit of trees on six rootstocks (different vigour classes). That was also the case for the 2005/06 fruit at harvest in Chapter 4 between trees on M793 and M7. It seems that a tree on a more dwarfing rootstock, for example M7, will be less vigorous and smaller than a tree on a vigorous rootstock such as M793. If a heavy crop load is experienced on a dwarfing rootstock, then fruit have to be smaller on the dwarfing

rootstock than on a more vigorous rootstock (as in Chapter 4). Higher Ca concentrations are found in smaller fruit due to a smaller dilution effect than found in large fruit.

From the literature it is known that K reduces Ca uptake by ion antagonism (Failla et al., (1990) and that the balance between these two elements is important in the susceptibility of fruit to bitter pit (Ferguson & Watkins, 1983). In the second season (2004/05) for fruit of Chapter 3 and 4 and for the 2004/05 season for fruit of Chapter 5 the K percentages in leaves were higher than the norm set by Kotzé (2001) for the K percentages in a nutrient solution. That might have contributed to the lower Ca concentration in fruits during those seasons.

Pre-harvest foliar Ca applications to increase fruit Ca content and reduce bitter pit incidence of 'Golden Delicious' apples were evaluated during the 2005/06 season. Eight applications of $Ca(NO_3)_2$ and Calcimax® applied between 40 dafb and 80 dafb were as effective in control of bitter pit as three applications of Ca acetate and Ca fulvate, once a month, from six dafb. $Ca(NO_3)_2$ was also the most affordable product to increase fruit Ca concentration and control bitter pit effectively. Furthermore, indications are that late applications (starting at 80 dafb) of $Ca(NO_3)_2$ and Ca acetate are more effective in increasing the Ca content of fruit at harvest than mid season applications (40-80 dafb). These results agreed with the results of Neilsen & Neilsen (2005). No satisfactory conclusions on the effectiveness of the treatments regarding bitter pit control could be drawn, due to low incidence of bitter pit during the 2005/06 season. However, results seem to point towards early (shortly after bloom) and middle (40 dafb to 80 dafb) applications with foliar Ca.

Pre-harvest ethylene forcing and Mg infiltration to predict bitter pit incidence for 'Golden Delicious' apples were also evaluated. The Mg infiltration method could correctly predict

45% of the actual bitter pit found, which was better than the potential 13% correct prediction found with ethylene treatment, because of the significance of these values. That was lower than that of Ratamales & Lepe (2000) for the incidence of Mg-induced pits and actual bitter pit after storage for 'Northern Spy' (R^2 = 0.73), and 40 days before harvest, for 'Braeburn' (R^2 = 0.93).

From this study the following concluding remarks regarding Ca uptake into apple fruit could be drawn. It is important in the first place that soil conditions are optimum for active root development, because it assures Ca uptake by roots (Terblanche et al., 1980). That includes all soil physical and chemical conditions (C.E.C, pH ect.), soil aeration and drainage. Secondly if management practices (irrigation, fertilization, pruning, tree training, thinning) are ideal, natural Ca uptake into fruit will be adequate. However, a few suggestions and recommendations can be made from this study. Experiments with Ca application strategies, timing and amounts should rather be done in sand culture with no preplant soil applications. Nevertheless, in any apple orchard preplant Ca rectification is an essential cultivation practise. Under the conditions of Experiment 1 and 2 it seems as if any additional Ca application even with a pulsating system where Ca is in solution around the roots, more Ca in the solution did not result in higher Ca accumulation in the fruit than a certain adequate threshold level.

Differences in Ca concentration in fruit of 'Brookfield Gala' (6.96-8.85 mg.100g^{-1} FW) and 'Reinders Golden Delicious' (4.72-5.49 mg.100g^{-1} FW) were found. However, the Ca content of both cultivars was similar. The results for the 2005/06 season of the first three trials predicted that well balanced nutrient solutions were applied to the trees for the yields of those trees. Faust (1980) stated that in such cases it is likely that the fruit received sufficient Ca and therefore high Ca concentrations are as reported in the first two trials especially.

Another recommendation is that the method of Ca analysis also needs some refinement/clarity. It is suggested that there maybe a difference in the outcome between Ca concentration in apple pulp and Ca concentration in the supernatant of apple juice after centrifuging. The first may include physiological inactive crystals whilst the latter may only be Ca in solution and therefore the actual Ca that we want to determine.

Finally, if the management of orchard conditions are not ideal, foliar applications of Ca, especially $Ca(NO_3)_2$, close to harvest (from 80 dafb) will still effectively increase Ca content in fruit at harvest to maintain fruit quality, but not necessarily reduce bitter pit incidence. Under South African conditions Terblanche et al. (1980) recommended that the minimum Ca concentration for 'Golden Delicious' apples for bitter pit control is 5.4 and 6.6 $mg.100g^{-1}$ fresh weight (FW) for unsprayed and Ca-sprayed fruit, respectively. From this study results points towards high Ca concentrations in fruit during early and mid season to be more important in the development of bitter pit and the development of the disorder are not necessarily influenced by the Ca levels just before harvest.

APPENDIX

Figure 1. Water and nutrient mixing tanks placed on a hill, 35 m above the 'Brookfield Gala' apple orchard, from where the water and nutrient solution was delivered gravitationally at 2.2 bar to the orchard.

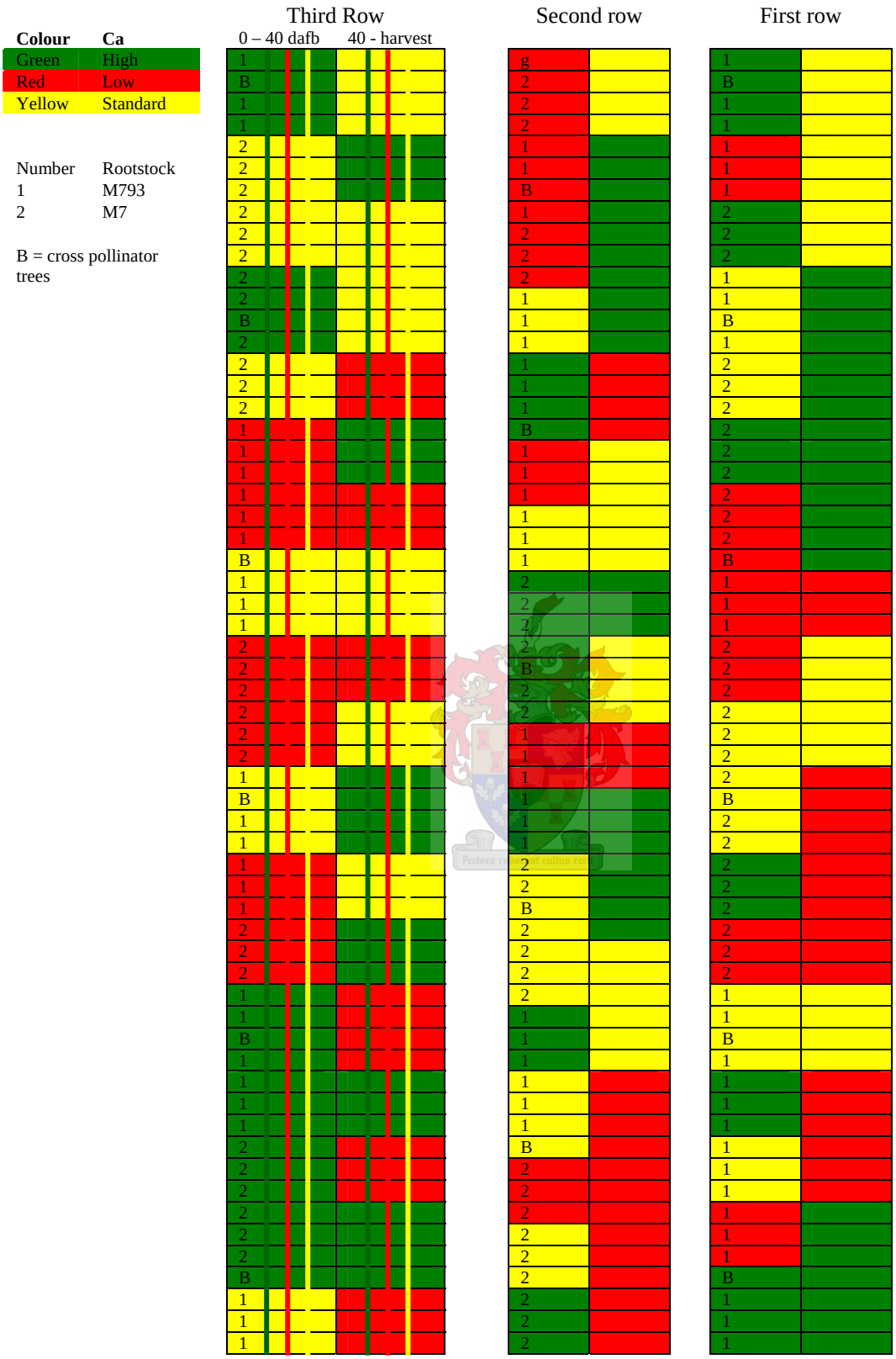


Figure 2. The randomized complete block lay-out of the experiment consisting of three Ca application levels applied to 'Brookfield Gala' apple trees planted on two rootstocks. Whenever water and nutrients were applied, drippers were open at the replicates of three trees where a certain Ca application level was applied, but the drippers were blank at the replicates where that Ca application level was not applied.

Pectora roborant cultus recti