Ca-metalosate as an alternative Ca formulation for decreasing Ca related disorders in fruit trees

By

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SUMMARY

Ca-metalosate as an alternative Ca formulation for decreasing Ca related disorders in fruit trees

Generally, calcium (Ca) foliar applications are used to improve the Ca status of fruit and control the incidence of Ca-related disorders, viz. bitter pit (BP) in apples and albedo breakdown (AB) in citrus. The main aim of the study was to determine whether Ca-metalosate as an alternative Ca formulation is effective in decreasing Ca-related disorders in fruit trees.

Firstly, the role of formulation of Ca and boron (B) foliar applications to improve fruit quality in ‘Golden Delicious’ apples was quantified. Secondly, evaluation of pre-harvest foliar applications of a chelated Ca and B combination to reduce AB in citrus was carried out. Thirdly, xylem functionality in developing fruitlets of different apple cultivars was determined, as it impacts on Ca transport into the fruit.

Ca concentration of fruit 80 days after full bloom (dafb), was significantly increased by Ca nitrate [$Ca(NO_3)_2$] foliar applications compared to Ca-metalosate and the control. The incidence of BP was also significantly reduced by $Ca(NO_2)_3$ foliar applications compared to the control, but not compared to Ca-metalosate. Results indicated that Ca foliar applications with a nitrate carrier, higher Ca concentration (active), lower point of deliquescence and molecular weight/size are more effective at increasing Ca concentration of fruitlets and reduce BP incidence in ‘Golden Delicious’ apples. This confirms previous findings that formulation has an effect on the efficiency and penetration of Ca foliar applications.

B-metalosate in combination with Ca-metalosate failed to significantly reduce the incidence of AB in both sweet orange cultivars (Turkey and Cara Cara). Further research under South-African conditions, with an amended protocol, including five or more Ca-metalosate foliar applications, starting from 81 dafb, is suggested to determine if metalosates can successfully reduce the incidence of AB. This protocol differs from the one used in this study, but was successful when applied as salt formulation foliar

application. Ca-metalosate and control indicated lower Ca (%) in the albedo tissue of creased fruit compared to non-creased fruit, indicating that Ca plays a role in AB.

At ±49 dafb, a steady decline in xylem functionality was observed in all six apple cultivars evaluated, supporting recommendations that additional Ca foliar applications should start before 40 dafb to decrease the incidence of Ca-related disorders in fruit trees. Less susceptible apple cultivars showed an earlier decline of xylem functionality (42 dafb) compared to susceptible apple cultivars (49 dafb). This is in contrast to previous findings. A relationship between Ca-related disorders and loss of xylem functionality early in the season could not be established in this trial. A slight recovery of xylem functionality in all six apple cultivars evaluated was observed later in the season, under both climatic areas and this has not been reported previously in apples.

Further research on xylem functionality under South African conditions should continue. Studies should commence earlier, starting at 28 dafb, and continue until harvest, to determine whether apple cultivars experiencing an earlier loss of xylem functionality are more prone to Ca-related disorders and whether xylem functionality slightly increases later in the season. By including microscopy studies during this period, the physical disruption of xylem bundles should be confirmed.

OPSOMMING

Kalsiummetalosaat as 'n alternatiewe kalsium formulasie vir die vermindering van kalsium verwante defekte in vrugtebome

Oor die algemeen, word kalsium (Ca) blaartoedienings gebruik om die Ca status van vrugte te verhoog en die voorkoms van Ca verwante defekte te beheer, nl. bitterpit (BP) in appels en kraakskil in sitrus. Die hoofdoel van hierdie studie was om te bepaal of kalsiummetalosaat, as 'n alternatiewe Ca formulasie, effektief is om kalsium verwante defekte in vrugtebome te verminder.

Eerstens is die rol van formulasie in Ca en boor (B) blaartoedienings gekwantifiseer om die vrugkwaliteit in 'Golden Delicious' appels te verbeter. Tweedens is gekombineerde Ca- en B-metalosaat geëvalueer as voor-oes blaartoedienings om kraakskil in sitrus te verminder. Derdens is xileemfunksionaliteit in ontwikkelende vrugte van verskillende appelkultivars bepaal.

Die Ca konsentrasie van vrugte op 80 dae na vol blom (dnvb) is betekenisvol verhoog deur kalsiumnitraat blaartoedienings teenoor kalsiummetalosaat en kontrole. Die voorkoms van bitterpit is ook betekenisvol verminder deur kalsiumnitraat blaartoedienings in vergelyking met die kontrole, maar nie in vergelyking met Ca-metalosaat nie. Resultate dui aan dat Ca blaartoedienings met 'n nitraatdraer, hoër Ca konsentrasie (aktief), laer POD (punt van deliquescence) en molekulêre gewig/grootte meer effektief is om die Ca konsentrasie van vrugte te verhoog en BP voorkoms in 'Golden Delicious' appels te verminder as Ca-metalosaat. Dit bevestig vorige bevindings dat formulasie 'n uitwerking het op die doeltreffendheid en penetrasie van Ca blaartoedienings.

Ca-metalosaat in kombinasie met B-metaalosaat het nie daarin geslaag om die voorkoms van kraakskil in beide kultivars (‘Turkey’ en ‘Cara Cara’) betekenisvol te verminder nie. Verdere navorsing onder Suid-Afrikaanse toestande met 'n alternatiewe protokol, vyf of meer Ca-metalosaat blaartoedienings vanaf 81 dnvb, kan oorweeg word om te bepaal of metalosate suksesvol sal wees in die vermindering van die voorkoms van kraakskil.

Hierdie protokol was suksesvol met die gebruik van soutformulasie blaartoedienings (Treeby and Storey, 2002; Storey et al., 2002; Pham et al., 2012). Ca-metalosaat en kontrole het 'n laer Ca (%) in die albedo weefsel van vrugte met kraakskil aangedui, wat bevestig dat Ca 'n rol in die voorkoms van kraakskil speel (Treeby and Storey, 2002; Storey et al., 2002).

Vanaf ±49 dnvb is 'n bestendige afname in xileemfunksionaliteit waargeneem in al ses appelkultivars wat geëvalueer is. Dit ondersteun vorige bevindings dat addisionele Ca blaartoedienings voor 40 dnvb moet begin om die voorkoms van Ca verwante defekte in vrugtebome te verminder (Lötze and Theron, 2006). Minder vatbare appelkultivars het vroeër 'n afname in xileemfunksionaliteit (42 dnvb) getoon in vergelyking met vatbare appelkultivars (49 dnvb). Dit is in teenstelling met vorige bevindings (Dražeta et al., 2004; Miqueloto et al., 2014). 'n Verhouding tussen Ca verwante defekte en verlies van xileemfunksionaliteit, vroeg in die seisoen, kon dus nie vasgestel word nie. Evaluasie het getoon dat xileemfunksionaliteit in al ses appelkultivars later in die seisoen herstel onder verskillende klimaatstoestande. Dit is nie voorheen in appels gerapporteer nie.

Verdere navorsing onder Suid-Afrikaanse toestande rakende xileemfunksionaliteit word aanbeveel. Studies moet vroeër begin, vanaf 28 dnvb tot oes, om vas te stel of appelkultivars wat vroeër 'n verlies van xileemfunksionaliteit ervaar, meer vatbaar is vir Ca verwante defekte en of die funksionaliteit van die xileem effens later in die seisoen weer toeneem. Xileem vaatbondel disintegrasie moet bevestig word deur mikroskopie studies gedurende die seisoen.

NOTE

This thesis is a compilation of chapters, starting with a literature review, followed by three research papers. Repetition or duplication between papers might therefore be necessary.

TABLE OF CONTENTS

GENERAL INTRODUCTION

The penetration and efficiency of foliar applications to reduce the incidence of calcium (Ca) related disorders in fruit trees is influenced by unique physio-chemical properties of mineral compounds (active elements) present in the formulation of foliar applications (Fernández et al., 2013). Physio-chemical properties of foliar applications include pH, point of deliquescence (POD), molecular size/weight, concentration (active) and solubility. Efficiency and penetration of foliar applications can be increased by the addition of specific additives. Chloride, nitrate, propionate and acetate are common macronutrient carriers used for Ca in salt formulation foliar applications. Salts can chelate or bind with compounds, for example Ca and/or be mixed with adjuvants in foliar applications (Val and Fernández, 2011).

Recently, chelates and complexes (amino acids, polyols, glucoheptonates, EDTA, ligonosulphonates, humic acids, fulvic acids etc.) are receiving more attention as alternative foliar applications compared to salt formulation foliar applications, for example Ca nitrate [$Ca(NO_3)_2$] and Ca chloride ($CaCl_2$) (Abadía et al., 2002; Fernández et al., 2013).

The main aim of the study was to determine whether Ca-metalosate as an alternative Ca formulation to Ca salts, is effective in decreasing Ca-related disorders in fruit trees. In Paper 1, the aim was to determine whether a chelated foliar formulation, viz. Ca-metalosate, is as efficient as the salt formulation $Ca(NO_3)_2$ in reducing bitter pit (BP) in 'Golden Delicious' apples. During 2016/17, two commercial orchards were selected for the trials in Elgin, South Africa. Fruit Ca concentration ($mg \cdot 100\ g^{-1}$) 80 days after full bloom (dafb), firmness, diameter, starch break down (%), mass (g), background colour and bitter pit (%) were determined. According to literature, concentration (Schlegel and Schönherr, 2002), solubility (Mengel, 2002), POD (Schönherr, 2002; Lötze and Turketti, 2014), pH (Blanpied, 1979) and molecular weight/size (Thalheimer and Paoli, 2002) have an effect on the penetration and efficiency of a foliar applications.

In Paper 2, the aim was to determine whether the chelated foliar formulation, viz. Ca-metalosate in combination with chelated boron (B), is effective in reducing albedo breakdown (AB) in ‘Cara Cara’ and/or ‘Turkey’ oranges. During 2016/17, two commercial orchards in Porterville (trial 1) and one commercial orchard in Simondium (trial 2), South Africa, were selected as trial sites. AB (%) for both trials and albedo mineral analysis (%) at harvest for ‘Cara Cara’ (trial 2) was determined. According to previous reports, the incidence of AB has been successfully decreased by $Ca(NO_3)_2$ and $CaCl_2$ foliar applications, indicating a definite role of Ca in AB (Treeby and Storey, 2002; Storey et al., 2002; Pham et al., 2012).

Paper 3 set out to determine whether a relationship exists between the loss of xylem functionality early in the season and Ca-related disorders in susceptible (Golden Delicious and Braeburn) and less susceptible cultivars (Fuji, Cripps Pink and Granny Smith). If a relationship exists, results can be applied to indicate the most effective time to start additional Ca foliar applications in susceptible cultivars to reduce the incidence of Ca-related disorders, such as BP. Similarly, this can aid towards breeding of new cultivars with early detection of BP susceptibility.

Fruit were sampled over two seasons from trees in commercial orchards on Applethwaite Farm in Elgin, Welgevallen Research Farm in Stellenbosch and/or Lourensford Estate in Somerset West. Xylem functionality was determined using a simple dye fuchsin (1%) technique. Weekly samples of ten fruit per cultivar were harvested at each location until a steady decline in the number of stained vascular bundles was noticed. According to literature, certain apple cultivars are less susceptible to Ca-related disorders due to a later loss of xylem functionality, leading to higher Ca concentrations at harvest (Dražeta et al., 2004; Miqueloto et al., 2014).

The results from these studies should broaden the understanding of the role of formulation in foliar applications and the impact it has on the penetration and efficiency of foliar applications. Secondly, results may broaden the understanding of xylem functionality development in developing fruitlets of different apple cultivars.

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LITERATURE REVIEW

Evaluating the effect of physio-chemical properties of calcium foliar applications and the effect on penetration

1. Introduction

Foliar applications have many advantages over soil applications. Micronutrients like boron (B), zinc (Zn), iron (Fe), manganese (Mn) and copper (Cu), and macronutrients like phosphorus (P), calcium (Ca), nitrogen (N), sulphur (S), potassium (K) and magnesium (Mg), are taken up more effectively when applied as foliar applications instead of soil applications (Obreza et al., 2010). This is because plants absorb the nutrients applied directly to leaves and fruit more readily, leading to a faster response. Chances of toxic symptoms caused by excessive soil accumulation of nutrients is also eliminated through the use of foliar applications. Micronutrients can become toxic more easily than macronutrients, because they are needed in much lower quantities by plants and fruit.

Once a foliar application is applied, penetration is influenced by the physio-chemical properties of the specific spray solution. A number of aspects involving the absorption of foliar applied nutrients take place on the surface of leaves and fruit (Fernández et al., 2013). These include the formulation of the nutrient being applied, atomization of the foliar solution and the transport of the foliar applied nutrient towards the plant surface (Young, 1979). These are influenced by the developmental stages of fruit and leaves, and by environmental conditions (Bukovac, 1985). These processes overlap and are interconnected. If one of these processes change, it will most likely influence the others. The wetting, spreading, rain fastness and retention of a foliar application are all controlled by the physio-chemical properties of the foliar application formulation.

Foliar applications are aqueous solutions containing active elements known as mineral compounds (Fernández et al., 2013). The nutrient(s) in the aqueous solution has physio-chemical characteristics for example, pH, molecular weight/size, concentration (active), point of deliquescence (POD) and solubility that may have an influence on the penetration of the nutrient into the leaf or fruit. Foliar applications are however modified by the addition of certain additives to the foliar formulation to help improve their performance and penetration (Fernández et al., 2013).

2. Formulation and Carriers

Two key components determine foliar application formulations, active ingredient(s) and inert material(s) or adjuvant(s) (Fernández et al., 2013). Foliar applications may be more effective with the use of adjuvants that increase spreading and/or persistence of materials and active ingredients in the formulation once applied to the surface of leaves and fruit. There are a few quality measures that should be kept in mind when looking at formulations: the form of the nutrient (salts or chelated forms), the kind of chelating agent being used, the degree of chelation (fully chelated, partly chelated, and non-chelated) and whether there are any additives present to increase efficiency (El-Fouly, 2002).

2.1 Salts

Macronutrients and micronutrients are both applied to plants and it is important to make a distinction between the two, because micronutrients are applied at lower rates and concentrations and are known to be less stable when applied as inorganic salts (Fernández et al., 2013). Common macronutrient carriers for Ca are chloride (Cl_2), propionate and acetate, and a common micronutrient carrier is sulphate (SO_4). Salts can chelate or bind with compounds and/or be mixed with adjuvants. Calcium, which is a macronutrient, can form compounds with the following salts, calcium nitrate [$Ca(NO_3)_2$], calcium chloride ($CaCl_2$), calcium propionate ($C_6H_{10}CaO_4$) and calcium acetate ($C_4H_6CaO_4$) (Val and Fernández, 2011). Other examples include magnesium sulphate ($MgSO_4$), zinc sulphate [$Zn(SO_4)$], magnesium nitrate [$Mg(NO_3)$] and potassium chloride (KCl) (Orlovius, 2001; Dordas, 2009; Lester et al., 2010). In the 1970’s foliar applications were dominated by the application of inorganic compounds (carriers), like SO_4 and Cl_2. Boaretto et al. (2002) studied different micronutrients used to carry Zn in a foliar application formulation and found that each micronutrient influenced the uptake of Zn into leaves. Chloride was a more effective carrier compared to SO_4. When SO_4 was used as the Zn carrier ($ZnSO_4$, LS-$ZnSO_4$ or EDTA-$ZnSO_4$), the total amount of Zn absorbed by leaves was only 6%. When Cl_2 was used as a Zn carrier, ($ZnCl_2$ or LS-$ZnCl_2$) the absorption of Zn increased to 92%.

The first synthetic fertilizer used was Norge saltpetre, also known as $Ca(NO_3)_2$ (Scheibler et al., 1991). Limestone dissolved in nitric acid, produces $Ca(NO_3)_2$. The liquid is then neutralized with powder from lime or line. This method is currently used however, ammonia is currently used for the neutralization process by mixing nitric acid (50-60%) and limestone. Calcium nitrate is also a by-product of the Odda Process-a process that takes place when rock phosphate and nitric acid are digested to produce $Ca(NO_3)_2$ and phosphoric acid. Calcium nitrate is then used as a fertilizer in plants and can be applied directly. The use of $Ca(NO_3)_2$ is declining worldwide and alternative fertilizers are being used, for example Ca chelates.

2.2 Chelates and Complexes

Chelates and complexes received attention later in the 1980's (amino acids, polyols, glucoheptonates, EDTA, lignosulphonates ext.) (Fernández et al., 2013). Other foliar applications (chelates and complexes) seemed more promising compared to the use of inorganic compounds. There is a wide variety of organic complexes to choose from, which can contain either lingo-sulfonates, humic, fulvic acids or amino acids (Abadía et al., 2002).

The most used synthetic chelate for the application of iron is EDTA (ethylene-diamine-tetra-acetic acid), followed by DTPA (diethylenetriaminepentaacetic), HEDTA (hydroxyethyl ethylenediamine) and EDDHA (ethylenediaminedi) (Piaggesi et al., 2002). Piaggesi et al. (2002) used three different chelated formulations namely LPCA (ligninpolycarboxylic acid), EDTA and DTPA, but found no difference between the three on the penetration of Ca in leaves.

EDTA is the most widely used in chelate in agriculture. Marginally alkaline water can degrade the foliar application Fe-EDTA. If the pH of the water used is above 6.5, DTPA is usually used, but low salt formulations should rather be used. The application of LPCA is not easily photo degraded and poses a low risk to scorching (Piaggesi et al., 2002). Therefore LPCA can be applied at higher rates. EDTA and their salts form water soluble complexes with heavy metal ions, for example $[Fe(EDTA)]^-$ (Hart and Grace, 1987). For every 1 g chelating agent (EDTA) there are 105 mg Ca ions present in the formulation (Hart, 1982). Various methods can be used for the synthesis of EDTA, but the most widely used is alkaline cyano-methylation of ethylene-diamine by using sodium cyanide and formaldehyde (Bersworth, 1945).

Metallic atoms are bound to organic molecules, forming chelates (Martell and Calvin, 1952). The organic molecule is protected by a claw formed by the metal. The metal holds the organic molecule at two points. Chelates have a ring-like structure, formed by elements and metals that are bound and have two or more donor groups available. There are two known natural chelates; haemoglobin found in blood and chlorophyll found in plants (Voet and Voet, 2004). Ligand molecules donate a pair of electrons to the metal (Bowman-James, 2005). This forms a bond, because they now share the same electrons. A study on rats proved that when a strong chelator is used with a metal, the availability of that metal decreases once it is absorbed, but when a weaker chelator (more natural) is bound with a metal, the availability of the metal increases once absorbed (Giroux and Prakash, 1977. Bioavailability of a metal is influenced by the binding effect of the ligand once absorbed by animals and plants (Dutta et al., 2010). Availability once absorbed (plants and animals) is determined by the binding strength between the ligand and metal (Pullman et al., 1963). The structure of substances is the main difference in effectivity of a chelate and it is still uncertain whether inorganic or organic minerals are the best (Hill, 2005). Chelates form when bonds are formed between amino acids and minerals as well as mixtures of amino acids and inorganic metals. Interaction and precipitation of nutrients (micronutrients and macronutrients) with substances are limited when added to fertilizers in the chelated form (Hart-Smith, 1982). The absorption process for the plant is made easier by the chelate, because cations are converted to anionic form.

3. Added substances to enhance uptake

3.1 Marine algae and seed extracts

Seed extracts (Cwojdiński et al., 1996) and/or marine algae (Briand et al., 2003) can be combined with minerals in foliar applications. Natural plant metabolites, for example peptide chains, phytohormones, organic acids, amino acids, sugars, and minerals can be found in seed extracts and marine algae. Commercial seaweed biostimulants should however be approached with caution. Lötze and Hoffman (2016) looked at the nutrient composition of various biological active compounds of three commercial seaweed biostimulants produced in South-Africa. The seaweed products were all manufactured from *Ecklonia maxima* (seaweed) and, marketed as equivalent products: Basfoliar®Kelp, Afrikelp® and Kelpak®. Afrikelp® and Basfoliarkelpak® had significantly higher concentrations of P and N compared to Kelpak®. Calcium, Mg and K concentrations were significantly higher in Kelpak®. However, the mineral levels of these kelp products were well below the fertilizer formulation levels and cannot be used to replace mineral nutrient products.

The marine ecosystem consists out of a number of seaweeds and consists out of three main groups Phaeophyta, Rhodophyta and Chorophyta (Critchley et al., 2009), better known as brown, red and green algae. Brown seaweed is more commonly used in agriculture today and consists of 2000 species (Blunden and Gordon, 1986). Improved crop performance, yield, seed germination, resistance to stress (biotic and abiotic) and post-harvest shelf-life are all beneficial effects of seaweed, when applied to plants (Beckett and van Staden 1990; Hankins and Hockey 1990; Norrie and Keathley, 2006).

Brown algae (*Ecklonia maxima)* is used to manufacture a liquid seaweed concentrate which is known as Kelpak® (Beckett and van Staden, 1990). Kelpak® is used in agriculture to maximize the penetration of foliar applications (nutrients), and reduce the number of foliar applications. Organic acids and methionine are organic molecules that are present in seaweed extract and can chelate minerals (Lynn, 1972). In a study by Beckett and van Staden (1990) using 1% or 5% Kelpak®, they reduced leaf burn of the minerals applied (Cu, Mn and Zn) possibly due to the chelating properties of Kelpak®.

Results however, did not show improved foliar uptake using seaweed concentrates in tomatoes grown under the normal commercial conditions (Beckett and van Staden, 1990).

Kelpak® maximizes the penetration of foliar applications (nutrients) through reducing the drying rate of droplets on the leaf surface (Beckett and Staden, 1990). This is possible through aliginates in Kelpak® that have gel forming properties. Lodolini et al. (2002) studied the effect of a combination of humic acids on the drop life of foliar applications on leaves. The surfactant (Etravon 0.05%) had a slightly shorter drop life compared to humic acid (Zymo 0.18 $g{\cdot}L^{-1}$). Therefore the wetting action of the humic acids may increase nutrient uptake in leaves.

In contrast, North and Wooldridge (2003) used 0.003% v/v Agral 90® mixed with $Ca(NO_3)_2$ sprays to reduce the incidence of bitter pit (BP), and to study the mineral composition in 'Braeburn' apples. Calcium nitrate sprays (117g Ca x 12 sprays) without Kelpak® had the highest fruit Ca content at harvest, although it was not statistically different compared to $Ca(NO_3)_2$ sprays (117g Ca x 12) applied with Kelpak®. It can therefore be concluded that the Kelpak® failed to increase the uptake of $Ca(NO_3)_2$ sprays and reduce the incidence of BP significantly.

Sáanchez (2002) assessed the effect of Auxym on leaf and fruit mineral status. Auxym is a natural product that consists of mineral nutrients, vitamins and natural amino acids. Calcium concentrations were higher in leaves, but not significantly so. Auxym had no influence on the mineral concentration of pear fruit. In a study by Malaguti et al. (2002), 'Gala' and 'Fuji' apples received foliar applied seaweed extracts (brown algae, *Fucus spp)* with fertilizer (N, P_2O_5 and K_2O). Foliar applications did not affect fruit yield, fruit weight, vegetative growth or nutrient concentrations in the fruit and leaves in either cultivar.

3.2 Sugars

Some multi-fertilizer companies advise adding sugars and amino acids to foliar applications to counter any stress plants may encounter (Smoleń, 2012). A solution of sucrose is mixed (0.5 – 2%) with foliar applications if plants experience long term, low intensity photosynthetically active radiation. Sugars are used by plants as an additional energy source for the fusion of minerals into organic compounds. Organic compounds are then used in metabolic processes, for example growth and development. Arzani et al. (2002) found that the application of 3% sucrose, 2% glucose and 0.5% fungicide to pistachios, resulted in a significantly higher leaf sugar content compared to the control, and that the foliar applied carbohydrates improved the quality of the nuts.

Assimilation of compounds with a small mass (sucrose), takes place in cuticular pores, and struggle to penetrate through the cuticular layer (Marschner, 1995). Transport proteins (sucrose/H^+ symport) are involved in the transport of sugars (sucrose) between the parenchyma cells of leaves (Starck, 2003). Sucrose synthesized during photosynthesis in parenchyma cells and exogenous sucrose applied through foliar applications are transported in the phloem to developing sinks in plants. Sugars also play an important role as signalling substances to inform cells and other areas of the plant if there is a need for photosynthetic products. Hormones and sugars activate the expression of certain genes (Smoleń, 2012). Foliar penetration does not seem to be enhanced by the addition of sugars to foliar applications, but seems to be an additional energy source for plants.

3.3 Hormones

Apart from containing sugars and proteins, marine algae and seed extracts may also contain dihydrozeatin or cytokinin (Cwojdzinski et al., 1996). Benzyl adenine (BA) is another phytohormone that belongs to the cytokinin group. Cytokinin plays an important role in regulating metabolic processes in plants by controlling enzymatic activity (Maliszewska et al., 1997). Liu et al. (2006) found that the foliar application of cytokinin increased chlorophyll levels in leaves. Increased assimilative potential caused by foliar applied cytokinin, may indirectly improve assimilation of N to organic compounds, and N uptake by plants (Smoleń et al., 2010). It should still be taken into consideration that the simultaneous foliar application of amino acids and cytokinin may decrease the metabolism of plants and N uptake. *Ascophyllum nodosum* (seaweed extract) is well known for its cytokinin content, but auxins, betaines and oligossacharides have also been identified (Jameson, 1993). Hormones may improve foliar penetration by enhancing both K^+ and Ca^{2+} fluxes at stromata level (guard cells) (Mancuso et al., 2006).

4. Adjuvants

Adjuvants are substances that are added to a formulation/foliar application, and changes the nutrient active-ingredient action or foliar application/formulation characteristics (Hazen, 2000). Activator adjuvants increase the activity, retention and spreading of the active ingredient, for example surface active ingredients (Penner, 2000). Utility adjuvants do not affect the efficiency of the formulation but modify the properties of the solution. Adjuvants increase uptake of foliar applied products through physical and chemical mechanisms (Fernández et al., 2013. Activators, penetrators and synergists are all adjuvants.

Moggia et al. (2002) studied the effect of adjuvants applied with different Ca sources, on the control of BP. Bitter pit incidence varied between treatments, therefore it was concluded that other mineral elements or adjuvants might have played a role in increasing Ca concentration and reducing BP incidence (Glenn et al., 1985). The same results were found by Haefs et al. (2002) who applied foliar applications of $CaCl_2$ (0.63 M) to 'Braeburn' trees to reduce Ca deficiency symptoms. Calcium chloride was applied either alone or in combination with 2 $g \cdot L^{-1}$ of rapeseed oil, alkylether, Ca-dodecylsulfonate or castor oil. Due to the foliar application of $CaCl_2$ in combination with the adjuvant Ca concentration significantly increased in fruit and BP incidence was reduced by 50% at harvest while the treatment of $CaCl_2$ alone reduced BP incidence with just 10%.

Buffering agents and neutralizers adjust the pH of foliar application solutions, while detergents, wetting agents and spreaders follow the same general principals. Stickers reduce the drying of foliar applications, while increasing the retention time and rain-fastness (Fernández et al., 2013). Luber et al. (2002) studied the penetration of a new, adjuvant Ferti-Vant and the effect on penetration of nutrients compared to other adjuvants (silicone based) and found that Ferti-Vant had a significant, long lasting effect that retained nutrients on citrus and olive leaves for a few weeks.

Compounds known as humectants have water binding properties and may be organic, for example carboxy-methyl cellulose or inorganic, for example $CaCl_2$ (Val and Fernández, 2011). Humectants are responsible for lowering the POD in a foliar application formulation that reduces drying and increases foliar penetration in areas having little to no rain (dry areas).

Surfactants, also known as active agents, are the most widely used adjuvants in foliar applications (Fernández et al., 2013). Surfactants can be used to increase the absorption of a foliar application, but the concentration and formulation of the foliar application may affect the efficiency of the surfactant (De Villiërs and Hanekom, 1977; Harker and Ferguson, 1991). Surfactants can also cause run-off that reduces the total weight of Ca on the surface of leaves and fruit, leading to decreased Ca uptake.

Surfactants are large molecules containing a nonpolar, hydrophobic area linked to a polar, hydrophilic group (Tadros, 1995). They are placed in three groups namely; non-ionic, ionic and zwitterionic where the non-ionic surfactants are mostly used in foliar applications, because they do not interact with other polar components in the formulation (Fernández et al., 2013). Table 1 gives a summary of adjuvants that are on the market today and their method of action, which helps improve the efficiency of foliar applications.

The primary function of surfactants is to lower the surface tension and increase the area of interaction between foliar applications (nutrients) and leafs/fruit (Fernández et al., 2013). This also lowers the concentration gradient after a foliar application between the outside and inside of fruit/leafs, leading to decreased penetration. Haefs et al. (2002) tried to enhance the cuticle penetration of tomato fruit and ‘Braeburn’ apples through the application of $CaCl_2$. $CaCl_2$ (0.2 M) was applied with surfactants namely rapeseed oil ethoxylates, alkylether, Ca-dodecylsulfonate and castor oil. All surfactants significantly increased Ca concentration in apple fruit.

5. Physio-chemical factors

Efficiency of foliar applications is influenced by physical-chemical factors, for example ion concentration, pH and the formulation of the compound being used (Schlegel and Schönherr, 2002). Penetration of minerals differ according to the abundance of lenticels, number of cracks in the fruit peel, and the developmental stage of the fruit (Haker and Ferguson, 1988). Furthermore, these physio-chemical factors may result in significant differences between penetration depth and Ca concentration in leaves and fruit, as shown with different Ca foliar application formulations on tomatoes (Lötze and Turketti, 2014).

5.1 Concentration

Fick's law states that solutes will move from a high to a low concentration, and is used as the cuticle diffusion model (Fernández et al., 2013). The concentration of the nutrient applied (foliar application) is higher in comparison to the concentration of the nutrient found inside the leaf/fruit, establishing a concentration gradient between the inside and outside. This leads to the absorption of the nutrient applied (foliar application) across the surface of the specific plant organ targeted (Fernández et al., 2013).

Schlegel and Schönherr (2002) recommended that, a foliar application with the highest possible active Ca concentration in a formulation should be used (without causing leaf burn) to obtain the highest efficiency (penetration) of a foliar product.

$Ca(NO_3)_2$ and $CaCl_2$ penetrated the apple peel at higher rates than other Ca foliar applications with a lower concentration of (active) Ca (Schlengel and Schönherr, 2002). Wilsdorf (2011) concurs in that the foliar application Calflo™ [$Ca(NO_3)_2$] caused a higher Ca concentration in 'Braeburn' apples compared to the other foliar applications GS™ and GG™. Calflo™ and Calcimax™ have a higher active Ca (12%) compared to Foliar GS™ and GG™ (10%).

Neilsen and Neilsen (2002) also evaluated the effectiveness of different Ca compounds on 'Fuji', 'Jonagold' and 'Gala', and found similar results. Calcium chloride (19 $ml \cdot L^{-1}$) foliar applications were the most effective for increasing fruit Ca concentration. Neither of the foliar treatments, Nutrical (8% organic chelated Ca) or Calcimax (8% organic chelated Ca) both applied at a rate of 4·5 mlL^{-1}, were as effective. When Nutrical was applied at the same rate as $CaCl_2$, the fruit Ca concentration for the two different foliar applications were similar.

Mayr and Schröder (2002) investigated the influence of different Ca concentrations, time of application, and combinations with prohexadione-Ca on nutrient concentration in ‘Boskoop’ and ‘Elstar’ apples. All $CaCl_2$ sprays increased the Ca content compared to the control. The best treatment was 10 kg $CaCl_2$ in 500 $L{\cdot}ha^{-1}$ applied every two weeks after June drop. This Ca treatment increased Ca content by 29% and 18%, respectively.

Increasing the concentration leads to higher penetration of Ca minerals as reported by Schönherr (2001), using isolated cuticles, and Zhang and Brown (1999), using intact leaves. Eichert et al. (2002), using isolated epidermal strips and anionic fluorescent dye uranine (sodium fluoresceinate), found that uptake increased as the concentration of the dye increased. It should however be taken into consideration that the relationship between foliar uptake and concentration is not yet fully understood.

Wójcik and Szwonek (2002) investigated the penetration of different Ca foliar applications (formulations) in improving apple quality. Trees received five sprays either with Rosatop Ca [22% Ca as $Ca(NO_3)_2$], Rosafos (4% Ca as $CaHPO_4$), Rosacal [19% Ca as $Ca(NO_3)_2$] and $CaCl_2$ (29% Ca). Rosatop Ca sprays resulted in the highest Ca content in ‘Szampion’. These results support the fact that increasing Ca concentration in a formulation has positive effects until a certain point and further increases may hinder Ca penetration. It is also important to prevent foliar damage during certain growth stages in plants and fruit when using high Ca concentrations in foliar applications.

Table 2 shows some commercial salts and chelates and their Ca content (Modified from Schönherr, 2002; Wójcik and Szwonek, 2002; Neilsen and Neilsen, 2002).

5.2 Solubility

Compounds in a foliar application should be fully dissolved before application (Fernández et al., 2013). Water is used as a solvent in most foliar applications and can contain different active chemical compounds including chelates, complexes and salts. Additives may change the solubility of a chemical compound in water (solvent) at a certain temperature. For foliar uptake to be effective, water solubility is an important factor to keep in mind since absorption of a nutrient can only take place once the compound is dissolved in a liquid phase. Most nitrates used for foliar application are quite soluble in water (Mengel, 2002). Table 3 shows some salts/chelates and their solubility in water (modified from Lide, 1991; pubchem.ncbi).

5.3 Number of foliar applications and developmental state

The developmental state of lenticels and their distribution on the fruit surface are important in determining the efficiency of a foliar application (Schlegel and Schönherr, 2002). Calcium chloride applied to 'Golden Delicious' fruit discs to assess the relationship between penetration and fruit developmental stage, found rapid penetration in young fruit 12-45 days after full bloom (dafb) due to the presence of trichomes and stomata. Trichomes and stomata later disappeared (after 50 dafb) and the main absorption site changed to the developing lenticels. Fruit receiving foliar applications at a later stage in development might show better absorption due to more lenticels. In the case of young leaves the epicuticular waxes form an almost impermeable layer for water soluble solutes (Mengel, 2002).

Lanauskas and Kvikliene (2006) found on 'Sinap Orlovskij' that $CaCl_2$ and $Ca(NO_3)_2$, Ca foliar applications applied seven times decreased BP incidence about twice as effectively in comparison with the control and the trees receiving only two foliar applications of $CaCl_2$. Apples that contained 170-230 mg $Ca{\cdot}kg^{-1}$ had a BP incidence of 35% and fruit that contained 340-460 mg $Ca{\cdot}kg^{-1}$ had a BP incidence of only 2%.

5.4 Point of deliquescence

The relative humidity (RH) in the air over the cuticle and the hygroscopicity of the salts in a formulation affect the penetration of foliar applications (Schönherr, 2002). Dissolution of a salt is required for penetration. Dry deposits are salt crystals or organic residues, for example amino acids and saccharides that remain on the surface of the leaf after the droplet has dried (Smoleń, 2012). Deliquescence is the conversion of a solid into a liquid due to the absorption of moisture or water vapour from the surrounding air. Rehydration of a salt, and RH in the air around the salt (foliar application) on fruit/leafs, determine the POD. RH in the air around a salt can be defined as POD. The salt residue on fruit/leafs dissolves if in the surrounding air is above POD, if below the salt residue remains solid and penetration stops (Schönherr, 2002).

Foliar applications that have a POD above 90%, have little chance of rehydration by the surrounding air (Smoleń, 2012), because they only penetrate at a RH close to 100% (Schönherr, 2002). In such a case the penetration of a foliar application will be determined by how long the leaf can remain wet after a foliar application. Lötze and Turketti (2014) reported that Ca formulations with a low POD for example, salts [$Ca(NO_3)_2$ and $CaCl_2$] penetrated better (depth and concentration) on tomatoes (leaves or fruit?) than foliar applications with a high POD (Ca-metalosate) . As temperature decreases, the absorption of Ca decreases due to the increase in viscosity of the solution. Supporting this statement, Schönherr (2002) found that the lower POD of a salt (inorganic) in comparison with other organic foliar applications (chelates) penetrated better. Table 4 shows some of the characteristics of salts and organic compounds used in foliar applications containing one or two mineral nutrients with respect to POD and the applicability for a foliar application (Modified from Schönherr, 2002).

Adjuvants can improve the penetration of Ca when applied with foliar applications by altering the POD (Blanco et al., 2010). The addition of adjuvants can improve the penetration of Ca in pre-harvest foliar sprays by altering the POD and distribution of Ca in the droplet.

5.5 pH

The pH of a solution is an important parameter to take into consideration with regards to the effectiveness of a foliar application (Smoleń, 2012). If the pH of a foliar solution is below or higher than certain values, absorption of nutrients may be poor and the solution may cause leaf damage (El-Otmani et al., 2000). Marschner (1995) reported that the use of a foliar application with a lower pH is less likely to cause leaf damage and that the internal Ca concentration of a foliar application is pH sensitive. Uptake of a foliar application is higher at pH 3 than at 11. Blanpied (1979) found using a Ca solution with a pH between 3.3 and 5.2 had the best absorption in apple leaves; however Lidster et al. (1977) found that using a solution pH of 7 for $CaCl_2$ had the highest absorption in sweet cherry. The optimum pH for a foliar application in citrus should be between 5 and 7.5 (Zekri and England, 2010), while an earlier study (also on citrus) found that the best pH for a solution of urea ranged from 5.5 to 6 (El-Otmani et al., 2000).

5.6 Molecular weight and size

The size of the nutrient molecule in the foliar application influences the rate of foliar penetration (Fernández et al., 2013). The estimated radius of cuticle aqueous pores in leaves is 0.3 to 0.5 nm and in fruit 0.7 to 1.2 nm (Beyer et al., 2005; Schönherr, 2006). The pectic matrix of the primary cell wall determines porosity (Mengel, 2002). Larger molecules, for example chelates will struggle to gain entry into the pectic matrix. Calcium, sucrose and potassium all have diameters of 0.82, 1.0 and 0.66, respectively.

Urea is a solute that is permeable through pores, but not so larger molecules for example synthetic chelates (eg. Me-EDTA, Me-DTPA, Me-EDDHA, Me-HEDTA, Me-EDDHMA, Me-EDDCHA, Me-EDDSHA), polysaccharides, peptides and humic acids (Marschner, 1997).Permeability through the cuticle is weight selective and compounds with a high molecular weight do not penetrate the cuticle as effectively as lower molecular weight compounds (Schreiber and Schönherr, 2009).

Ca-EDTA, Ca-acetate and Ca-propionate Ca foliar applications all have lower penetration compared to other Ca foliar applications [$CaCl_2$ and $Ca(NO_3)_2$] with a lower molecular weight formulation (Schönherr, 2002). Table 5 shows the molecular weight ($g \cdot mol^{-1}$) of salts and chelates (modified from Schönherr, 2002 and pubchem.ncbi).

Thalheimer and Paoli (2002) assessed foliar absorption of Mn using different foliar products. Significant differences in leaf Mn was found between different foliar products. $MnSO_4$ (100 $g \cdot hl^{-1}$) had the highest Mn concentration followed by $MnSO_4$ (50 $g \cdot hl^{-1}$). Commercial products for example Mantrac, Mn Chelal, Meda F2, Manganbetter had satisfactory leaf concentrations although lower than the $MnSO_4$. The lowest leaf uptake was observed by the chelated products Mn chelal and Manganbetter. Supporting the fact that chelated compounds have a larger molecular weight that may prevent them from entering the hydrophilic pores within the cuticle of the leaf (Marschner, 1997).

Furuya and Umemiya (2002) investigated fifteen different amino acids and found that the rate of foliar N penetration increased as molecular weights of the amino acids decreased. There was however, an exception for 2 amino acids namely arginine and L-lysine that showed significantly higher rates of penetration compared to other amino acids that had similar molecular weights.

6. Conclusion

The carrier used in a foliar application will influence the penetration of Ca. Chloride and nitrate [$CaCl_2$ and $Ca(NO_3)_2$] seem to be the most effective inorganic compound used to deliver Ca to plants according to literature. After selecting a carrier, a foliar application with the highest possible (active), Ca concentration (considering leaf burn), lowest POD, lowest molecular weight (size), and a pH between 3 and 5, should be selected.

Marine algae (Kelpak®) and seed extracts seem to reduce leaf burn (caused by Ca foliar applications) and like sugar (sucrose) have beneficial effects in plants (biotic and abiotic resistance). However, increasing Ca foliar penetration is unlikely. Adjuvants (surfactants) lower the surface tension and POD, increasing foliar penetration. However, lowering the surface tension lowers the concentration gradient (Fick’s law). Therefore, adding surfactants give variable results.

7. References

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8. Tables

Table 1. Adjuvants that are available on the market and their mode of action (Modified from Fernández et al., 2013).

Adjuvant name	Mode of action
Surfactant	Lowers surface tension
Wetting agent	Lowers surface tension
Detergent	Lowers surface tension
Spreader	Lowers surface tension
Sticker	Increased retention and rain fastness
Retention aid	Increased retention and rain fastness
Buffering agent	pH buffer
Neutraliser	pH buffer
Acidifier	Lowering the pH
Penetrator	Increasing penetration (solubilizing cuticle)
Synergist	Increasing rate of foliar penetration
Activator	Increasing rate of foliar penetration
Compatibility agent	Improving formulation compatibility
Humectant	Retarding drying by lowering the POD
Drift retardant	Increased spray targeting
Bounce and shatter minimizer	Increased spray targeting

Table 2. Salts/chelates and nutrient content compared with one other (Modified from Schönherr, 2002; Szwonek, 2002; Neilsen and Neilsen, 2002 and Wójcik, 2014).

Salts/chelates	Ca content (%)
$CaCl_2$	18.3
$Ca(NO_3)_2$	10.3
Ca propionate	19.6
Ca lactate	13.0
Ca acetate	25.4
Calcimax (organic chelate)	8.0
Rosafos (metalosate)	4.0
Ca-metalosate (amino acid chelate)	6.0

Table 3. Salts and their solubility in water (modified from Lide, 1991; pubchem.ncbi).

Salts/chelates	Solubility $g \cdot kg^{-1}$ H_2O
$CaCl_2$	2790
$Ca(NO_3)_2$	6600
Ca propionate	490
Ca lactate	31
Ca acetate	374
Calcium EDTA complex	1000000

Table 4. Salts and organic compounds used in foliar applications that consist of one or two nutrients, with respect to POD, and applicability for Ca foliar application (Modified from Schönherr, 2002).

Mineral nutrients	Salt compound	POD %	Applicability for foliar application
Ca	$CaCl_2$ (inorganic salt)	33	Very good
	Lactate (organic salt)	97	Bad
	Propionate (organic salt)	95	Bad
	Acetate (organic salt)	100	Bad
Ca and N	$Ca(NO_3)_2$ (inorganic salt)	56	Very good

Table 5. Salts and their molecular weight $g \cdot mol^{-1}$ (modified from Schönherr, 2002 and pubchem.ncbi).

Salts	Molecular weight $g \cdot mol^{-1}$
$CaCl_2$	219
$Ca(NO_3)_2$	236
Ca propionate	204
Ca lactate	308
Ca acetate	158
Calcium EDTA complex	330.306
Ca-DTPA	500.38

PAPER 1

The role of formulation of calcium and boron foliar applications to improve fruit quality in 'Golden Delicious' *(Malus domestica* Borkh.*)* apples.

Abstract

Bitter pit (BP) is a physiological disorder in apple (*Malus domestica* Borkh.) fruit caused by a calcium (Ca) deficiency. Calcium plays an important role in the cell wall functioning and Ca deficiency is closely associated with development of BP lesions. Foliar applications of Ca, i.e. Ca-nitrate [$Ca(NO_3)_2$] and Ca-metalosate are reported to increase the Ca concentration of fruit and decrease BP incidence. Commercially available foliar Ca products often have different formulations that vary due to unique physio-chemical properties. The aim of this study was to determine whether a chelated foliar formulation, viz. Ca-metalosate, is as effective as the salt formulation $Ca(NO_3)_2$ in reducing BP in 'Golden Delicious' apples. Two commercial orchards with a history of high BP incidence were selected for the trials in Elgin, South Africa. Eight foliar applications starting from 42 days after full bloom (dafb) were applied to single-tree replicates in a randomised complete block design (n=10), on 'Golden Delicious' trees on seedling rootstock. The treatments consisted of two different Ca formulations, viz. $Ca(NO_3)_2$ and Ca-metalosate, as well as an untreated control that received no Ca. In Trial 1, Ca concentration in fruit treated with $Ca(NO_3)_2$ was significantly (P=0.0311) higher (8.4 mg·100 g^{-1}) 80 dafb compared to the other two treatments, viz. Ca-metalosate (6.9 mg·100 g^{-1}) and the control (7.05 mg·100 g^{-1}). In fruit treated with $Ca(NO_3)_2$ BP incidence was significantly (P=0.0241) lower (0.0%) compared to the control (1.3%), but not with Ca-metalosate (0.2%). In the second trial, there were no significant differences in the Ca concentrations between treatments at 80 dafb, and no BP occurred. Results from these trials confirmed reports that formulation of Ca foliar applications play a

role in determining the efficacy of these Ca products in increasing Ca concentration in fruitlets 80 dafb, as well as their efficacy in controlling BP.

Keywords: Bitter pit, calcium chelate, calcium nitrate, boron, physio-chemical factors

Introduction

Calcium and boron (B) are important mineral nutrient elements in growth and development of fruit trees (Kadir, 2005). Calcium deficiency was linked to many physiological disorders in fruit as early as the 1930's. One such physiological disorders, bitterpit (BP), is caused by low Ca levels in fruit, either as a result of low Ca levels in trees or the soil (Shear, 1975; Kirkby and Pilbeam, 1984). Bitter pit in apple is a major problem in export-orientated industries, as markets only tolerate a BP incidence lower than 2% (Lötze and Theron, 2006).The main symptom of BP is distinct pitting of the cortical flesh, and the breakdown of the outer-most cells that lead to small depressions on the surface of the fruit (Jackson, 2003). These depressions usually takes on a much deeper brown and/or green colour than the rest of the peel, as by the time pitting is visible to the naked eye, plasmolysis of the cytoplasm has already occurred. Localized reduction in Ca content in the fruit cells beneath the peel cause brown pits, which occur predominantly at the calyx-end of the fruit (Hopfinger et al., 1984).

Calcium plays an important role in cell wall integrity, as it increases structural rigidity of cell walls by creating cross-links inside the pectin polysaccharide matrix, which delays degradation (Tobias et al., 1992; Easterwood, 2002). Calcium also helps to increase membrane stability in plant cells (Kirkby and Pilbeam, 1984; Tobias et al., 1992). Thus, fruit with higher Ca levels have stronger cell walls that may also increase resistance to fungi and bacterial infection, in addition to a lower susceptibility of the fruit to BP (Kirby and Pilbeam, 1984; Tobias et al., 1992; Easterwood, 2002). Fruit Ca concentration at harvest should be above the critical threshold of 4 to 4.5 mg·100 g fresh weight $(FW)^{-1}$ to attain good fruit quality (Terblanche, 1985; Neilsen et al., 2005). If fruit Ca concentration is above 4.5 mg·100 g FW^{-1}, BP incidence is highly unlikely to occur (Terblanche, 1985).

Calcium has a low mobility rate in plants and is primarily transported in the xylem (Kirkby and Pilbeam, 1984; Rethwisch et al., 2000). In the early developmental stages of fruit up until 40 dafb, Ca assimilation by fruit occurs via xylem transport from the roots of apple trees and this leads to high fruit Ca concentrations (Kirkby and Pilbeam, 1984). Until 40 dafb, absorption of foliar applied Ca can occur through the trichomes, cuticle and the stomata of the fruit peel (Schlegel and Schönherr, 2002). Thereafter, Ca absorption declines until 28 to 42 days before harvested (Casero et al., 2002). During the last stage, exogenous foliar Ca applications can be absorbed through the lenticels and cuticle cracks of the peel (Schlegel and Schönherr, 2002). The timing of foliar Ca applications therefore contributes significantly to its efficacy and should be taken into consideration when targeting applications in commercial apple production.

Lötze and Theron (2006) reported that 12 Ca foliar applications increased fruit Ca concentration in 'Golden Delicious' apples, and reduced BP incidence significantly. An initial increase in Ca concentration in both the control and treatment from 40 dafb until 70 dafb (first peak) was followed by no increase in Ca concentration from 77 to 84 dafb. Thereafter, another increase in Ca concentration followed for a period of four weeks, confirming results from Schlegel and Schönherr (2002). During the first Ca peak, Ca concentration in fruit increased until 60 dafb (end of cell division), while the fruit are developing and are still strong sinks (Kirkby and Pilbeam, 1984) and Ca is primarily obtained from reserves. During this period, Ca is transported via the xylem to strong sinks that have high transpiration rates. The second peak in Ca concentration in fruit occurred from 80 dafb when the leaf and shoot growth ceased and the fruit became stronger sinks (Harker and Ferguson, 1988).

Soluble inorganic Ca foliar applications like $Ca(NO_3)_2$ and $CaCl_2$ have been used successfully to increase Ca concentration in fruit, with resulting reduction in Ca related disorders (Bangerth, 1979; Easterwood, 2002). Recently, more attention has been given to chelates and complexes (EDTA, glucoheptonates, polyols, amino acids, lignosulphonates, humic, fulvic etc.) that seem more effective as alternative foliar applications compared to the use of inorganic compounds [$CaCl_2$, $Ca(NO_3)_2$] (Abadía et al., 2002; Fernández et al., 2013). Chelated formulations such as EDTA, DTPA and LPCA increased Ca in leaves and were not influenced by the nature of the chelating agent (Piaggesi et al., 2002). Chelates are formed when organic molecules are bound to a metallic atom (Martell and Calvin, 1952). The metal is bound by the organic molecule at two points, holding and binding it like a claw to protect the organic molecule. Chelates are elements that are bound with a metal that has two or more donor groups so that rings can be formed to give the chelate a ring-like structure. The bond is formed when the atom of the ligand molecule donates a pair of electrons to the metal and then share the same electrons (Bowman-James, 2005). The binding of the ligand with a metal affects the bioavailability of the metal and can reduce or increase the availability of the metal once it is absorbed by plants and animals (Giroux and Prakash, 1977 Dutta et al., 2010). This means that the binding strength between the metal and the chelate plays a very important role in the availability of the metal once plants have absorbed it (Pullman et al., 1963). It is still unclear whether organic or inorganic minerals are the best, but the main difference in efficacy of a chelate is the structure of the substances (Hill, 2005). Mixtures of inorganic metals and amino acids can occur as well as minerals that form bonds with amino acids that form chelates.

Micronutrients and macronutrients are added to fertilizers in the chelated form to help limit the interaction and the precipitation that these nutrients can have with other substances (Hart-Smith, 1982). Cations are thus converted to an anionic form by the chelate to make the absorption processes easier for the plant and decrease the binding of minerals with other substances.

Lötze and Turketti (2014) reported variation in penetration depth and concentrations between foliar applied Ca formulations in tomatoes. Most of the chelated formulations (Carbology Cal, CAL-PRO, Gluco-Calcium and LBCa5) were outperformed by Ca salt formulations [Quatro Calbormo, Pitstop, Quatro Calcium, Mainstay Calcium, Manni-plex Ca and $Ca(NO_3)_2$].

Foliar formulations commonly consist of at least two major components: the active ingredient and the inert material(s) or adjuvant(s) (Fernández et al., 2013). The micronutrient used as the source to carry zinc (Zn) was found to have an influence on the uptake of Zn into leaves (Boaretto et al., 2002). Physio-chemical factors influenced the efficiency and penetration of foliar applications (Schlegel and Schönherr, 2002). To obtain the highest efficiency of a foliar product, a foliar application with the highest possible active Ca concentration in a formulation should be used. Fick’s law is used as the cuticle diffusion model and the law states that solutes will move from a high to a low concentration (Fernández et al., 2013). This means that a concentration gradient is established between the inside and outside of the fruit and $Ca(NO_3)_2$ establishes a larger concentration gradient in comparison to Ca-metalosate. $Ca(NO_3)_2$ and $CaCl_2$ penetrates the apple peel at higher rates than Ca foliar applications with a lower active Ca (Schlegel and Schönherr, 2002).

The point of deliquescence (POD) of a foliar application has an effect on penetration. $Ca(NO_3)_2$ has a lower POD, and may therefore have increased penetration compared to other foliar applications, for example Ca-metalosate (Schönherr, 2002; Lötze and Turketti, 2014). Schönherr (2002) found that the lower POD of a salt (inorganic) than chelated (organic) foliar applications increased penetration. The POD determines the likelihood of rehydration of a salt and relative humidity (RH) over the salt residue on the leaf. If RH in the surrounding air is above POD, the salt residue on the cuticle of the leaf dissolves, if below, the residue remains solid and penetration stops (Schönherr, 2002).

Similarly, Schönherr (2002) showed that $CaCl_2$ has a higher Ca penetration compared to Ca foliar applications like Ca-EDTA, Ca-acetate and Ca-propionate, which can be associated with the lower molecular weight of the $CaCl_2$ formulation. $Ca(NO_3)_2$ has a lower molecular weight in comparison to Ca-metalosate and may improve penetration. This supports the findings in a number of studies that foliar applications with a lower molecular weight has increased penetration (Schönherr, 2002; Thalheimer and Paoli, 2002; Furuya and Umemiya, 2002). Porosity of the primary cell wall is determined by the pectic matrix (Mengel, 2002). The primary cell wall has spaces with a diameter of < 5 nm. Potassium, Ca and sucrose have a diameter of 0.66, 0.82 and 1.0 nm, respectively. It should therefore be taken into consideration that the pectic matrix will act as a barrier for larger molecules, for example chelates.

Boron has often been implied in an increase in Ca uptake in fruit. Peryea et al. (2003) reported that B mixed with $CaCl_2$ and sprayed at pink stage was useful in controlling BP incidence in 'Scarlet Gala'. The mixing of the two nutrients did not interfere with the Ca status in the fruit and increased both the leaf and fruit sodium concentrations. Calcium foliar applications have been combined with B applications to reduce the risk of BP incidence and to increase fruit set in apple orchards. According to Shear and Faust (1971) it is likely that B improves the movement of Ca in leaves.

The aim of this study was to determine whether the chelated foliar formulation, Ca-metalosate, is as efficient as the salt formulation $Ca(NO_3)_2$, in reducing BP in 'Golden Delicious' apples.

Materials and Methods

Trial layout and site selection

The trials were conducted in Elgin, South Africa on Applethwaite Farm (34.1570° S, 19.0152° E) during 2016/17. Two commercial orchards of 'Golden Delicious' (GD) on seedling rootstock were used (Fig. 1). The orchards have a history of high BP incidence of up to 20% (personal communication, Mr. J. Fourie). A randomized complete block design (RCBD) was used with 3 treatments and 10 single-tree replications (n=10). Buffer trees and rows were left to ensure no cross contamination of spray drift from one treatment onto another. The foliar applications were applied to entire trees for ±30 s until point of run-off using a Stihl motorised knapsack-sprayer.

Calcium-metalosate was applied at a rate of 1 $ml \cdot L^{-1}$ water, and B-metalosate was initially added (first 4 dates) at a rate of 0.5 $ml \cdot L^{-1}$ water to further increase efficiency of Ca uptake. $Ca(NO_3)_2$ was applied at a rate of 6.5 $g \cdot L^{-1}$, without any B addition. The control received no foliar application and was left untreated. Details for each treatment are shown in Table 1.

Fruit, leaf and soil mineral analysis

Fruit were sampled ±80 dafb on 30 Dec. 2016 to determine the Ca concentration in the fruitlets. A sample of six fruit of similar sizes was collected from each tree. The core and seeds of the fruit were removed as it generally has a high Ca content, but was of no interest in this study. Leaf and soil mineral analysis of the orchard were provided by Applethwaite Farm (personal communication, Mr. J. Fourie) to determine if the orchards had any history of Ca deficiency (Table 2).

Fruit yield and maturity

Due to accidental premature commercial harvesting of trees in Trial 2, only Trial 1 will be discussed in terms of BP incidence. Fruit samples were harvested on 28 Feb. 2017, ±140 dafb (optimum commercial maturity). Ten apples of similar size were randomly collected from each tree and transported to the Department of Horticulture Science at Stellenbosch University for evaluation of important fruit quality attributes.

Fruit size (length and diameter) was measured with an EFM (Electronic Fruit Size Measure) and the firmness of the fruit was measured on both pared sides of the fruit using a FTA (Fruit texture analyser, GUSS Manufacturing (Pty) Ltd, Strand, South Africa) and a 11.1 mm tip. The mass of the fruit was determined with an electronic scale (GUSS Manufacturing (Pty) Ltd, Strand, South Africa). Fruit ground colour was evaluated using a colour chart (Ground colour: Unifruco Research Service (Pty) Ltd. Colour chart score for this apple cultivar is 0.5 = green, and 5 = yellow). Starch breakdown was visually assessed subsequent to staining with a 1% iodine solution (Deciduous fruit board (Pty) Ltd).

For evaluation of BP incidence, approximately 18 kg fruit (±100 fruit) were sampled separately from each replicate on 28 Feb. 2017. The apples were stored at -0.5°C for 2 months in regular atmosphere to induce development of BP symptoms. After the 2 months, the apples were stored at 15°C for 14 days to enhance ripening. Visual evaluation of individual fruit indicated presence or absence of BP and incidence was scored as either pitted (Fig. 2) or non-pitted.

Statistical Analysis

Statistical analysis was performed in a Two-way analysis of variance procedure (ANOVA) using Statistical Analysis System (SAS) programme (SAS Institute Inc, 2004, Cary, NC). A logit transformation was performed on percentage data (Snedecor and Cockran, 1997).

Results

Fruit mineral analysis

Fruit Ca concentration differed significantly between treatments (Table 3). $Ca(NO_3)_2$ treated fruit had the highest fruit Ca concentration (80 dafb) and differed significantly from the control and Ca-metalosate treatment, while the latter did not differ significantly. In Trial 2, no significant differences were found in fruit CA concentration (Table 4). Leaf and soil mineral analysis indicated no severe Ca deficiency (Table 2).

Fruit quality at harvest

Fruit firmness, mass, diameter or starch breakdown did not differ significantly in Trial 1 (Table 3). Ground colour differed significantly with $Ca(NO_3)_2$ treated fruit having the greenest ground colour.

Bitter pit evaluation

The analysis of the logit transformed BP data showed a significant difference between treatments (Table 3). $Ca(NO_3)_2$ treated fruit had a significantly lower BP incidence (0.0%) compared to the control (1.3%), but not compared to Ca-metalosate (0.2%).

Discussion

The aim of this study was to determine whether the chelated foliar formulation, Ca-metalosate, is as efficient as the salt formulation $Ca(NO_3)_2$ in reducing BP in 'Golden Delicious' apples.

Fruit mineral analysis

In Trial 1, fruit treated with 6.5 $g.L^{-1}$ $Ca(NO_3)_2$ had a significantly higher Ca concentration at 80 dafb in comparison to the other two treatments. $Ca(NO_3)_2$ at 6.5 $g.L^{-1}$ therefore penetrated fruit more efficiently than 1 $ml.L^{-1}$ Ca-metalosate and increased Ca concentrations. A number of previous studies showed that Ca concentration in a foliar application (active) had a positive effect on the penetration. Therefore foliar applications with a higher Ca concentration (active) penetrated fruit more efficiently than foliar applications with a low Ca concentration (active), for example Ca-metalosate (Mayr and Schroder, 2002; Neilsen and Neilsen 2002; Schlegel and Schönherr, 2002; Wilsdolf et al., 2011; Lötze and Turketti, 2014).

$Ca(NO_3)_2$ has a low POD, and may therefore explain increased penetration in comparison to other foliar applications, for example Ca-metalosate in Trial 1 (Schönherr, 2002; Lötze and Turketti, 2014). $Ca(NO_3)_2$ has a lower molecular weight in comparison to Ca-metalosate which may explain the increased penetration in Trial 1. This supports the findings in a number of studies that showed that foliar applications with a lower molecular weight has increased penetration (Furuya and Umemiya, 2002; Schönherr, 2002; Thalheimer and Paoli, 2002). In Trial 2, Ca concentrations at 80 dafb did not differ significantly between treatments.

Fruit quality at harvest

Fruit quality was not altered due to the treatments, other than the slightly greener peel ground colour following the $Ca(NO_3)_2$. This was previously also found by (Wilsdorf, 2011) and is probably due to the nitrogen component. However, this difference is too small to the naked eye to have an economic effect and will not be further discussed.

Bitter pit evaluation

In Trial 1, the average Ca concentration of fruit were 2.43 (control), 2.98 [$Ca(NO_3)_2$] and 2.59 mg·100 g^{-1} (Ca-metalosate), respectively if the values obtained at 80 dafb was extrapolated to harvest. These were all below the threshold Ca concentrations Terblanche (1985) suggested as optimal to prevent BP. Still, $Ca(NO_3)_2$ and Ca-metalosate had a very low BP incidence of 0.0% and 0.2%, respectively, questioning the value of the extrapolation of Ca concentration obtained at 80 dafb to determine possible Ca concentrations at harvest. It also questions the use of Ca concentration threshold values at harvest to predict BP. However, there are no existing threshold values for Ca concentrations in fruit 80 dafb on order to predict susceptibility of fruit to BP after harvest.

$Ca(NO_3)_2$ treated fruit had a significantly lower BP incidence compared to the control in Trial 1, whereas in the control fruit it did not differ significantly from the BP incidence of the Ca-metalosate treatment. Ca-metalosate thus failed to significantly decrease the incidence of BP compared to the control.

Chelated B in combination with Ca-metalosate early in the season, failed to improve the concentration of Ca in fruit (80 dafb). This does not support the statement made by Shear and Faust (1971) that B may improve the mobility of Ca in plants and therefore increase Ca concentrations.

Conclusion

In Trial 1, eight 6.5 g.L-1 $Ca(NO_3)_2$ applications significantly increased the Ca concentration of fruit 80 dafb compared to the control and Ca-metalosate treated fruit. $Ca(NO_3)_2$ also significantly reduced the incidence of BP compared to the control. Thus, it was possible to increase the Ca concentration in fruitlets early in the season with a foliar $Ca(NO_3)_2$ application which resulted in a significant reduction of BP compared to the control. However, no significant differences in Ca concentration of fruit at 80 dafb was found in Trial 2. Our results indicate that the formulation with a nitrate carrier, higher Ca concentration (active), lower POD and lower molecular weight [$Ca(NO_3)_2$], was more successful in increasing Ca concentration of fruitlets and reducing BP incidence and in ‘Golden Delicious’ apples, confirming the importance of formulation in evaluation of efficacy of Ca foliar applications to reduce BP.

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Tables and Figures

Table 1. Time of application (dafb) and the number of foliar applications for each treatment in Trial 1 and 2 on 'Golden Delicious' on Applethwaite farm, Elgin during 2016/17.

Treatment	Foliar application	dafb
1	Ca-metalosate + B first 4 dates Ca-metalosate on last 4 dates	42, 49, 56, 63, 70, 77, 84, 91
2	$Ca(NO_3)_2$	42, 49, 56, 63, 70, 77, 84, 91
3	Untreated control (UTC)	No foliar application

Table 2. Leaf and soil mineral analysis results provided by Applewaite Farm (personal communication Mr. J. Fourie).

Year	% Ca (Leaf analysis)		Ca $cmol.kg^{-1}$ (Soil analysis)	
	Trial 1	Trial 2	Trial 1	Trial 2
2015	-	-	2.1	5.1
2016	1.59	1.40	-	-
2017	1.30	1.26	3.11	4.91
Industry norms	0.7-1.6	0.7-1.6	2-15	2-15

Table 3. Ca concentration of fruit at 80 dafb, quality parameters at harvest and bitter pit evaluation for Trial 1 after storage for ‘Golden Delicious’ apples from Applethwaite, Elgin during 2016/17.

Treatments	Fruit Ca Conc. ($mg.100g^{-1}$)	Firmness (Kg)	Diameter (mm)	Starch Break down (%)	Mass (g)	Background colour	Bitter pit (%)	Bitter pit logit
Control	7.05 b	7.52 ns	67.76 ns	32.55 ns	124.06 ns	1.93 b	1.3 b	4.68
$Ca(NO_3)_2$	8.4 a	7.51	67.45	32.2	122.26	1.83 a	0.0 a	5.51
Ca-metalosate	6.9 b	7.47	66.62	37.0	118.05	1.94 b	0.2 ab	5.25
P-value	0.0311	0.9180	0.2131	0.5037	0.2167	0.0165		0.0241

Table 4. Ca concentration of fruit at 80 dafb for Trial 2 on ‘Golden Delicious’ on Applethwaite farm, Elgin during 2016/17.

Treatments	Fruit Ca Conc. ($mg.100g^{-1}$)
Control	7.37 ns
$Ca(NO_3)_2$	7.95
Ca-metalosate	7.34
P-value	0.5089

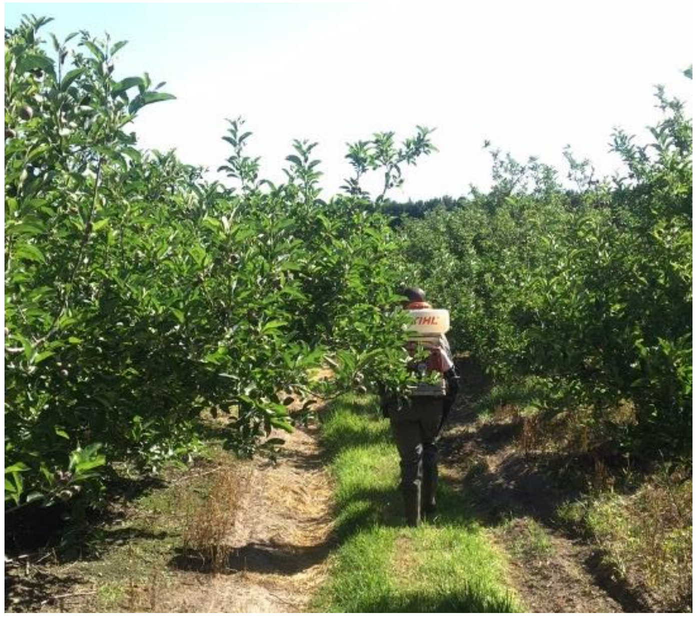

Fig.1. 'Golden Delicious' on seedling rootstock (Trial 1 and 2).

Fig. 2. 'Golden Delicious' apple showing bitter pit lesions as indicated with an arrow.

PAPER 2

Evaluation of pre-harvest foliar applications of a chelated Calcium and Boron combination to reduce albedo breakdown in citrus.

Abstract

Albedo breakdown (AB) is a physiological rind disorder prone to develop in *Citrus* spp. Cells of the white albedo tissue of the rind start to fracture and continue to separate during the fruit expansion period. The major cause of AB is changes in the cell wall cohesion of adjoining cells at the middle lamella. Normal development of the albedo tissue may therefore be dependent on calcium (Ca) and boron (B) in cell adhesion. The aim of this study was to determine whether the chelated foliar formulation, viz. Ca-metalosate in combination with chelated B, is effective in reducing AB in 'Cara Cara' and/or 'Turkey' oranges. Two commercial orchards in Porterville (Trial 1) and one commercial orchard in Simondium (Trial 2), South Africa were selected for the trials. Ca-metalosate and B-metalosate were applied with an untreated control, receiving no Ca or B. Three foliar applications, from 40% bloom, were applied to single-tree replicates in a randomized complete block design (n=10), on 'Cara Cara' and 'Turkey' trees on 'Carrizo Citrange' rootstock. Ca-metalosate in combination with B-metalosate failed to reduce the incidence of AB significantly in both 'Turkey' (P=0.4704) and 'Cara Cara' (P=0.2191; P=0.0682).

Keywords: albedo breakdown, 'Cara Cara'; creasing; mineral analysis; timing; 'Turkey'

Introduction

Oranges are prone to develop creasing, a physiological rind disorder, also known as albedo breakdown (AB) (Storey et al., 2002). Albedo breakdown manifests as creases or puffiness on the surface of the fruit rind, and causes fruit to be unsuitable for fresh-fruit markets, which leads to large economic losses (Jones et al., 1967; Treeby and Storey, 2002).

Albedo breakdown generally develops after colour break. According to Storey et al. (2002), the cells of the white albedo tissue of the rind start to fracture and continue to separate during the fruit expansion period. This causes localized undulations to develop on the surface of the rind. Genotype (Jones et al., 1967), climate, rootstock (Treeby et al., 1995), crop load (Jones et al., 1967), rind thickness, irrigation (Agustí et al., 2004), mineral nutrition (Bower, 2004), ethylene (Singh and Hussain, 2015a) and polyamines (Singh and Hussain, 2015b) have all been associated with the incidence of AB.

It has been suggested that the major cause of AB is due to changes in the cell wall cohesion of adjoining cells at the middle lamella at the end of stage two in fruit development (Jona et al., 1989; Storey and Treeby, 1994; Treeby and Storey, 2002). Reduced cohesion may be due to changes in the chemistry of the cell wall of albedo tissue (Monselise et al., 1976). The cell walls of albedo cells have a high pectin content and Ca salts are the main component of the middle lamella. The normal development of the albedo tissue may therefore be dependent on Ca for cell adhesion (Storey and Treeby, 1994).

Fruit affected by AB have increased pectin-methylesterase activity and water soluble pectins (Monselise et al., 1976) and disintegration of pectins leads to AB (Monselise et al., 1976; Jona et al., 1989; Li et al., 2009). Singh et al. (2014) found higher activity of pectinesterase, endo-1, 4-B-D-glucanase, exo-polygalacturonase and endo-polygalacturonase in the albedo of creased fruit compared to non-creased fruit. These enzymes appear to enhance the loss of pectins and starch in the cell walls of the albedo, leading to cell wall loosening.

Albedo breakdown has also been associated with lower levels of Ca in oranges (Storey et al., 2002; Treeby and Storey, 2002). Storey et al. (2002) reported that Ca ion concentration in Navel and Valencia are significantly lower in creased than non-creased fruit. Calcium foliar applications therefore have been successfully used to decrease the incidence of AB in a number of studies (Treeby and Storey, 2002; Storey et al., 2002; Pham et al., 2012). Calcium foliar applications increased Ca concentrations [mmol·g dry weight (DW^{-1})] in the albedo at harvest from 0.137 to 0.184 ($CaCl_2$) and 0.159 [$Ca(NO_3)_2$] (Storey et al., 2002).

It seems that Ca is required during the early developmental stages of fruit, during the cell wall formation process (Treeby and Storey, 2002). Since Ca is xylem and not phloem mobile, it is important that foliar applications should be applied directly to the surface of the fruit when required to improve penetration and efficiency, as is the case with other fruit, for example, apple (Schlegel and Schönherr, 2002; Saure, 2005).

Foliar Ca applications of 2% Ca as $CaCl_2$ or $Ca(NO_3)_2$ applied to individual 'Leng' Navel oranges increased unaffected fruit from 11% to 65-85%. Fruit were sprayed from late December to June at 10-14 day intervals. A significant decrease in the number of fruit classified as severe and moderate AB were found. There were no significant difference between the two foliar applications (Storey et al., 2002). In a similar study, five weekly foliar applications of 0.11% or 0.33% Ca [$CaCl_2$ or $Ca(NO_3)_2$], at an early stage of fruit development (81 dafb) increased Ca concentration in the rind, albedo and lead to a significant decrease in AB in 'Bellamy' Navel oranges. However, $Ca(NO_3)_2$ did not cause any leaf burn or fruit drop and was preferred (Treeby and Storey, 2002).

Foliar applications of 2% $Ca(NO_3)_2$ and surfactants except 'Triton X100' starting from 81 dafb at 10 day intervals (five applications), significantly reduced the incidence of AB in 'Washington' navel compared to the control and Ca only treatment (Pham et al., 2012). Calcium nitrate lead to a significantly thicker albedo compared to the control. Textural properties of the rind for example, hardness, cohesiveness, tensile strength, adhesiveness, and firmness was improved. In all these studies, Ca foliar applications started at 81 dafb and five or more foliar applications were used to reduce the incidence of AB.

Boron, like Ca, is a structural element of the plant cell wall (Pham, 2009). A single foliar application of B was reported to increase B concentrations in the leaf, rind and pulp and reduced the incidence of AB. The movement of Ca in leaves, may be improved by B (Shear and Faust, 1971). Furthermore, gibberellic acid (Monselise et al., 1976; Gilfillan et al., 1982) and exogenous applications of ethylene biosynthesis inhibitors such as AVG (Aminoethoxyvinylglycine) and $CoSO_4$ sprayed at golf ball stage significantly reduced the incidence of AB (Singh and Hussain, 2015a).

The aim of this study was to determine whether the chelated foliar formulation, Ca-metalosate in combination with chelated B, can reduce AB in ‘Cara Cara’ and/or ‘Turkey’ oranges.

Materials and Methods

Trial layout and site selection

The first trial was conducted in Porterville, South Africa at Dasbosch Farm (lat. 33.02°S; long. 18.99°E) during 2016/17. Two commercial sweet orange [*Citrus sinensis* (L.) Osbeck] orchards, viz. ‘Cara Cara’ and ‘Turkey’ on ‘Carrizo Citrange’ [*C. sinensis* L. (Osb.) × *Poncirus trifoliata* (L.) Raf.] rootstock (Fig. 1) were used as trial sites. The orchards have a history of high AB incidence of up to 15% (personal communication, Mr. G. Valentine). The second trail was conducted in Simondium, South Africa at Nieuwe Sion Farm (lat. 33.50°S; long. 18.57°E) during 2016/17. At this site, one commercial orchard, viz. ‘Cara Cara’ on ‘Carrizo Citrange’ rootstock, was used. This orchard has a history of high AB incidence up to 10% (personal communication, Mr. F. Du Toit).

A randomized complete block design was used with two treatments and 10 single-tree replications (n=10). Buffer trees were left between trees to ensure that no cross-contamination of spray drift from one treatment to another could occur. Each treatment was applied to entire trees for ±30s until point of run-off using a Stihl motorised knapsack-sprayer. Ca-metalosate was applied at a rate of 6 $L \cdot ha^{-1}$ (13 ml $Ca \cdot L^{-1}$ water) and B-metalosate at 1.5 $L \cdot ha^{-1}$ (0.3 ml $B \cdot L^{-1}$ water). The control received no foliar application and was left untreated. Details for each treatment are presented in Table 1.

Albedo mineral analysis

In Trial 2, conducted in Simondium, albedo samples were taken at harvest from ‘Cara Cara’. Six creased and six non-creased samples were taken at random from each treatment. Albedo was sampled by removing strips of rind from the fruit. The albedo was separated from the flavedo with a razor blade. The albedo was cut into small pieces and mineral analysis was done by a commercial laboratory [Bemlab (Pty) Ltd, Strand, South Africa].

Creasing analysis

Fruit samples for AB analysis were harvested on 10 May 2017 ‘(Cara Cara’, Trial 1), 31 May 2017 (‘Cara Cara’, Trial 2) and 19 Jun. 2017 (‘Turkey’, Trial 1). Approximately 16 kg fruit (±60 fruit) were sampled separately from each single-tree replicate. Creasing evaluation was done at the Department of Horticultural Science, Stellenbosch University. Albedo break down classification was done visually on individual fruit, classifying fruit as either creased (Fig. 2) or non-creased.

Statistical Analysis

Statistic analysis was performed in a two-way analysis of variance procedure (ANOVA) using Statistical Analysis System (SAS) programme (SAS Institute Inc, 2004, Cary, NC). A logit transformation was performed on percentage data (Snedecor and Cockran, 1997).

Results

Albedo mineral analysis

A lower Ca concentration was found in the albedo tissue of creased fruit compared to non-creased ‘Cara Cara’ fruit in Trial 2 (Table 2).

Albedo breakdown evaluation

No significant differences in AB was found between treatments in any of the trials (Table 3).

Discussion

The aim of this study was to determine whether the chelated foliar formulation, Ca-metalosate in combination with chelated B, is effective in reducing AB in ‘Cara Cara’ and/or ‘Turkey’ oranges.

Albedo mineral analysis

Albedo breakdown mineral analysis showed lower Ca concentration in the albedo tissue (treatment and control) of creased fruit compared to non-creased fruit in Trial 2 (Table 2). This supports Storey et al. (2002) who reported that Ca ion concentration in Navel and Valencia is significantly lower in creased than non-creased fruit. Navel had a Ca-ion concentration (mmol·g DW^{-1}) of 0.246 for non-creased and 0.151 for creased fruit. Valencia had a Ca-ion concentration (mmol.g DW^{-1}) of 0.225 for non-creased and 0.171 for creased fruit. Thus, Ca seems to play a role in the incidence of creasing (AB).

Albedo breakdown evaluation

Ca-metalosate in conjunction with B failed to significantly reduce the incidence of AB in ‘Turkey’ and/or ‘Cara Cara’ oranges in our trials. This is in contrast with reports where foliar applications of $Ca(NO_3)_2$ and $CaCl_2$ have been successfully used to decrease the incidence of AB (Treeby and Storey, 2002; Storey et al., 2002; Pham et al., 2012). In the present study foliar applications were applied only three times during bloom and this may partly explain why Ca-metalosate in combination with B failed to reduce the incidence of AB. The major cause of AB is due to changes in the cell wall cohesion of adjoining cells at the middle lamella at the end of stage two in fruit development (Jona et al., 1989; Storey and Treeby, 1994; Treeby and Storey, 2002). This may explain why Ca foliar applications applied at 81 dafb can be successful to reduce the incidence of AB. Another possible reason for no result with the Ca-metalosate application may be due to the formulation.

Foliar applications of chelates and complexes (humic, glucoheptonates, amino acids, lignosulphonates, EDTA, fulvic, polyols etc.) are widely used (Abadía et al., 2002). Inorganic foliar applications, for example $CaCl_2$ and $Ca(NO_3)_2$ are also an alternative.

However, penetration depth and concentration vary between different Ca formulations in tomatoes (Lötze and Turketti (2014). Therefore, although different Ca formulations are used for foliar applications on various crops, this chelate formulation may have been less efficient in the case of citrus with regards to e.g. penetration as physio-chemical factors have been reported to influence the efficiency and penetration of foliar applications (Schlegel and Schönherr, 2002).

Conclusion

In both trials, Ca-metalosate in combination with B-metalosate failed to significantly reduce the incidence of AB in 'Turkey' and 'Cara Cara' oranges. Five foliar applications of $Ca(NO_3)_2$ and $CaCl_2$ have been used successfully to reduce the incidence of AB. Therefore, further research under South-African conditions should be encouraged with an alternative protocol, similar to the ones reported above. This should entail five or more applications of Ca-metalosate, starting later in the season (81 dafb), when the disintegration of the albedo starts. Albedo mineral analysis indicated that AB fruit (treatment and control) had a lower Ca concentration compared to non-creased fruit in Trial 2, indicating a definite role of Ca in this defect. It will thus be essential to include a complete albedo mineral analysis in future trials to determine whether the chelate penetrates and increases the Ca concentration in the albedo to the same levels reported in trials where Ca salts successfully reduced AB. This could confirm the efficiency of formulation on the penetration of foliar applications and the use of alternative foliar applications for addressing AB in citrus.

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Tables and Figures

Table 1. Ca-metalosate and B treatment application dates during 2016/17.

Ca Treatments	‘Cara Cara’ (Trial 1)	‘Turkey’ (Trial 1)	‘Cara Cara’ (Trial 2)
1st application date	80% full bloom	60% full bloom	40% full bloom
2nd application date	Full bloom	80% full bloom	70% full bloom
3rd application date	Petal drop	Petal drop	Petal drop

Table 2. Albedo mineral analysis at harvest for ‘Cara Cara’ during 2016/17 (Trial 2).

Treatments	P%	K%	Ca%	Mg%	N%
Control non-creased	0.05	0.41	0.69	0.07	0.49
Control creased	0.05	0.39	0.61	0.07	0.55
Ca non-creased	0.05	0.38	0.76	0.06	0.49
Ca creased	0.04	0.36	0.63	0.08	0.50

Table 3. AB evaluation results for each treatment in trial 1 and 2, during 2016/17.

	‘Cara Cara’ (Trial 1)		‘Turkey’ (Trial 1)		‘Cara Cara’ (Trial 2)	
Treatments	Creased %	Creased logit	Creased %	Creased logit	Creased %	Creased logit
Control	19.8 ns	0.77	3.3 ns	3.53	21.6 ns	1.51
Ca-metalosate	15.3	3.21	3.5	3.21	27.9	1.48
P-value		0.2191		0.4704		0.0682

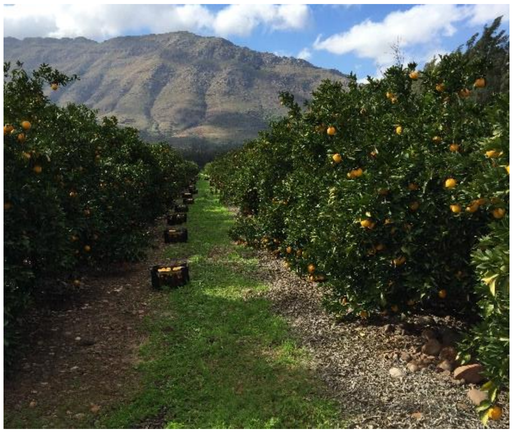

Fig.1. ‘Turkey’ orange trees on ‘Carrizo Citrange’ rootstock at the Porterville site.

Fig.2. ‘Turkey’ orange showing albedo breakdown used for visual classification of fruit.

PAPER 3

Xylem functionality in developing fruitlets of different apple cultivars.

Abstract

Calcium (Ca) is primarily transported to fruitlets via the xylem. In order for Ca to enter fruit, the xylem must remain functional throughout fruit development. Fruit high in Ca have stronger cell walls and increased membrane stability that reduces the incidence of Ca-related disorders, such as bitter pit (BP). The aim of the study was to determine whether a relationship exists between the loss of xylem functionality early in the season, and Ca-related disorders in susceptible and less susceptible apple cultivars. If a relationship exists, this can be used to indicate the most effective time to start additional Ca foliar applications in susceptible cultivars to reduce Ca-related disorders. Fruit were selected from trees in commercial orchards on Applethwaite Farm in Elgin, Welgevallen Research Farm in Stellenbosch and Lourensford Estate in Somerset West. Weekly samples of ten fruit per cultivar were harvested until a steady decline in number of stained vascular bundles was noticed. Xylem functionality was determined using a simple dye fuchsin (1%) technique. In season 2, a steady decline of xylem functionality was observed in all six cultivars from ±56 days after full bloom (dafb)-supporting the recommendation that Ca foliar applications should start before 40 dafb. Results indicated an earlier loss of xylem functionality for 'Granny Smith' and 'Fuji' (less susceptible) at 49 dafb than for 'Braeburn' (susceptible) at 56 dafb. Xylem functionality slightly recovered throughout the season before a total loss of xylem functionality was reached later in the season. No relationship between loss of xylem functionality early in the season and Ca-related disorders could be established.

Keywords: bitter pit; calcium; dye fuchsin; foliar applications; *Malus domestica*

Introduction

Nutrients are transported to growing tissues in apple fruit (*Malus domestica)* through vascular bundles (Dražeta et al., 2004). According to Dražeta et al. (2004) fruit vascular bundles are divided into two systems known as the cortical vessels and carpels. The fruit carpel is surrounded by ten primary bundles to form the cortical vascular system. The primary bundles branch inward towards the epidermis and form a secondary cortical vasculature system. Five dorsal and ten ventral bundles are found in the carpel vascular system. These bundles appear from the penduncle and pass through the carpels in anastomosis and end in the pistil. Vascular bundles are composed of two vascular tissues namely xylem and phloem (Dražeta et al., 2004).

Transport of water and inorganic solutes occurs in the xylem, which consists of dead cells (Miqueloto et al., 2014). Calcium is highly mobile in the xylem of plants and highly immobile in the phloem (Himelrick and McDuffie, 1983). As apple fruit mature, the number of xylem vessels start to break down (Lang and Ryan, 1994). The rate of xylem flow is therefore reduced and a larger contribution is made by the phloem (Lang, 1990). However, only five percent of Ca is re-allocated from the leaves to the fruit via the phloem, rendering this contribution inefficient to supply in the fruit's demand for Ca (Saure, 2005).

Calcium transport is negatively influenced by the loss of xylem functionality in a number of species as the fruit matures (Lang, 1990; Lang and Ryan, 1994; Dichio et al., 2003; Dražeta et al., 2004; Miqueloto et al., 2014). The increase in number (Rančić et al., 2010) and/or elongation of parenchyma cells compresses the xylem vessels as the fruit matures (Lang and Ryan, 1994) and may cause loss of xylem functionality. Microscopy revealed that expansion of the fruit flesh caused the xylem to physically disrupt (Dražeta et al., 2004). The expression of aquaporin genes has been found to change throughout the season and have a significant effect on the hydraulic conductance of water in grape berries (Choat et al., 2009). This however, does not affect the functionality of the phloem. In contrast, Clearwater et al. (2012) found that the xylem and phloem contribute to the growth of kiwifruit and both participate in the inflow of water and remain functional, even in the later stages of fruit development.

Dražeta et al. (2004) recorded weekly intervals of xylem functionality in apples, until 133 dafb. On each occasion, fifteen fruit were evaluated for xylem functionality, using a simple dye infusion technique (1% aqueous acid fuchsin) that stains the active xylem. At the base of the spur fruit were cut, under distilled water, to avoid any air embolism prior to immersion. Fruit were placed in a vial containing dye for 2h under standard transpiration conditions. Fruit were sectioned equatorially to score dye intensity. Xylem disintegration can start as early as 45 dafb, depending on the cultivar (Dražeta et al., 2004). The loss of xylem functionality in 'Granny Smith' started as late at 67 dafb, but for 'Braeburn', it was as early as 45 dafb. This may have an effect on bitter pit (BP) incidence (Dražeta et al., 2004; Neilsen et al., 2005), as 'Braeburn' is a very susceptible cultivar, whereas 'Granny Smith' is not prone to BP. Miqueloto et al. (2014) reported that 'Fuji' apples (lower susceptibility to BP) have higher fruit Ca concentrations at harvest compared to 'Catarina' apples (higher susceptibility to BP). This may be partly explained by the loss of xylem functionality that occurs later during the season in 'Fuji' compared to 'Catarina'.

In kiwifruit, it was suggested that the amount of water that enters the xylem is reduced over time, due to the anatomical changes that take place in the xylem vessels and on the fruit epidermis (Morandi et al., 2009). This is due to the suberization of the cell wall and loss of trichome functionality on the fruit epidermis later in the season (Hallett and Sutherland, 2005). Furthermore, the functionality of the xylem recovered throughout the season (Dichio et al., 2003). A reduction in xylem functionality was found around 20, 55 and 90 dafb. Recovery between these three stages has been noticed (50-90%), and a complete loss of xylem functionality was noticed around 120 dafb. This has not been reported in apples so far. However, less susceptible apple cultivars may experience a later loss of xylem functionality due to a greater number/variability of procambial cells (Chatelet et al., 2008). New xylem (protoxylem and metaxylem) is formed by procambial cells that may replace disrupted xylem and increase xylem functionality in apples.

This study was conducted to determine whether a relationship exists between the loss of xylem functionality early in the season and Ca-related disorders for susceptible (Golden Delicious and Braeburn) and less susceptible cultivars (Fuji, Cripps Pink and Granny Smith) under South African conditions. If a relationship can be established, results can be applied to indicate the most effective time to start additional Ca foliar applications in susceptible cultivars to reduce the incidence of Ca-related disorders, such as BP.

Materials and Methods

Trial layout and site selection

During 2015/16, fruit were sampled from 'Braeburn' and 'Fuji' trees in commercial orchards on Applethwaite Farm in Elgin (34.1570° S;19.0152° E), 'Royal Gala' trees on Welgevallen Research Farm in Stellenbosch (33.9427° S; 18.8664° E) and 'Golden Delicious', 'Granny Smith', 'Cripps Pink' and 'Cripps Red' at Lourensford Estate in Somerset West (34.0719° S; 18.8885° E). During 2016/17, fruit were sampled from 'Braeburn', 'Fuji', 'Golden Delicious' and 'Granny Smith' trees in commercial orchards on Applethwaite Farm in Elgin, and 'Cripps Pink' and 'Royal Gala' trees at Welgevallen Research Farm in Stellenbosch. Different areas were selected to determine whether climate would affect xylem functionality noticeably. Weekly samples of ten fruit per cultivar were harvested on selected dates until a steady decline in xylem functionality was noticed. Average size fruit on spurs were selected. Apples were evaluated at the Department of Horticulture Science, Stellenbosch University.

Determination of active xylem

The cultivars were harvested on different dates and sampling times were indicated as days after full bloom (dafb) (Tables 1 and 2). Apples were harvested randomly from x trees early in the morning when plant transpiration was minimal, using a pruning secateurs and removing the branch adjacent to the fruit cluster, with cuts at least 15 cm from fruit, to avoid any air from gaining entry into the xylem. Apples were placed in polyethylene plastic bags and transported in a cool box to further prevent air embolism.

Xylem functionality was determined using a simple dye fuchsin technique that stains the active vascular bundles (Drażeta et al., 2004). To determine the active xylem, 1 g of fuchsin was mixed with 100 ml distilled water (1%). Individual apples were cut from the spur at the base of the stalk under water to further avoid any air embolism prior to immersion. Fuchsin (1%) was then absorbed through the peduncle of active xylem. This was carried out under normal laboratory conditions. Apples were left for 45-60 min in a laminar flow bench before xylem evaluation (Fig. 1). A minora blade was used to section ten fruit per cultivar equatorially and minimize any tissue damage. The ten vascular bundles in transverse sections of each fruit were clearly visible and stained bundles were counted. This was done visually on individual fruit (Fig. 2). At each sampling date, fruit diameter was measured with an electronic Vernier calliper.

Statistical Analysis

Statistics in this study was performed using the Standard error procedure in Microsoft Excel (2016).

Results and Discussion

Tables 1 and 2 represent the dafb (not actual dates) of sampling, and the mean number of active xylem bundles out of a possible 10 per cultivar for 2015/16 and 2016/17. Table 3 represent the dafb (not actual dates) of evaluation, and the mean diameter (mm) for each cultivar during 2016/17.

Season 1(2015/16)

During season 1, no complete loss of xylem functionality occurred in any of the seven cultivars evaluated, for any of the sites (Table 1). This is in contrast to previous findings in a number of fruit species (Lang, 1990; Lang and Ryan, 1994; Dichio et al., 2003; Dražeta et al., 2004; Miqueloto et al., 2014). Results in the first season was however difficult to evaluate due to challenges with the solution (dye) used in the first few weeks. Excessive ‘bleeding’ was caused by 1% fuchsin with 70% alcohol mixed with 30% distilled water as recommended (Miqueleto et al., 2014). This however made visual evaluation very difficult. The solution was then changed to a 1% fuchsin with 30% distilled water during 2016/17 that improved evaluation (Draźeta et al., 2004).

Season 2 (2016/17)

In spite of climatic differences, fruit from Stellenbosch and Elgin (Table 4 and 5) indicated similar progression of loss of xylem functionality (Table 2).Results indicated a steady decline in xylem functionality in all six cultivars evaluated. This supports previous findings in a number of species (Lang, 1990; Lang and Ryan, 1994; Dichio et al., 2003; Dražeta et al., 2004; Miqueloto et al., 2014). Our results indicated an earlier loss of xylem functionality for 'Granny Smith' and 'Fuji' (less susceptible cultivars to BP) after 49 dafb, than for 'Braeburn' (susceptible) at 56 dafb. This is in contrast to previous findings (Dražeta et al., 2004; Miqueloto et al., 2014). Therefore, no clear relationship between loss of xylem functionality early in the season and Ca-related disorders for susceptible (Golden Delicious and Braeburn) and less susceptible cultivars (Fuji, Cripps Pink and Granny Smith) was found.

A slight increase in xylem functionality occurred between 63-70 dafb for all cultivars. This may indicate a recovery of the functionality of the xylem after 63 dafb. This concurs, with Dichio et al. (2003) who reported recovery between breakdown stages (20, 55 and 90 dafb) in kiwifruit and Chatelet et al. (2008) who reported that new xylem (protoxylem and metaxylem) can be formed by procambial cells that later replace disrupted xylem and slightly increase xylem functionality.

Findings in this study indicated that all cultivars evaluated started to experience a loss of xylem functionality at ±56 dafb, supporting findings by Dražeta et al. (2004) that xylem disintegration can start as early as 45 dafb. All apple cultivars indicated a rapid expansion of fruit flesh ±49 dafb (Table 3). As expansion of the fruit flesh causes physical disruption of the xylem, this could have led to the loss of xylem functionality starting ±56 dafb as reported by Dražeta et al. (2004). Calcium movement into fruit may therefore be negatively influenced after this stage.

Conclusion

Results obtained from Season two indicated a steady decline in xylem functionality from ±56 dafb in all apple cultivars evaluated and support previous findings in a number of species (Lang, 1990; Lang and Ryan, 1994; Dichio et al., 2003; Dražeta et al., 2004; Miqueloto et al., 2014). This supports the recommendation by Lötze and Theron (2006) that additional Ca foliar applications should start before 40 dafb to decrease the likelihood of Ca-related disorders in apple due to xylem dysfunction.

No relationship between loss of xylem functionality early in the season and Ca-related disorders could be established, and contrasts previous reports (Dražeta et al., 2004; Miqueloto et al., 2014). Results indicated that xylem functionality slightly recovers later in the season before a total loss of xylem functionality is reached late in the season under both climatic conditions (Dichio et al., 2003).

Further research under South African conditions should establish whether apple cultivars showing an earlier loss of xylem functionality (Braeburn and Golden Delicious) are more prone to Ca-related disorders. These studies should commence earlier, at 28 dafb, and be continued for longer, until harvest, to determine whether xylem functionality decreases and later slightly increases throughout fruit development. Microscopy studies will assist in confirming physical disruption of xylem bundles in the fruit cortex as primary cause for loss of xylem functionality.

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Tables and Figures

Table 1. Evaluation dates per cultivar (DAFB) and the average number of active xylem bundles out of a possible 10 for each cultivar during 2015/16, showing the mean followed by the standard deviation (±).

DAFB	'Braeburn'	'Cripps Pink'	'Cripps Red'	'Fuji'	'Golden Delicious'	'Granny Smith'	'Royal Gala'
47				6±1.07			
54							6±1.04
61	1±0.90						
68				2±0.43			
75					2±0.50	1±0.27	
82	5±1.24	2±0.4		5±0.75			
89							3±0.68
96	6±0.86		2±0.25	6±1.11	4±0.48	2±0.28	
103		4±0.85		5±1.18			5±1.05
110	5±0.90						7±0.81
117	5±0.57		2±0.47		7±0.84	5±0.55	
124		5±1.0			3±0.91	4±1.03	
131		9±0.72	6±0.82		3±1.03	7±1.21	
138		8±0.65	5±0.86				
145			6±1.07				

Table 2. Evaluation dates per cultivar (DAFB) and the average number of active xylem bundles out of a possible 10 for each cultivar during 2016/17, showing the mean followed by the standard deviation (±).

DAFB	'Braeburn'	'Cripps Pink'	'Fuji'	'Golden Delicious'	'Granny Smit'	'Royal Gala'
42			9.6±0.27		7.4±0.86	
49	9±0.33	9.9±1.0	4.3±0.42	7.5±0.91	6.2±0.55	9.8±0.13
56	7.6±0.35	4±0.49	2±0.38	3.5±0.62	1.3±0.33	5.8±0.61
63	1.9±0.35	1.8±0.44	4.4±0.79	1.6±0.27	1.8±0.29	2.4±0.45
70	2.2±0.36	2.4±0.48	3.9±0.38	2.2±0.29	2.3±0.45	4.6±0.67
77	3.1±0.53	1.9±0.43		1.8±0.42		4.4±0.48

Table 3. Evaluation dates per cultivar (DAFB) and the average diameter (mm) for each cultivar during 2016/17, showing the mean followed by the standard deviation (±).

DAFB	'Braeburn'	'Cripps Pink'	'Fuji'	'Golden Delicious'	'Granny Smit'	'Royal Gala'
42			25.8±2.92		27.5±1.24	
49	37.4±1.80	29.2±0.66	29±0.83	25.1±0.74	31.4±1.37	33.9±0.54
56	41.1±0.67	33.4±0.77	34.1±0.42	31.2±0.59	33.5±1.06	31.9±0.55
63	42.5±0.66	37.8±1.52	41.9±0.83	31.7±0.80	35.9±0.97	35.9±0.72
70	45.2±0.37	41.5±1.07	41.3±0.80	35±0.55	40.5±0.79	38.8±1.32
77	45.4±0.61	39.2±0.67		39.2±0.36		34.5±0.82

Table 4. Temperature (ºC) and rainfall (mm) for Stellenbosch, during 2015/16 (HORTEC, 2017).

	Minimum Temp		Maximum Temp		Average Temp		Total Rainfall	
Month	2015	2016	2015	2016	2015	2016	2015	2016
September	4.4	4.1	31.3	26.9	14.5	13.3	33	53
October	5.1	5	37.1	33.7	16.9	15.8	12	17
November	6.5	6.9	35.2	34.3	17.6	18.7	22	5
December	8.8	9.1	39.9	34.2	20.5	20.2	35	18

Table 5. Temperature (ºC) and rainfall (mm) for Elgin, during 2015/16 (HORTEC, 2017).

	Minimum Temp		Maximum Temp		Average Temp		Total Rainfall	
Month	2015	2016	2015	2016	2015	2016	2015	2016
September	3.8	3.6	30.6	24.5	13.1	12.3	56	75
October	3.6	5.1	34.4	31.5	16	14.7	14	25
November	6.3	6.5	33.4	31.4	16	16.6	44	14
December	8.5	9.8	35.4	31.7	19.3	19	19	42

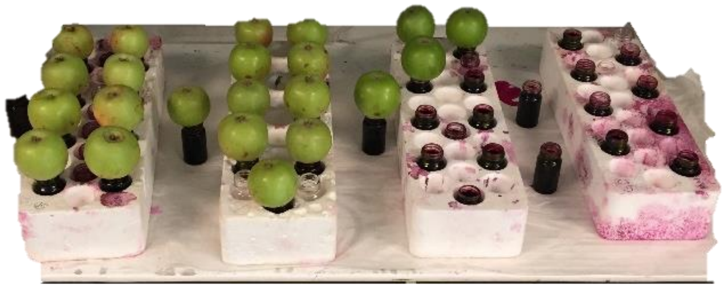

Fig.1. Apples left for 45-60 min in a laminar flow bench before xylem evaluation.

Fig.2. Fuchsin (1%) dye showing two active (2016/17), no active (2016/17) vascular bundles and excessive bleeding (2015/16) in ‘Golden Delicious’ apples

GENERAL DISCUSSION AND CONCLUSION

Calcium (Ca) foliar applications are used to improve the Ca status of fruit and control the incidence of Ca-related disorders, for example bitter pit (BP) in apples (Kirkby and Pilbeam, 1984) and albedo breakdown (AB) in citrus (Treeby and Storey, 2002). After applying Ca foliar applications, penetration and efficiency are influenced by unique physio-chemical properties of the minerals found in the foliar applications (Fernández et al., 2013). Point of deliquescence (POD), pH, concentration, molecular size/weight and solubility are all physio-chemical factors that influence the penetration into leaves and fruit.

The main aim of the study was to determine whether Ca-metalosate as an alternative Ca formulation, is effective in decreasing Ca-related disorders in fruit trees.

In the first paper, the aim was to determine whether a chelated foliar formulation, viz. Ca-metalosate, is as effective as the salt formulation, calcium nitrate [$Ca(NO_3)_2$] in reducing bitter pit (BP) in 'Golden Delicious' apples. In Trial 1, at 80 days after full bloom (dafb), fruit treated with $Ca(NO_3)_2$ had a significantly higher Ca concentration compared to the Ca-metalosate treatment and control (no foliar applications). Calcium concentration of fruit was increased more efficiently by $Ca(NO_3)_2$ than Ca-metalosate foliar applications (penetration). The active Ca concentration in a foliar application therefore has an effect on the penetration of foliar applications. This supports findings in a number of studies that foliar applications with a lower active Ca concentration, for example Ca-metalosate, penetrate fruit less efficiently compared to foliar applications with a higher active Ca concentration, for example $Ca(NO_3)_2$ (Mayr and Schroder, 2002; Neilsen and Neilsen, 2002; Schlegel and Schönherr, 2002; Wilsdolf et al., 2011; Lötze and Turketti, 2014).

Calcium nitrate foliar applications have a lower POD compared to Ca-metalosate foliar applications, and this may explain increased penetration 80 dafb (Schönherr, 2002; Lötze and Turketti, 2014). Calcium metalosate foliar applications have a higher molecular weight compared to $Ca(NO_3)_2$ foliar applications, which may explain the decreased penetration 80 dafb. This supports findings in a number of studies that foliar applications with a higher molecular weight have less effective penetration compared to foliar applications with a lower molecular weight (Schönherr, 2002; Thalheimer and Paoli, 2002; Furuya and Umemiya, 2002).

In Trial 2 the Ca concentration of fruit at 80 dafb did not differ significantly between treatments. This does not support previous findings that certain foliar applications have decreased penetration due to lower Ca concentration (active), higher POD and molecular weight/size. Treatments did not alter the quality of fruit. Calcium nitrate did however cause a significantly greener peel ground colour, confirming previous reports (Wilsdorf, 2011). The nitrogen component in $Ca(NO_3)_2$ probably caused the greener colour.

The average Ca concentration of fruit (Trail 1) from the different treatments were 2.98 [$Ca(NO_3)_2$], 2.43 (control), and 2.59 mg·100 g^{-1} (Ca-metalosate), respectively at harvest, after extrapolation of results 80 dafb. As suggested by Terblanche (1985), these Ca concentrations at harvest were well below the threshold value for low BP incidence. Never less, Ca-metalosate (0.2%) and $Ca(NO_3)_2$ (0.0%) had a very low BP incidence. This raises the question whether Ca concentration at 80 dafb should not be used to predict BP incidence instead of the final Ca concentration at harvest. However, no existing threshold values for prediction of BP at 80 dafb exist.

Bitter pit incidence did not differ significantly between $Ca(NO_3)_2$ and Ca-metalosate treatments in Trial 1, although $Ca(NO_3)_2$ had a significantly lower BP incidence compared to the control. Bitter pit incidence between the Ca-metalosate and control did not differ significantly. Calcium concentration in fruit 80 dafb was not increased by the Ca-metalosate in combination with chelated B. In this trial, B failed to improve the mobility of Ca in the fruit significantly as measured by fruit Ca concentration, which disagrees with Shear and Faust (1971).

In the second paper, the aim was to determine whether the chelated foliar formulation, viz. Ca-metalosate in combination with chelated B, is effective in reducing albedo breakdown (AB) in ‘Cara Cara’ and/or ‘Turkey’ oranges. The albedo tissue of AB fruit showed a lower Ca (%) in the treatment and control compared to non-AB fruit in Trial 2. This supports previous findings in Navel and Valencia that Ca-ion concentration in the albedo tissue (mmol·g DW^{-1}) of AB fruit is lower than in non-AB fruit (Storey et al., 2002). Therefore, AB incidence is linked to Ca.

In both trials, the incidence of AB in 'Turkey' and/or 'Cara Cara' oranges was not significantly reduced by Ca-metalosate in combination with B foliar applications. However, five or more $Ca(NO_3)_2$ or $CaCl_2$ foliar applications, starting at 81 dafb were used to successfully decrease the incidence of AB in oranges (Treeby and Storey, 2002; Storey et al., 2002; Pham et al., 2012). In this study, foliar applications of Ca-metalosate in combination with chelated B was applied only three times during bloom, as recommended by the suppliers (protocol). This may partly explain why Ca-metalosate failed to reduce the incidence of AB in this study.

The development of AB takes place at the end of stage two in fruit development and is due to changes in the cell wall cohesion of adjoining cells at the middle lamella (Jona et al., 1989; Storey and Treeby, 1994; 2002). Therefore, to reduce the incidence of AB, it is recommended that foliar applications should be applied 81 dafb and re-evaluated with Ca-metalosate. The formulation of Ca-metalosate foliar applications differs from that used in previous studies (salts). In tomatoes, different Ca formulations applied as foliar application resulted in differences in penetration depths and concentrations in the tomato peel (Lötze and Turketti, 2014). The penetration and efficiency of foliar applications have also been reported to be influenced by physiochemical-factors (Schlegel and Schönherr, 2002). In the case of citrus, this chelate formulation (Ca and B) may have been less efficient, although different Ca formulations are being used for foliar applications on various crops in practice.

In paper 3, the aim of the study was to determine whether a relationship exists between the loss of xylem functionality early in the season and Ca-related disorders for susceptible (Golden Delicious and Braeburn) and less susceptible apple cultivars (Fuji, Cripps Pink and Granny Smith). If a relationship exists, it can be applied to indicate the most effective time to start additional Ca foliar applications in susceptible cultivars to reduce the incidence of Ca-related disorders, such as BP, or to identify new cultivars that may be susceptible to BP early during evaluation.

During 2015/16, all seven apple cultivars evaluated indicated no complete loss of xylem functionality and does not support previous studies (Lang, 1990; Lang and Ryan, 1994; Dichio et al., 2003; Dražeta et al., 2004; Miqueloto et al., 2014). During 2016/17, all six apple cultivars evaluated indicated a steady decline in xylem functionality and supports previous studies (Lang, 1990; Lang and Ryan, 1994; Dichio et al., 2003; Dražeta et al., 2004; Miqueloto et al., 2014). In this study Granny Smith and Fuji apple cultivars (less susceptible) indicated an earlier loss of xylem functionality at 49 dafb than for Braeburn (susceptible) at 56 dafb. This is in contrast with previous findings (Dražeta et al., 2004; Miqueloto et al., 2014).

Thus, no relationship between the loss of xylem functionality early in the season and Ca-related disorders in susceptible (Golden Delicious and Braeburn) and less susceptible cultivars (Fuji, Cripps Pink and Granny Smith) could be established in this study. In both seasons, in Elgin and Stellenbosch, similar progression of loss of xylem functionality was reported despite climatic differences. All six apple cultivars indicated a slight increase in xylem functionality between 63-70 dafb, supporting the statement made by Dichio et al. (2003) that xylem functionality may slightly recover in between the breakdown stages (20, 55 and 90 dafb) as in kiwifruit. Procambial cells form protoxylem and metaxylem (new xylem) that later replace disrupted xylem and increase xylem functionality (Chatelet et al., 2008).

All six cultivars evaluated indicated a steady loss of xylem functionality ±56 dafb, supporting Dražeta et al. (2004) who reported that xylem disintegration can start as early as 45 dafb, depending on the cultivar. Vascular bundles failed to show any dye movement (staining) as the season progressed due to physical disruption of the xylem caused by the expansion of the fruit flesh ±49 dafb (Dražeta et al., 2004). After this stage the movement of Ca into the fruit may be negatively influenced. Therefore, additional Ca foliar applications to reduce the incidence of Ca-related disorders should start before 40 dafb as motivated by Lötze and Theron. (2006).

This study contributed towards our understanding of Ca-metalosate as an alternative Ca formulation for decreasing Ca related disorders in fruit trees. Unique physio-chemical properties of Ca foliar applications seem to influence the efficiency and penetration of Ca into fruit and leaves. Findings in this study support reports that Ca foliar applications with the highest possible Ca concentration (active), lowest POD, lowest molecular size/weight, and a pH between 3 and 5, should be selected for decreasing Ca-related disorders in fruit trees. The physio-chemical properties of $CaCl_2$ and $Ca(NO_3)_2$ foliar applications seem to increase the likelihood of increased penetration and efficiency, while that of Ca-metalosate foliar applications did not achieve this goal in the presented trials.

Secondly, this study contributed towards our understanding of xylem functionality in developing fruitlets of different apple cultivars. Around ±56 dafb, apples experience a steady decline of xylem functionality. This supports previous findings and the recommendation that additional Ca foliar applications should start before 40 dafb. However, further research under South African conditions should be performed to determine whether apple cultivars experiencing an earlier loss of xylem functionality, are more prone to Ca-related disorders.

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