Aspects of mineral nutrition affecting fruit quality of 'Nadorcott' mandarin

by

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Summary

Mineral nutrition of *Citrus* is one of the key controllable factors influencing fruit quality. The mineral nutrients known to have the greatest impact on *Citrus* fruit quality, in order of magnitude of effect, are nitrogen (N), potassium (K) and phosphorous (P). The timing of application together with the amount of fertiliser applied is of critical importance in determining the impact it will have on *Citrus* fruit quality, including any possible long-term effects on tree growth, development and phenology. Three experiments were conducted to study fruit quality of 'Nadorcott' mandarin, taking both these above-mentioned considerations into account. The first experiment explored the known influence of P on decreasing citric acid content of *Citrus* fruit. In production areas with cold winters, harvest dates can be delayed by high fruit acid content, to such an extent that flowering is detrimentally impacted and hence also the fruit yield of the following season. Results obtained indicated that the citric acid content of fruit was significantly decreased by mono-ammonium phosphate (MAP) sprays, applied at seven and/or eight weeks after full bloom (WAFB). The second and third experiments concentrated on the influence of late N application on 'Nadorcott' fruit quality when studied in combination with different irrigation regimes and under different climatic conditions, respectively. The second experiment showed that, when the influence of N is considered in combination with irrigation, it has a more pronounced effect on *Citrus* fruit quality than exerted by N alone. Over-irrigation can have the most serious negative effect on fruit quality when all the factors studied were considered, therefore it is proposed that irrigation is likely to be the main determinant for success when managing N fertilisation. In the third experiment where the effect of late N in different forms on fruit quality was studied, the influence thereof was found to be insignificant. In addition, none of the N applications negatively affected rind quality, including that of colour development or the incidence of disorders. Beneficial effects on flowering for the following season were, however, also not recorded. Currently it can be concluded that since the application of late N had negligible effects on fruit quality in this study it may be used in an orchard with a known N deficiency, later in the season, subject to the prerequisite that the orchard is not over-irrigated. This study confirmed the complexity of mineral nutrition in citriculture, based on the large number of factors that influence fruit quality. Future research should attempt to establish the ideal time of MAP application, also taking different concentrations and production areas into account. Late N application trials should be expanded to an in-depth study of the effect of the N status of the tree on flower initiation, whilst establishing the production conditions under which this practice should be recommended.

Opsomming

Die minerale voeding van *Citrus* is een van die vernaamste beheerbare faktore wat vruggehalte beïnvloed. Die drie minerale nutriënte wat die grootste invloed op *Citrus* se vruggehalte het, is, in volgorde van die impak, stikstof (N), kalium (K) en fosfor (P). Tydsberekening asook die hoeveelheid kunsmisstowwe wat toegedien word, bepaal in ’n groot mate watter uitwerking dit op *Citrus*-vruggehalte en die langtermyngroei, ontwikkeling en fenologie van die boom sal hê. Met die inagneming van die bogenoemde was daar drie eksperimente uitgevoer om vruggehalte van ‘Nadorcott’-mandaryne te bestudeer. Die eerste eksperiment was gebaseer op die erkende feit dat P die sitroënsuurinhoud van *Citrus* verlaag. ’n Hoë vrugsuurinhoud kan problematies wees in koue produksie-areas weens die geassosieerde uitstel van die oesdatum. Gevolglik kan dit blomtyd en vrugopbrengs vir die komende seisoen nadelig beïnvloed. Die sitroënsuurinhoud was beduidend verlaag deur mono-ammoniumfosfaat (MAP) blaarspuite wat sewe en/of agt weke na volblom (WNVB) toegedien was. Die tweede en derde eksperimente het onderskeidelik gefokus op die invloed van laat N-toediening op vruggehalte wanneer dit gekombineerd was met verskillende besproeiingsvolumes, asook soos toegedien in verskillende klimaatsomstandighede. In die tweede eksperiment is bevind dat die invloed van N op *Citrus*-vruggehalte groter is in kombinasie met besproeiing as wanneer N alleen in ag geneem word. Oorbesproeiing kan die grootste nadelige uitwerking op vruggehalte hê, nadat alle faktore oorweeg was. Besproeiing blyk die belangrikste bepalende faktor te wees in die bestuur van N-bemesting. In die derde eksperiment was slegs die effek van laat N toediening in verskillende vorms op vruggehalte ondersoek en die invloed daarvan was nie beduidend nie. Verder is daar met geen van die N-toedienings ’n nadelige invloed op skilkwaliteit, insluitend kleurontwikkeling en die voorkoms van skildefekte, aangeteken nie. Daar was egter ook geen voordelige uitwerking op blom vir die daaropvolgende seisoen waargeneem nie. Die gevolgtrekking is dus dat die toediening van laat N ’n weglaatbare effek op vruggehalte het en in ’n boord met ’n N-tekort aanbeveel kan word, op die voorwaarde dat nie oorbesproei word nie. Die kompleksiteit van die minerale voeding van *Citrus* weens al die bydraende faktore betrokke by vruggehalte is weereens gestaaf deur die resultate verkry in hierdie studie. Voorgestelde toekomstige navorsing kan fokus om die ideale tyd van MAP-toediening vas te stel, asook om verskillende konsentrasies daarvan en ook verskeie produksie areas in te sluit. Laat N-toedieningsproewe kan uitgebrei word tot ’n in-diepte studie van die uitwerking van die N-status van die boom op blom-inisiasie, asook in watter produksie kondisies dit ’n aanbevole praktyk kan wees.

Note

This thesis is a compilation of chapters, starting with a literature review, followed by four research chapters. The referencing and formatting style in this thesis is written according to the requirements, in general, of the Journal of the American Society for Horticultural Science.

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Chapter 1: General introduction

The high financial returns associated with 'Nadorcott' mandarin have led to this high value cultivar being the most planted *Citrus* hybrid in South Africa over the past few years (CGA, 2016). However, as competition became more intense with the increase in production of this profitable mandarin, the demand for high fruit quality of 'Nadorcott' also became increasingly more important (Stander and Cronjé, 2016). Many factors, both controllable and uncontrollable, influence *Citrus* fruit quality during production, with one of the most important factors under the control of the producer, being mineral nutrition.

Mineral nutrition in *Citrus* has been researched extensively and as a result, various guidelines and recommendations in fertilisation regime have been developed, as nutrition requirements may differ significantly between *Citrus* cultivars due to variation on genetic basis and harvest date. Some examples of publications covering mineral nutrition of *Citrus* include Chapman (1968), Smith (1966) and Ting and Attaway (1971) and more recently fertilisation manuals focusing on specific growing areas such as "Nutrition of Florida Citrus Trees" by Obreza and Morgan (2008), or countries such as "Fertilization of Citrus" by Coetzee (2007). Previous research focused primarily on conventional soil applications. More recently the use of foliar sprays which target specific phenological stages, at times when the greatest impact could be expected, received attention as certain vegetative and reproductive growth phases will have different demands for specific nutrients (Lovatt, 2009).

The mineral nutrition of 'Nadorcott' mandarin cannot be studied in isolation as various physiological processes during the phenology have a significant impact on *Citrus* fruit quality. One such process is the phenomenon of alternate bearing, for which this cultivar is infamous for, as fruit quality is decreased in both 'on' and 'off' years (Stander and Cronjé, 2016).

Both external and internal quality of *Citrus* fruit is important when determining fruit quality. However, consumers perceive the external quality or appearance of *Citrus* fruit as the primary quality parameter (Abbott, 1999; Agustí et al., 2002). External *Citrus* fruit quality is determined by the rind and aesthetic quality thereof, but also refers to fruit size and shape, rind colour, firmness and the presence or absence of disorders. Internal *Citrus* fruit quality comprises of juice content, soluble solids content (SSC), titratable acidity (TA), seed content and ratio of SSC to acid. In addition to fruit quality, shelf life is also of high importance in an export driven citrus industry such as is the case in South Africa. The role of mineral nutrition on all these quality aspects collectively as well as yield should therefore be considered when conducting a study. To illustrate: increasing nitrogen (N) and over-irrigation may impact negatively on *Citrus* fruit quality, but both are paramount for high *Citrus*

fruit production (Koo, 1988). Nitrogen is recognized as the single mineral element that has the largest impact on *Citrus* fruit quality and production. However, increasing N fertilisation may decrease *Citrus* fruit size and mass, adversely affect rind colour and quality by increasing the number of green fruit, delaying degreening and increasing rind thickness (Reitz and Koo, 1959; Smith, 1966; Koo, 1988). Similarly, over-irrigation's effect on *Citrus* fruit quality is known to be detrimental, whereas adequate irrigation results in ideal fruit quality characteristics (Koo, 1988).

The aim of this study was thus to evaluate the effect of mineral nutrition, specifically N and phosphorus (P), on 'Nadorcott' mandarin fruit quality as relevant to specific fertilisation applications at particular fruit growth stages, where a beneficial or detrimental impact is likely to occur.

The first objective was to study the role of nutrition in the reduction of citric acid content to facilitate earlier harvest. The efficacy of mono-ammonium phosphate (MAP) foliar sprays to reduce the citric acid content of 'Nadorcott' mandarin was compared to calcium arsenate (Ca-As) and potassium nitrate (KNO_3), both known to decrease and increase citric acid content respectively. The second objective of the study was to evaluate the interaction of different irrigation rates and additional N on 'Nadorcott' mandarin fruit quality. A third objective was to determine the influence of additional N as limestone ammonium nitrate (LAN) and foliar urea sprays when applied late in stage II of fruit growth on 'Nadorcott' mandarin fruit quality, in both a mediterranean and subtropical production region. The final objective was to evaluate using scanning electron microscopy (SEM) and fluorescence microscopy as techniques to determine the distribution and content of magnesium (Mg) and calcium (Ca) in *Citrus* rind.

The outcome of this study regarding possible negative effects on fruit yield and quality will guide future research on the use of new approaches to nutrition for the manipulation of tree growth and development. The focus was therefore on the impact of various nutrition applications on 'Nadorcott' mandarin fruit quality, but always in consideration of long-term effect on tree growth, development and phenology.

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Chapter 2: Literature review - The influence of mineral nutrition on *Citrus* fruit quality and fertilisation considerations

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Introduction

Mineral nutrition in horticulture has been studied extensively, still many industry questions remain unanswered and unresolved. Though the basic principles in mineral nutrition remain constant, the new focus is on how the environment is affected by various fertilisation practices. In addition, an expectation is placed on the ability of improved mineral nutritional practices to assist in meeting the demand for food security imposed by an ever-growing human population. In citriculture expansion of production areas, new irrigation trends that influence root systems, novel cultivars and the effect of nutrition on pests and diseases calls for a renewed effort to evaluate the impact of nutrition on production. In addition, the increasing demand for top quality fruit by consumers and the growing need for sustainable utilisation of resources requires a re-evaluation of mineral nutrition of *Citrus* trees to ensure maximized sustainable high quality yields.

Mineral nutrition and fertilisation is central to *Citrus* fruit quality and production. According to Dasberg (1988) and Koo (1979, 1988a) the two main nutrients affecting *Citrus* fruit quality is Nitrogen (N) and potassium (K), as these elements are required in the largest amounts. Embleton et al. (1978) added phosphorous (P) to N and K as the prime elements of commercial significance, not only regarding *Citrus* fruit quality, but also considering yield. Of these macro-elements, N is identified as the mineral nutrient having the greatest impact on *Citrus* fruit quality and production (Koo, 1988a; Smith, 1966a). Therefore, although fruit quality will be detrimentally affected if any of the other essential mineral nutrients are deficient or excessive, the effects of these mineral nutrients compared to N and K will be negligible, unless severely deficient (Koo, 1988a).

The cost of optimum fertilisation of *Citrus* is minor compared to the total budget of production, yet it has a pronounced effect on the profitability. This literature review will therefore focus on the impact of mineral nutrition, with emphasis on N, P and K, on the internal and external quality of *Citrus* fruit and their role to maximize the yield of high quality fruit.

Well-known publications covering mineral nutrition of *Citrus* include Chapman (1968), Smith (1966a) and Ting and Attaway (1971). A recent review published focusing on the influence of mineral nutrition on *Citrus* fruit quality by Aular et al. (2017) states that K has been the most evaluated macronutrient, yet makes no mention of well-known research done with N fertilisation. Furthermore, the review states that information regarding mineral nutrition on *Citrus* fruit quality is lacking. The difficulty on this subject is however not lack of information, but rather contrasting and inconsistent results due to the many factors influencing the effect mineral nutrition on fruit quality in *Citrus* as well as the unpredictable effect of other less quantifiable factors on fruit quality. A large contribution to literature today on mineral nutrition and fruit quality is based on experiments done in the 1950s and 1960s in Florida, and those studies often failed to deliver consistent results due to the many factors influencing fertilisation effects (Quaggio et al., 2006).

Citrus phenology and fruit growth

Phenology is defined as the different stages of vegetative- and reproductive growth and development, including that of the various fruit growth stages. Nutrient requirements differ depending on the phenological stage, with certain stages such as the period of rapid fruit growth, having a high demand of mineral nutrients, which in turn has important consequences for fertilisation practices and recommendations.

Citrus fruit growth development, originally defined by Bain (1958) for ‘Valencia’ orange, has a sigmoidal growth pattern that is classically divided into three stages that are applicable to most commercial *Citrus* cultivars. The length of stage I is the shortest and of similar duration for all *Citrus* cultivars. Stages II and III are, however, cultivar dependent (Rabe, 2000). An additional stage was suggested by Davies and Albrigo (1994) to occur between stage I and II, separating cell division and differentiation. This literature review will however only refer to the standard three stages, as is used by most authors.

Growth and development of *Citrus* trees are complex and is controlled by an interaction of internal factors which are inherent and cannot be altered with external factors which can be controlled (El-Otmani et al., 2000a). The influence of these factors on *Citrus* fruit growth will be discussed shortly in the following section.

Albrigo et al. (2001) states that the concept of best management practices (BMPs) which provide guidelines to the most efficient system, is based on the understanding of the various stages of vegetative and reproductive development, with required application of certain cultural practices at critical stages of development. Hence, the nutrient demand would differ between the different growth stages, at the fruit and the whole tree level. Consideration of the drastic change of fruit quality parameters during the different stages of phenology is of importance when constructing a fertiliser program, especially in the use of foliar applications to manipulate fruit quality (El-Otmani, 2000).

Stage I is described as the cell division stage (Bain, 1958) where the ultimate fruit size is determined (Rabe, 2000). The next stage, stage II, is known as the cell enlargement phase or phase of rapid fruit growth. Acidity in the pulp increases during stage II until the fruit is approximately 50% of the final size (Erickson, 1968). Thereafter, acidity decreases gradually during the season, mostly due to a decline in citric acid (Sadka et al., 2000a, b). By comparison, sugar content (carbohydrates) increase throughout fruit development. Reviews on the early changes associated with organic acids and sugars in *Citrus* fruit by Albertini et al. (2006) as well as on fruit development throughout the season by Iglesias et al. (2007) provide good insights into *Citrus* fruit physiology. The final phase, stage III, is the maturation phase (Fig. 1) which is characterized by rind colour changes, retardation of fruit growth, a continuous increase in soluble solids content (SSC), albeit slowly, and a rapid decrease in total acidity (TA) (Bain, 1958).

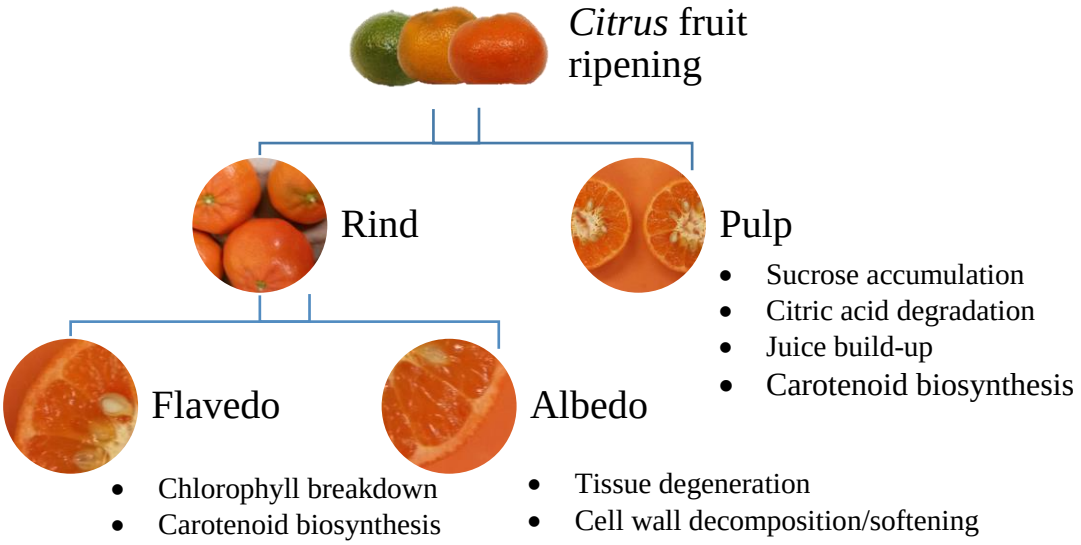

Figure 1. Basic principles of *Citrus* fruit maturation (Goldschmidt, 2000).

To summarize this section: *Citrus* fruit size increases throughout development although its maximum capacity is determined within the first growth phase (Fig. 2). *Citrus* fruit rind colour

changes from green to orange, which is due to the conversion of chloro- to chromoplasts, entailing the gradual loss of chlorophylls and accumulation of carotenoids (Huff, 1983; 1984). This is a complex process and cannot be simplified with respect to fruit growth stage. Organic acid quantity at harvest is determined by the total accumulation during stage II as well as the decrease thereof during the rest of the season. Sugar content, on the other hand, increases throughout *Citrus* fruit growth (Fig. 2).

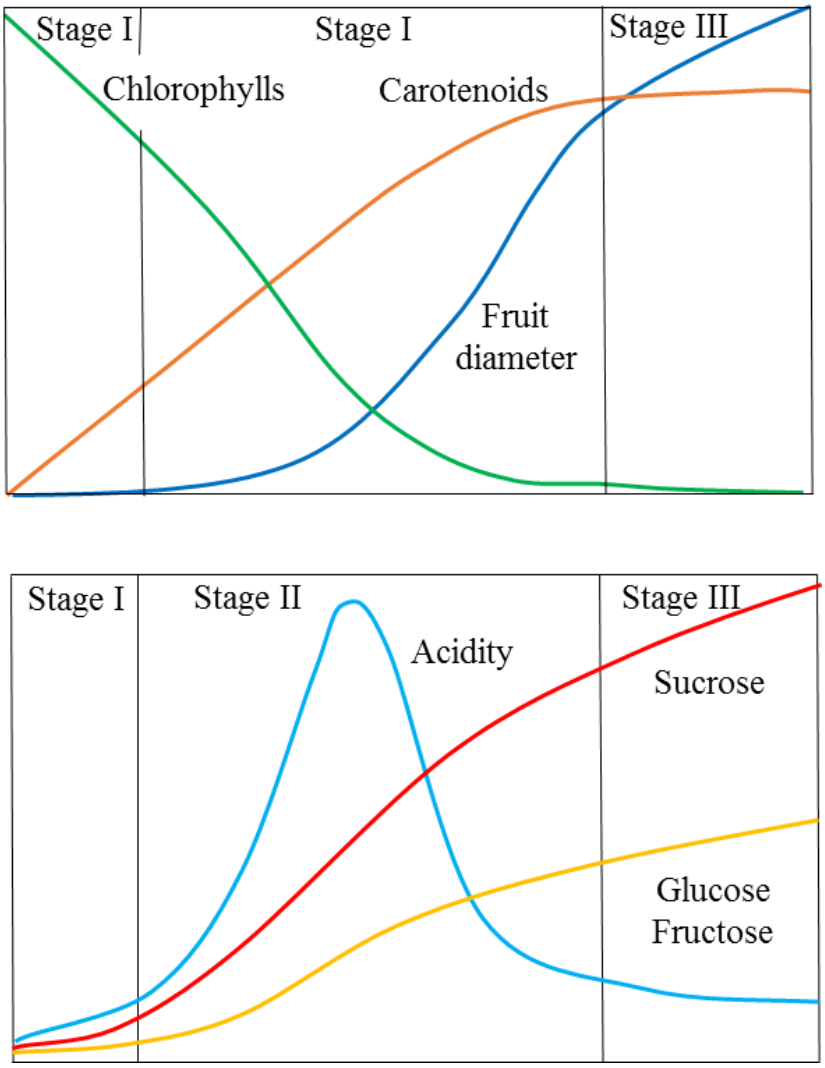

Figure 2. *Citrus* fruit growth stages and quality changes as occurring throughout fruit development (adapted from Iglesias et al., 2007).

Citrus fruit quality

Citrus fruit quality refers to, amongst others, the appearance of the product (Abbott, 1999). Desired characteristics differs from product to product, but both internal and external fruit quality parameters determine the value of *Citrus* fruit (Agustí et al., 2002). External appearance, such as rind colour, lack of superficial blemishes and fruit size, is however mostly emphasized as it is the main quality factors directly perceived by consumers (Abbott, 1999). If the internal quality, or palatability, as well as the appearance of fruit is excellent, the fruit value is very high (El-Otmani, 2000). In addition to fruit quality, a long shelf life is an important criterion, especially in the South African export-driven citrus industry.

In most citrus producing countries fruit quality standards are defined commercially by a set of characteristics, with a minimum standard that is legally enforced (Soule and Grierson, 1986). *Citrus* fruit quality standards applicable for the fresh export market, are qualified by percentage juice content, SSC, titratable acidity (TA), the SSC:TA ratio, fruit size, rind colour and seed content. In addition, the fruit rind should be without any visible lesions or physiological disorders (Lado et al., 2014). Maturity standards usually include a minimum juice content, rind colour standards and SSC:TA ratios, with some additional standards (DAFF, 2011) that may be required, depending on the species and cultivar in question.

Internal Citrus fruit quality

Internal *Citrus* fruit quality is defined by percentage juice content, SSC, TA, seed content and ratio of SSC to acid. Generally, fruit with a high sugar-to-acid ratio is preferred by consumers, whereas the acceptable seed content depends on the cultivar and market it is destined for. With respect to percentage juice content, a too high or too low juice content is undesirable for fresh fruit consumption.

Titratable acidity (TA)

Total acidity of *Citrus* juice is a collective term referring to all the free and combined organic acids present, whilst TA is a measure of only the free organic acids present (Ulrich, 1970). Citric acid is the main contributor to acidity in *Citrus* (Degu et al., 2008; Sadka et al., 2000a; Sinclair, 1984), comprising the largest amount of the organic acids. Titratable acidity thus correlates strongly with citric acid content (Degu et al., 2008; Sadka et al., 2000a; Sinclair, 1984) so that the citric acid percentage of TA has been determined as between 70-90% (Davies and Albrigo, 1994) or as high as 80-90% (Iglesias et al., 2007). Other organic acids present are malic, oxalic, succinic, malonic, quinic

and tartaric acid (Davies and Albrigo, 1994). The respective acid contents are vastly different between the various *Citrus* cultivars and may even differ drastically within the same cultivar, depending on cultivation conditions (Spiegel-Roy and Goldschmidt, 1996).

Citric acid content can reduce fruit quality when its level is either too high or too low at maturity (Degu et al., 2008). As previously discussed, the TA of *Citrus* fruit decreases gradually after reaching a peak during fruit growth stage II when the fruit is approximately 50% of its final size. This decrease in acidity is mostly due to citric acid breakdown during fruit respiration (Ulrich, 1970). Citric acid accumulates in the cells of the juice-sacs and has a major influence on *Citrus* fruit quality through its sensory attributes. A too high citric acid content is undesirable and a too low citric acid content leads to tasteless fruit or a very sweet fruit, if sugars are high (Degu et al., 2008).

Soluble solids content (SSC)

Soluble solids content includes both the organic acids and sugars present in the fruit, of which they form the major compounds (Tucker, 1993). Glucose, sucrose and fructose are the main sugars in the soluble solids found in *Citrus* fruit (Albertini et al., 2006). Other compounds comprising the SSC include carbohydrates, proteins, fats and minerals (Erickson, 1968).

Ratio of soluble solids content to titratable acidity

Organoleptic quality or taste of fruit is determined by the sugar and acid content as well as the volatiles in the juice (Tucker, 1993). The ratio of SSC to TA determines the edibility, flavour and palatability of the fruit as well as possible aromatic or bitter properties. Fruit with a high SSC:TA ratio and high SSC is generally desired, having a sweet taste (Davies and Albrigo, 1994).

Health-promoting bioactive compounds

Studies have shown that *Citrus* fruit reduce the risk of cancer and cardiovascular diseases (Patil et al., 2009). *Citrus* fruit are rich in vitamin C (ascorbic acid) and bioactive compounds such as flavonoids, coumarins, limonoids and carotenoids, which all have antioxidant properties, known to be beneficial to human health (Patil et al., 2009; Yu et al., 2005). *Citrus* fruit are by far the most well-known for its vitamin C content, with a recent review by Magwaza et al. (2017) covering important preharvest factors influencing the vitamin C content of *Citrus* fruits.

External *Citrus* fruit quality

External fruit quality refers to the aesthetic quality of the fruit as is defined by the appearance such as the fruit size and shape as well as the overall rind quality. Rind quality is determined by rind

colour, firmness, the presence or absence of physiological disorders as well as the softening and senescence of the rind (Agustí et al., 2002; El-Otmani, 2000).

Fruit size and mass

Large fruit are preferred by consumers and consequently fruit size is an important fruit quality parameter to consider (Agustí et al. 2002). The role of mineral nutrition as one of many factors that influence fruit size (Guardiola and García-Luis, 2000) will be discussed later on in this literature review.

Rind colour

Rind colour is used by consumers to assess *Citrus* fruit quality and a vivid rind colour is therefore desirable. Pigments in the rind and pulp of *Citrus* fruits produce the characteristic colour thereof. A comprehensive review on pigments found in *Citrus* fruit has been done by Alquézar et al. (2008). Pigments which may be present in various combinations include chlorophyll, carotenoids, lycopene and anthocyanins. Chlorophyll is responsible for the green colour of immature fruit, but also gives the fruit the ability to photosynthesize. The major group of pigments leading to the characteristic, desired orange colour at harvest, are the carotenoids. These pigments also have well-known, nutritional and antioxidant qualities (Alquézar et al., 2008).

Rind physiological disorders

Physiological rind disorder incidences reduce *Citrus* fruit quality and can occur pre- as well as post-harvest. In *Citrus*, well-known disorders include creasing (Du Plessis and Maritz, 2004), splitting (García-Luis et al., 1994), puffing (Ibáñez et al., 2014), cold or rind pitting (Medeira et al., 1999; Vercher et al., 1994) as well as rind staining and rind breakdown (Agustí et al. 2001). With creasing the rind is weak and a puffy rind forms, due to cracking of the albedo cells (Storey et al., 2002). Splitting occurs when the rind and pulp do not grow simultaneously and growth is disrupted (Erickson, 1968; García-Luis et al., 2001). Another disorder related to the disruption between rind and pulp is the separation of the rind and pulp which then results in ‘puffing’ (Ibáñez et al., 2014). Pitting, which can be seen either as brown or black lesions, can occur pre- or postharvest, although it is mostly associated with postharvest conditions (Medeira et al., 1999; Vercher et al., 1994). During the rind breakdown disorder, sunken areas on the rind are initially formed, which later change to reddish-brown areas. Rind stain or rind breakdown is a physiological disorder that commonly affects Navel oranges (Agustí et al. 2001, 2002). Physiological rind disorders in *Citrus* has been extensively

covered in publications (Agustí et al., 2002, 2004; Lafuente and Zacarías, 2006; Magwaza et al., 2013), but these physiological problems persist and this remains an area that requires research.

Factors affecting *Citrus* fruit quality

Various factors, in addition to mineral nutrition, influence *Citrus* fruit quality. Factors can be divided into two broad groups namely, controllable factors, such as cultural practices, and uncontrollable factors which can be exogenous (external) like the climate and its impact on the scion and rootstock cultivar characteristics and the scion-rootstock combination, or may be of an endogenous (internal) nature, such as the reserve status of the tree (El-Otmani et al., 2000a). A better understanding of these factors individually as well as their interactions and their subsequent influence on *Citrus* fruit quality is of great importance.

Climate has a large influence on *Citrus* fruit quality and determines where successful cultivation can occur. For example, due to the significant impact of climate on fruit size, South Africa's citrus production areas can be classified into 'small fruit' and 'large fruit' areas (Du Plessis, 1996). In general, hot areas typically have larger fruit whereas cooler areas may usually produce smaller fruit (Du Plessis and Koen, 1996). Furthermore, respiration sustained by the breakdown of organic acids (Ulrich, 1970) occurs at different rates, depending on temperature, thus leading to vast differences in the rate of acid content reduction and consequently influences harvest dates between production areas. In addition to the climate, the scion and rootstock characteristics also play an important role in determining the chemical composition of the fruit (Ting and Attaway, 1971). The effect of the various rootstocks on *Citrus* fruit quality can be explained by inherent differences and the influence thereof on soil-plant water relations (Romero et al., 2006). Furthermore, the performance of scion and rootstock combinations are known to differ under varying environmental conditions (Marsh et al., 2000).

Controllable aspects affecting fruit quality include cultural practices such as irrigation (Koo, 1988a), the use of external plant growth regulators (Erner et al., 2004) and pruning (Krajewski and Pittaway, 2000). Irrigation is important for *Citrus* fruit production, but in large volumes it is detrimental to *Citrus* fruit quality (Koo, 1988a). Strategies such as deficit irrigation can however be used to manipulate *Citrus* fruit quality desirably and irrigation effect on *Citrus* fruit quality has been studied and documented extensively (Kriedemann and Barrs, 1981; Doorenbos and Kassam, 1979).

Pruning and canopy manipulation is not essential for *Citrus* production, but selective pruning has the potential for improving both yield and fruit quality due to improved light levels within the canopy and possible modifications regarding partitioning of photosynthate, amongst others

(Krajewski and Pittaway, 2000). *Citrus* fruit quality can also be improved by pruning during flowering (Zaragoza et al., 2000).

Externally applied plant growth regulators influence *Citrus* fruit quality pre- and postharvest depending on the hormone applied and the growth stage for pre-harvest applications. For example, auxin when applied at the right growth stage is known to increase *Citrus* fruit size (Erner et al., 2004). The use of plant growth regulators on *Citrus* has been reviewed by El-Otmani et al. (2000a).

Mineral nutrients essential to *Citrus* production

Classification

Seventeen elements, of which fourteen are mineral nutrients, are required by green plants to complete their life cycle, due to the critical role of the element in the structure and/or metabolism of the plant. The mineral nutrients can be subdivided into macronutrients and micronutrients, according to the amount required by the plant of that particular element. The macronutrients, needed in large amounts, are N, P, K, calcium (Ca), magnesium (Mg), and sulphur (S). The micronutrients, needed in much smaller amounts, are iron (Fe), zinc (Zn), manganese (Mn), boron (B), copper (Cu), molybdenum (Mo), nickel (Ni), and chlorine (Cl) (Briskin and Bloom, 2010; Kirkby, 2012). *Citrus* trees require very much the same mineral nutrients as other plant life (Smith, 1966a), with twelve of these fourteen mineral nutrients, excluding the micronutrients Ni and Cl, considered essential (Davies and Albrigo, 1994). Other classifications of mineral elements exist (Briskin and Bloom, 2010; Kirkby, 2012), however the classification into macro- and micronutrients will be used in this review paper as it is the most common classification method.

Balance between mineral nutrients within the plant must be maintained and the growth, yield and quality is dependent on the most limiting essential element. This implicates that if one nutrient is deficient it will detrimentally affect growth, yield and quality, despite sufficient amounts of all the other mineral nutrients. The same holds true for an excess amount of an element, as such conditions would also influence the normal physiology and functioning of the plant. Furthermore, the deficiency or excess of one element might induce an excess or deficiency of another (Smith, 1966a, b; Briskin and Bloom, 2010).

Mobility in the plant

Mobility of elements, which refers to the translocation of a mineral nutrient within the xylem and/or phloem, differ, with some elements being classified as mobile, whilst others are viewed as

immobile (Table 1). This mobility factor of nutrients has implications with respect to the choice of preferred fertilisation application methods. Mineral nutrients that are phloem mobile and can move to other parts of the plant can be applied effectively with the use of foliar fertilisation. These mobile nutrients are N, K, Mg, P, Cl, Na, Zn, and Mo (Briskin and Bloom, 2010; Smith, 1966b), with P, Mg (Chapman, 1968; Spiegel-Roy and Goldschmidt, 1996) and K considered particularly mobile (Smith, 1966b). The immobile nutrients are Ca, S, Fe, B, and Cu (Briskin and Bloom, 2010; Marschner et al., 1996), with Fe being the least mobile element of all the essential elements (Chapman, 1968). Boron is also known for having limited mobility (Boaretto et al., 2004a). However, according to Boaretto et al. (2004b) the mobility of B within *Citrus* trees is determined by the nutritional status of the tree. Although Mn is not mentioned as either mobile or immobile above, Embleton et al. (1988) reported translocation thereof from sprayed to unsprayed *Citrus* leaves as minimal.

Table 1. The common mobility of mineral nutrients in plants (Briskin and Bloom, 2010).

Mobile	**Immobile**
Nitrogen	Calcium
Potassium	Sulphur
Magnesium	Iron
Phosphorous	Boron
Chlorine	Copper
Sodium	
Zinc	
Molybdenum	

Mineral nutrient status, norms and sampling

Maximizing yield and fruit quality requires optimal leaf mineral nutrient content (Embleton et al., 1978; Raveh, 2013). Therefore, leaf analysis along with soil analysis is used when making fertiliser recommendations (Smith, 1966b). In this literature review, only leaf analysis will be discussed. These recommendations are based on leaf mineral element norms which have been developed as guides for producers to ascertain whether their trees are healthy in terms of mineral nutrient levels (Embleton et al., 1978, 1996; Raveh, 2013). The concentration of a mineral element present can be grouped as either deficient, low, optimum, high or excessive (Table 3). If an element is deficient it indicates that it will lead to a reduction in both yield and fruit quality, whilst if levels are low, it is not adequate to sustain high productivity. A high level is when there is more than the required amount of the element present, while an excess amount is likely to have detrimental effects on the leaf mineral metabolism (Smith, 1966b). However, each orchard and production year differ, therefore norms should be used with this consideration in mind, whether production aim is either

optimum fruit quality and/or maximum yield (Embleton et al., 1996; Quaggio et al., 2006). The mineral element that will have the greatest impact on realising an increased income should be the focus when deciding on a fertilisation regime, as no orchard is ever likely have the optimal content of all mineral nutrients in their leaves (Fig. 3, 4 and 5) (Embleton et al., 1996).

Mineral element deficiencies and toxicities can be determined with leaf analyses before symptoms appear. Furthermore, if a deficiency is present, foliar symptoms are usually the first indication (Smith, 1966b). In some instances, however, fruit quality can be detrimentally affected before leaf deficiency symptoms appear. By using mineral nutrient norms in *Citrus* leaves, the health of the trees can be maintained at the optimal level without the occurrence of deficiencies or toxicities, thereby reducing a possible detriment to fruit yield and quality.

Mineral nutrient leaf analysis norms were first established in the USA, using 4- to 6-month-old spring-flush leaves from non-bearing terminals (Raveh, 2013). In South Africa, as well as Israel, Morocco and Brazil, however, fruit-bearing terminals are sampled in late summer (Table 2). The use of fruit-bearing terminals simplifies leaf sampling by making identification of the leaves to sample easier (Raveh, 2013).

In countries where the fruit-bearing terminals are used, mineral nutrient norms are established for every cultivar, whereas in countries where non-bearing terminals are used, the same norms tend to be used for all cultivars (Raveh, 2013). Studies have, however, shown that norms might differ between cultivars and species, for example, Intrigliolo and Intelisano (1997) reported that lemon has a lower optimal leaf N concentration than oranges. Mineral nutrient content of leaves on fruit-bearing terminals will be, as expected, lower than those of leaves on non-bearing terminals, due to the sink strength of the fruit (Raveh, 2013). Leaf N content is always required for the interpretation of leaf analysis as the status of the other nutrients are influenced by it (Smith, 1966b). The ‘critical value’ approach is the most common and simplest method of judging leaf mineral nutrient content. Another approach is the so-called DRIS approach which uses the ratio between the elements (Du Plessis and Koen, 1988).

Using the DRIS method, the N:K ratio of leaves in fruit-bearing ‘Valencia’ orange twigs were shown to be a more accurate prediction of yield and fruit size than the absolute values of these elements (Du Plessis and Koen, 1988). The level of N and K have contrasting effects on fruit size, decreasing and increasing fruit size respectively (Embleton et al., 1996). A higher yield will be obtained with a high N level, but due to the negative effect of increasing N levels on fruit size (Du Plessis and Koen, 1988), the ideal fruit size will be obtained with a lower N level. The N:K ratio

effect on fruit quality needs to be considered in certain circumstances as a high N level might detrimentally affect fruit size, even though the level of both N and K nutrients in the leaves are in the correct range (Embleton et al., 1996). Another such example is described by Du Plessis and Koen (1988) where a hot and cool area (specifically the temperatures experienced during phase I and beginning of phase II fruit growth) was compared for its effect on fruit size, but also for its differential effect on the optimal N:K ratio (Table 4). With optimum fruit quality as a main goal, it is important to consider that maximum yield will not lead to maximum income, especially when assessing N and K leaf levels (Du Plessis and Koen, 1988).

Table 2. Shoots used in sampling for *Citrus* leaf analysis in different regions and countries.

Area or country	Shoots for leaf sampling	Reference
Argentina	Non-bearing spring (4-month-old) or summer (8-month-old)	Perez, 1996
Australia	5-7 month-old non-bearing spring flush	Gallasch, 1996
Brazil	6- to 8-month-old fruit-bearing	Quaggio et al., 1996
California, USA	5- to 7-month-old non-bearing and non-flushing	Embleton et al., 1996
Florida, USA	4-6 month-old non-bearing	Obreza et al., 1996
Israel	Spring flush from fruit-bearing terminals	Lavon and Erner, 1996
Morocco	7- to 8-month-old fruit-bearing	Lekchiri, 1996
South Africa	7 month-old fruit-bearing	Du Plessis and Koen, 1996
Texas, USA	5-7 month-old non-bearing	Swietlik, 1996b

Nutrient relationships

Various nutrients affect each other and consequentially also fruit quality and/or yield (Du Plessis and Koen, 1988). An excess or deficiency of one nutrient can induce an excess or deficiency of another (Table 5), for example, Zn deficiency can be increased in the presence of excess P (Spiegel-Roy and Goldschmidt, 1996) and excess K can reduce Ca and Mg uptake (Quaggio et al., 2006). A high P content will reduce the N content of leaves and create Zn or Cu deficiencies (Embleton et al., 1978). Element ratios within the plant can also affect the availability thereof through the effect on nutrient mobility, for example, Fe mobility is affected by high P, as well as low K and Mg (Chapman, 1968).

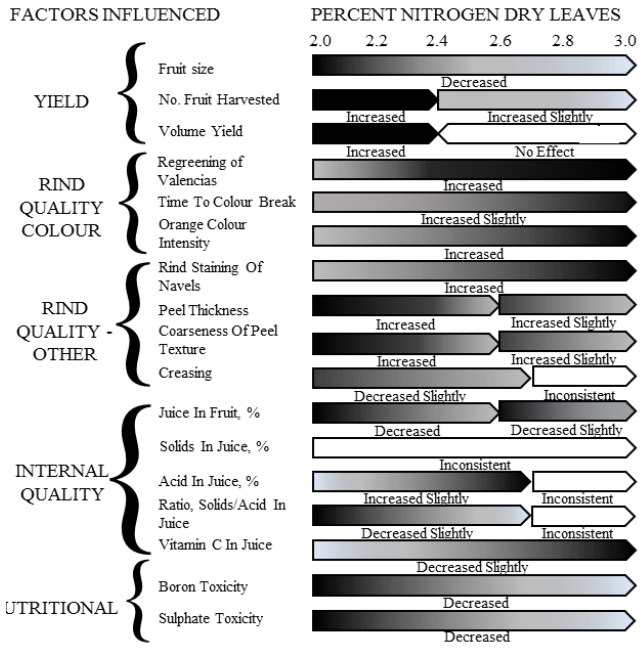

Figure 3. The influence of the percentage (%) N in five- to seven-month-old, spring-flush orange leaves from non-fruiting shoots on various production and fruit quality factors. The darker the bar, the greater the effect (Embleton et al., 1996).

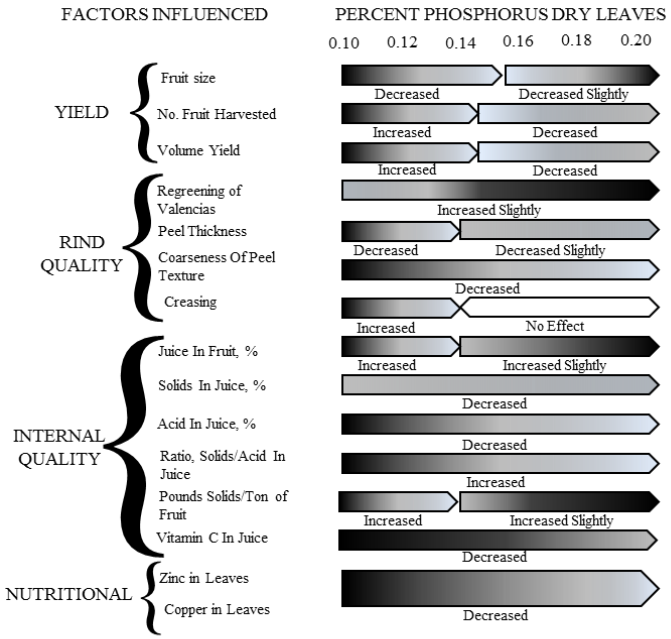

Figure 4. The influence of the percentage (%) P in five- to seven-month-old, spring-flush orange leaves from non-fruiting shoots on various production and fruit quality factors. The darker the bar, the greater the effect (Embleton et al., 1996).

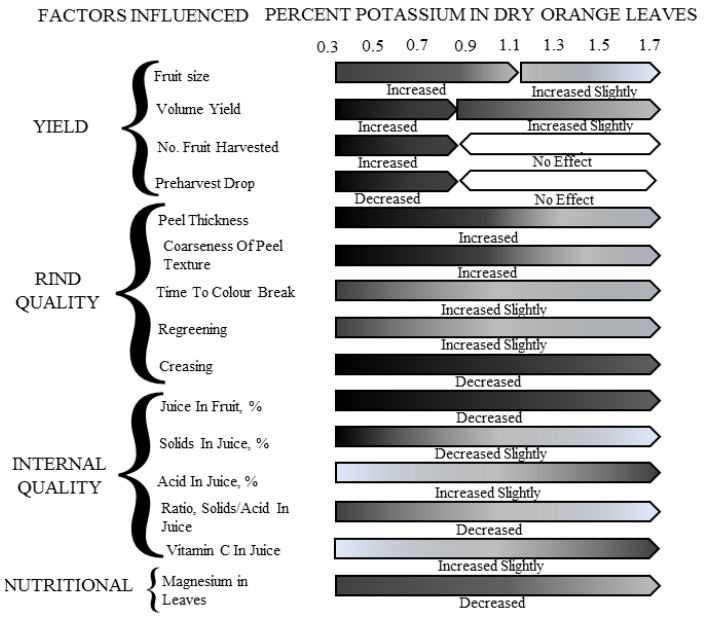

Figure 5. The influence of the percentage (%) K in five- to seven-month-old, spring-flush orange leaves from non-fruiting shoots on various production and fruit quality factors. The darker the bar, the greater the effect (Embleton et al., 1996).

Table 3. Leaf analysis norms, sampled from 5- to 7-month-old, terminal, spring-cycle leaves from non-fruiting and non-flushing shoots, for mature 'Valencia' and 'Navel' Orange (Embleton et al., 1996) (*from fruiting shoots).

Element		Ranges				
	Dry	Deficiency	Low range	Optimum	High	Excess
N	%	< 2.2	2.2-2.3	2.4-2.6	2.7-2.8	> 2.8
P	%	< 0.09	0.09-0.11	0.12-0.16	0.17-0.29	> 0.30
K	%	< 0.40	0.40-0.69	0.70-1.09	1.10-2.00	> 2.30
Ca	%	< 1.6	1.6-2.9	3.0-5.5	5.6-6.9	> 7.0
Mg	%	< 0.16	0.16-0.25	0.26-0.6	0.7-1.1	> 1.2
S	%	< 0.14	0.14-0.19	0.2-0.3	0.4-0.5	> 0.6
B	ppm	< 21	21-30	31-100	101-260	> 260
Fe	ppm	< 36	36-59	60-120	130-200	> 250
Mn	ppm	< 16	16-24	25-200	300-500	> 1000
Zn	ppm	< 16	16-24	25-100	110-200	> 300
Cu	ppm	< 3.6	3.6-4.9	5-16	17-22	> 22
Mo*	ppm	< 0.06	0.06-0.09	0.10-3.0	4.0-100	> 100
Cl	%	unknown	unknown	< 0.3	0.4-0.6	> 0.7
Na	%	unknown	unknown	< 0.16	0.17-0.24	> 0.25
Li	ppm	n/a	n/a	< 3	3-35	> 35
As	ppm	n/a	n/a	< 1	1-5	> 5
F	ppm	n/a	n/a	< 1 to 20	25-100	> 100

Table 4. The optimal leaf analysis norms for the South African citrus industry by Du Plessis and Koen (1996).

Element		Valencia		Navel	Grapefruit	Lemon	
		Cool area	Hot area			Yield	Fruit size
N	%	2.0-2.4	2.1-2.7	2.4-2.8	2.3-2.6	1.90-2.20	< 2.0
K	%	0.95-1.50	0.70-1.20	0.9-1.1	0.9-1.6	1.1-1.4	1.2-1.6
N/K		1.6-2.2	3.0-4.5	2.2-3.1	1.6-2.9	1.6-2.0	< 1.6
P	%	0.11-0.16				0.11-0.15	0.13-0.15
Ca	%	3.5-5.5				-	
Mg	%	0.30-0.55					
Cu	$mg.kg^{-1}$	5 - 16					
Zn	$mg.kg^{-1}$	20 – 70					
Mn	$mg.kg^{-1}$	30 – 150					
B	$mg.kg^{-1}$	50 – 150					
Fe	$mg.kg^{-1}$	80 - 300					

Table 5. The effect concentration increases of elements have on the other elements in *Citrus* leaves* (Embleton et al., 1996).

	N	P	K	Ca	Mg	S	B	Fe	Mn	Zn	Cu	Mo	Cl	Na
N	+	-	-	+	+	-	-	0	0	0	0			
P	-	+	-	+	+		-	0	+	-	-			0
K	-	0	+	-	-		+		-	0	0			
Ca	+	0	-	+	-									
Mg	0	0	-	-	+		0		+	+	-			
S	-	-	-	+	-	+								-
B	0	-	+	-	-	0	+		-	0	0			
Fe		-	+	+				+						
Mn	0	0	0	0	-		0	0	+	0	-			
Zn	0	0	+	-	-		0	0	0	+	-			
Cu	0	0	+	0	0		0		-	-	+			
Mo												+		
Cl													+	
Na														+

*This table was adapted from Chapman (1949) and Smith (1966b) by Embleton et al. (1996) for California. Blank spaces indicate that the effect is unknown.
- = decrease in concentration
+ = increase in concentration
0 = no effect

Mineral nutrition and *Citrus* fruit quality

Application of fertiliser is intended to replace nutrients, most notably those removed by the fruit crop annually, but also to balance nutrient ratios (Chapman, 1968) for optimal efficiency. The purpose of fertilisation should be optimum tree nutrition status and not the current fruit yield (Dasberg, 1987). According to Menino et al. (2004a) N fertilisation's focus should be on adequate vegetative development, however the effects of mineral nutrition on *Citrus* fruit quality are also important. Nitrogen and K are the most influential nutrient elements in *Citrus* fruit production and quality (Koo, 1979, 1988a), whilst other nutrient elements are of minor importance unless severely deficient (Koo, 1988a).

Macronutrients

Nitrogen

Nitrogen is the mineral nutrient having the single greatest influence on both fruit yield and quality, directly or indirectly (Dasberg et al., 1984; Koo, 1988a; Smith, 1966a). Therefore, it is required and applied in the highest amount of all the mineral nutrients (Chapman, 1968; Dasberg et al., 1983; Smith, 1966a) as consistent production and high fruit yields are dependent on N fertilisation.

Fruit quality is strongly influenced by N, with increasing rates of N fertilisation strongly associated with decreasing *Citrus* fruit size and mass (Chapman, 1968; Dasberg et al., 1983; Du Plessis and Koen, 1988; Koo, 1988a; Quaggio et al., 2006; Reitz and Koo, 1959; Smith, 1966a). This decrease in fruit size is partly due to the increased yield brought about by a higher fruit set (Quaggio et al., 2006). Also, increasing levels of N typically decreases the K content of leaves (Du Plessis and Koen, 1988). Potassium is known for its positive effect on fruit size (Du Plessis and Koen, 1988) and consequently high N levels can suppress the role of K within the fruit and tree. Potassium's effect has, however, been found to have a larger impact on fruit size than N, when present at higher levels (Du Plessis and Koen, 1988; Reitz and Koo, 1959). Therefore, the negative effect of N on fruit size is likely secondary due to its effect on K levels within the tree. In lemons, an increase in N level does not have the same consistent effect on fruit size, with some studies claiming that it does not affect fruit size (Koo et al., 1973), whilst Quaggio et al. (2002) found lower N fertilisation to be beneficial for fruit size in Sicilian lemons.

Rind colour is adversely influenced by increasing N fertilisation, as it delays degreening and increases the number of green fruit at harvest (Koo, 1988a; Dasberg, 1983; Reitz and Koo, 1959; Smith, 1966b). This effect on rind colour can also be caused by delayed N fertilisation (Quiñones et al., 2004). The detrimental effect of N on rind colour can be a direct effect through the chloro- to chromoplast conversion, as well as the reversion of chromo- to chloroplast during regreening. Rind colour development involves the gradual loss of chlorophyll and increased synthesis of carotenoids (Huff, 1983; 1984). High N fertilisation decreases carotenoid content and prevents chlorophyll disappearance, whilst low N fertilisation increases carotenoid content. Nitrogen suppresses degreening and as soon as rind N content decreases, the conversion or degreening process is promoted by the accumulation of sugars within the rind (Huff, 1983; 1984; Iglesias et al., 2001). In grapefruit, it was also found that high N delays the breakdown of chlorophyll in the rind (Ting and Attaway, 1971) leading to less colouration at harvest (Fig. 6) (Jones et al., 1945).

Other effects of increasing N fertilisation includes an increase in percentage juice content (Embleton et al., 1978; He et al., 2008; Koo, 1988a; Quaggio et al., 2006; Smith, 1966a), SSC (He et al., 2008; Koo, 1988a; Quaggio et al., 2006), TA (Koo, 1988a; Reitz and Koo, 1959; Smith, 1966a) and rind thickness (Fig. 6) (Dasberg, 1983; Embleton et al., 1978; Koo, 1988a; Smith, 1966a). Furthermore, it has also been found to decrease the SSC to acid ratio (He et al., 2008). The influence of fruit size on internal quality, including SSC and juice content, is visible where smaller fruit caused by higher N rates naturally have higher SSC and percentage juice content due to the concentration effect (Quaggio et al., 2002; 2006). Higher SSC values were recorded by Koo (1979) with an increasing N rate in 'Valencia' and 'Pineapple' orange. This is an example of the indirect effect N can have on *Citrus* fruit quality. According to Quaggio et al. (2006), N fertilisation could be said to decrease SSC in an 'off' year and increase it in an 'on' year. The effects on lemons are similar for TA, SSC and rind thickness (Intrigliolo and Intelisano, 1997; Koo et al., 1973). With respect to aesthetic quality, increasing N fertilisation intensifies the incidence of creasing and scab, but decreases the incidence of wind scar and other rind blemishes. Thus, the most important consideration with N fertilisation is the effect thereof on the acid content and rind colour, as both can delay *Citrus* fruit ripening and the harvest date (Koo, 1988a).

Figure. 6 The influence of increasing N fertilisation (left to right) on grapefruit rind colour and rind thickness. Increasing N fertilisation led to a less desirable rind colour and thicker rind (Jones et al., 1945).

Fruit yield increases with increasing N fertilisation (Quaggio et al., 2006). High fruit production requires adequate N, although excessive N fertilisation will have a detrimental impact (Koo, 1988a; Smith, 1966a). Fruit yield may be directly influenced through the availability of N for flowering and the following season's fruit set. The promotive effect of N can also indirectly affect flowering, in the form of endogenous ammonia, as flowering is promoted by foliar urea sprays (El-Otmani et al., 2000c; Lovatt et al., 1988; Rabe, 1990). The N requirement of trees can partially be met with urea (El-Otmani et al., 2000c). Nitrogen's effect on yield is greater than its influence on fruit size (Dasberg et al., 1983). It is important that the negative effect on fruit size with increasing N levels-off, before its beneficial effect on yield plateaus, thus creating a trade-off between these two desirable outcomes (Du Plessis and Koen, 1988).

The inverse relationship between yield and fruit size should therefore always be a key consideration in N fertilisation practises (Monselise and Goldschmidt, 1982). Producers may be tempted to apply excessive N fertiliser to achieve a minor yield increase, however this will detrimentally influence fruit quality (Chapman, 1968). Yet, for fresh fruit markets, optimum fruit quality is the main goal and N levels should thus be kept low enough to ensure that fruit quality is not diminished.

Discrepancies exist in literature, with some N fertilisation studies yielding unexpected results regarding both fruit quality and yield. Castel and Ginestar (1996) found no significant differences in any fruit quality parameters of 'Clementine' mandarin after comparing N rates of 120 and 210 kg $N.ha^{-1}$ per year. In another study, there was no significant differences in fruit number, fruit size, rind colour, rind thickness, SSC, total acidity or maturity indexing between two N rates used: 178.5 kg N ha^{-1} (300g per tree) and 297.5 kg N ha^{-1} (500g per tree) (Montaña et al., 2004). In several orchards observed by Johnston (1950) there was no connection between N and fruit size. Sometimes *Citrus* fruit quality parameters were influenced as expected, whilst other times it remained unaffected. Reitz and Koo (1959) observed only an increase in green fruit and acidity along with a decrease in fruit size, whilst Menino et al. (2004a) reported that only SSC and rind thickness to be affected as anticipated, with a decrease in acidity in young 'Lane Late' Navel orange under a mediterranean climate, contrary to the general expectancy. Furthermore, the best rind colour was observed at the highest N rate. When comparing different cultivars, Quaggio et al. (2006) reported all *Citrus* fruit quality parameters to be influenced by N fertilisation as expected in 'Valencia' orange, but with SSC and juice content to decrease in 'Pêra' orange. It can therefore be concluded that all *Citrus* fruit quality parameters are not always influenced to the same extent or trend, when exposed to a comparable same level of N fertilisation. These differences can be due possibly to the cultivar

studied, the environmental factors, the N already available, the type of irrigation used, the timing of the fertiliser application and the type of fertiliser used or a combination of any these factors. Alva et al. (2008) ascribed the lack of a response or deviations from the expected response in soil-applied N fertilisation studies to the short time span allowed for observations, as is applicable in most studies, for example, Quaggio et al. (2002) only found fruit quality differences after two years.

Nitrogen reserves are considerably remobilised within the tree, where for example, N moves from older to younger leaves. A large amount of N is also stored in the woody parts of mature trees (Alva et al., 2008). Nitrogen present in spring flush leaves comes mainly from N reserves and not from soil uptake (Dasberg et al., 1983). The quantity of remobilized N within young *Citrus* trees is dependent on the availability of N from other sources, with more N being translocated when the applied N fertilisation is lower (Martínez-Alcántara et al., 2011). The management of N fertilisation, at every stage of tree growth, remains crucial as both excessive and deficient levels thereof can negatively influence various tree parameters, including fruit quality (Intrigliolo and Intelisano, 1997). Nitrogen fertilisation can only be managed properly if the N status of the plant is known (Menino et al., 2004b).

Large amounts of N, between 100 and 400 kg N per ha, are applied to *Citrus* orchards annually (Dasberg, 1987). Many studies (mostly yield-orientated) have suggested widely varying amounts of N per ha for optimal yield and production, for example, fertiliser rate recommendations for N in Florida, varied from 135-282 $kg.ha^{-1}$ for oranges, depending on tree age, and from 135-180 $kg.ha^{-1}$ for grapefruit (Boman et al., 2008). Yield has been maximised by 189 kg $N.ha^{-1}$ in ‘Pêra’ orange and 193 kg $N.ha^{-1}$ in ‘Valencia’ orange (Quaggio et al., 2006). The amount of 202 kg $N.ha^{-1}$ for maximising yield has been suggested by Reese and Koo (1975) for ‘Hamlin’, ‘Pineapple’ and ‘Valencia’orange and for ‘Troyer citrange’ and ‘Valencia’ by Koo (1988b). The amount of 200 kg $N.ha^{-1}$ was considered adequate for good tree development and yields (Dasberg, 1987).

However, each orchard and its unique factors, for example, rootstock N-uptake ability and irrigation, should be considered separately. Moreover, the unpredictable and constantly changing effect of the environment makes establishing N application rates difficult (Smith, 1966b). Long term studies should be conducted prior to fertiliser recommendations, to allow for sufficient time whereby N growth and development responses can manifest in the trees along with any changes in the nutrient reserves in the woody parts of the tree (Alva et al., 2008).

Potassium

Potassium, after N, is the mineral nutrient that has the largest influence on *Citrus* fruit quality (Koo, 1988a), also as *Citrus* fruit have a larger K than N content (Dasberg, 1988). According to Ting and Attaway (1971), K and not N, is the mineral nutrient that has the largest influence on juice quality. Increasing K fertilisation increases the acid content of *Citrus* fruit (Alva et al., 2006; Du Plessis and Koen, 1988; Erner et al., 2004; Moss, 1972; Reitz and Koo, 1959), thereby delaying the harvest. The ratio of SSC to acid content of many cultivars is decreased with increasing K fertilisation (Quaggio et al., 2006). The higher acid content associated with increasing K fertilisation is the cause of the decreasing SSC to acid ratio (Alva et al., 2006; Koo, 1988a). Juice content is also detrimentally affected as it is reduced by high K fertilisation (Alva et al., 2006; Koo, 1988a).

Potassium is, however, mostly recognised and used for its promotive effect on fruit size and mass (Fig. 7) (Alva et al., 2006; Embleton et al., 1996; Koo, 1988a; Morgan et al., 2005; Quaggio et al., 2002, 2006). Low or deficient levels of K can lead to unacceptably small fruit (Smith, 1966b) with thin rinds (Moss, 1972). Rind thickness is increased at high levels of K fertilisation, forming a thick, coarse rind (Alva et al., 2006; Koo, 1988a; Smith, 1966b). Potassium's effect on the fruit rind may be the cause of increasing K fertilisation rates decreasing the SSC (Koo et al., 1979; Quaggio et al., 2002, 2006; Reese and Koo, 1975). Potassium fertilisation can also adversely affect rind colour (Smith, 1966b), increasing the amount of green fruit at harvest (Alva et al., 2006; Koo, 1988a). Potassium fertilisation decreases the incidence of rind disorders (Bower and Wolstenholme, 1996), reduces the incidence of both creasing and plugging (Embleton et al., 1996; Koo, 1988a), whilst also preventing fruit splitting, which is prevalent in fruit with thinner rinds, and typical formed under conditions of low K availability (Morgan et al., 2005). According to Moshe et al. (2000) K deficiency results in water loss due to malfunction of membranes in 'Shamouti' orange leading to rind pitting. Therefore, optimum K levels extends the postharvest shelf life of *Citrus* fruits by lowering the incidence of rind disorders during storage.

The most important effects of K, however, is its impact on fruit size and acidity (Alva et al., 2006; Du Plessis and Koen, 1988; Erner et al., 2004), with the other effects on fruit quality of much less importance (Reitz and Koo, 1959). Trees receiving higher K fertilisation have improved stomatal control, resulting in slower water loss, with stomata being open for longer and consequently allowing for more CO_2 uptake (Bower and Wolstenholme, 1996). Furthermore, K is considered important in both the process of photosynthesis in leaves and other chlorophyll-containing structures and the translocation of the photosynthetic products (Bower and Wolstenholme, 1996).

Yield is increased at elevated levels of K, according to Koo (1988a) and will not be negatively affected by high K levels, unless it interferes with the uptake of other elements (Smith, 1966b). However, in a long-term, six-year study, low K fertilisation did not influence fruit yield for the first five years of the study (Reitz and Koo, 1959). It is therefore considered less important to maximise yield, compared to N, but rather more important for adequate fruit size.

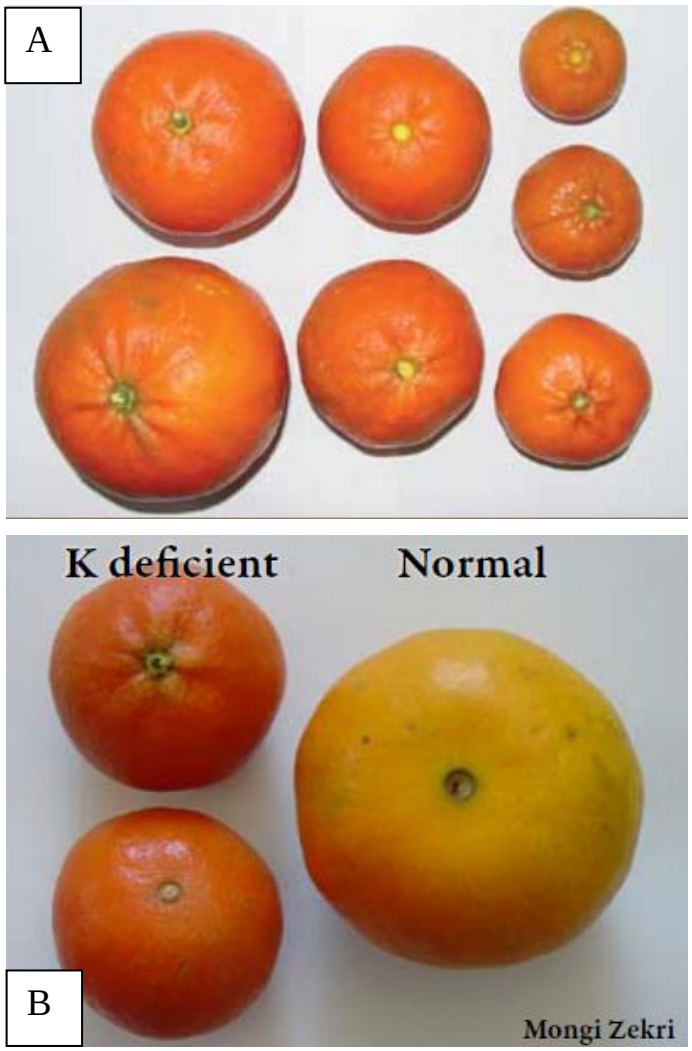

Figure 7. Potassium deficiency in *Citrus* is characterised by small fruit as can be seen in both photos (A - Zekri and Obreza, 2012a; B - Obreza and Morgan, 2008).

Phosphorous

After N and K, the next macronutrient that has the largest influence on *Citrus* fruit quality and yield is P. According to Quaggio et al. (2006) the influence of P on *Citrus* fruit quality is not as clear in the literature as is that of N and K. Rind colour is generally improved at low P levels (Fig. 8B), but generally *Citrus* fruit quality is detrimentally affected, according to Smith (1966b). At high P levels, juice quality is slightly affected, by registering a lowered SSC and acid content (Anderson,

1966; Jones and Parker, 1949; Moss, 1972; Smith et al., 1963) and an increase in the SSC to acid ratio (Koo, 1988a). The decrease in SSC and acid content at high P nutrition levels is more distinct in 'on' years (Quaggio et al., 2006). Percentage juice content can also be increased at high P levels (Moss, 1972). Externally, under the influence of high P nutrition, fruit colour is perceived unsatisfactory, the development thereof delayed and regreening of oranges can occur (Obreza, 2000; Smith, 1966b), leading to more green fruit (Koo, 1988a). Furthermore, rind thickness (Fig. 8A and 8B) and percentage russet fruit is reduced, whilst wind scarring is more prominent, but storage decay is unaffected (Koo, 1988a). The influence of P on yield depends on the available P in the soil, with areas of low availability, responding to increasing P fertilisation by increased yields (Quaggio et al., 2006).

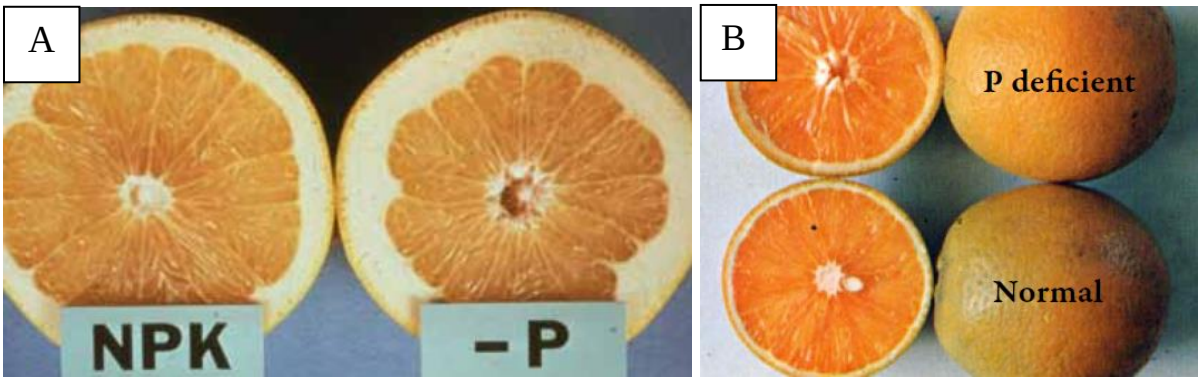

Figure 8. Phosphorous deficiency leads to fruit with a thick rind and hollow core as illustrated in photos A and B. Rind colour can, however, be improved slightly at low P levels as seen in B (Obreza and Morgan, 2008).

Other macronutrients

Calcium is the most abundant element in the structure of mature *Citrus* trees (Chapman, 1968), where it plays a role in metabolite transport, enzyme functions as well as cell wall structure (Davies and Albrigo, 1994). However, apparently, Ca has little effect on juice quality and external fruit quality (Koo, 1988a) and is very rarely limiting in *Citrus* (Dasberg, 1988). When deficient, Ca leads to small and misshapen fruit (Fig. 9). Similarly, Mg has little effect on fruit quality except when severely deficient (Koo, 1988a). At high rates, this element increases SSC and consequently SSC:TA ratio (Koo, 1988a). Externally deficient levels of Mg slightly increase fruit size and mass, whilst also decreasing rind thickness (Koo, 1988a). As with Ca, Mg has a role in enzyme reactions within the tree, but is also rarely limiting (Davies and Albrigo, 1994). Sulphur, although a macronutrient and required in large amounts as part of proteins and enzymes (Smith, 1966b), is seldom deficient in *Citrus*, with deficiency symptoms only known from soil pot culture experiments.

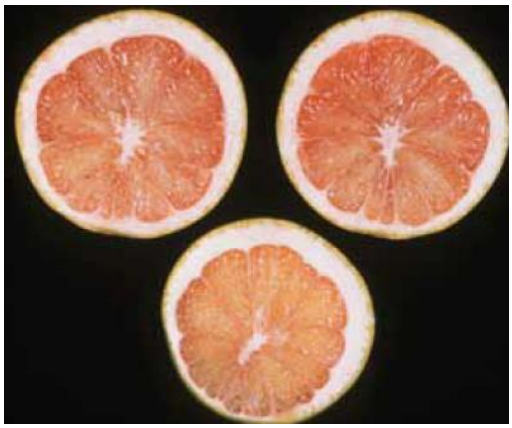

Figure 9. Calcium deficiency leads to small misshapen fruit (Zekri and Obreza, 2012b).

Micronutrients

Micronutrients (Fe, Zn, Mn, B, Cu, Mo, Ni and Cl) seldom influence *Citrus* fruit quality (Koo, 1988a), unless severely deficient (Fig. 10A, 10B, 10C, 10D and 10E). The influence of micronutrients on *Citrus* fruit quality should, however, not be ignored (Embleton et al., 1988) and can be of high importance on a light soil type (Smith, 1966a). After identification of Zn and Mn foliar deficiency symptoms in California in the 1930's, the correctional use of these elements by including them in pesticide foliar sprays became common practice (Embleton et al., 1988).

Zinc deficiency is apparently widespread, but most studies found no economically significant effects on *Citrus* fruit quality, most likely because only mild deficiencies were present. Swietlik (1996a) reported an increase in yield of grapefruit trees following a Zn foliar spray two months before anthesis, when 20% or more leaves had deficiency symptoms (Fig. 10F), yet fruit quality remained unaffected. Similar, studies with 'Valencia' orange found fruit yield to be unaffected despite correcting Zn foliar deficiency symptoms (Embleton et al., 1988). Even when Zn is applied as a foliar application, translocation from sprayed to unsprayed leaves is generally poor (Swietlik, 1996a).

Manganese has been found to decrease fruit size as well as to increase the incidence of creasing, the ratio of SSC to acid content and vitamin C in juice (Embleton et al., 1988). Similar to Zn, Mn has very limited translocation following foliar application, although the correction of Mn foliar deficiency symptoms with the use of foliar sprays, has been reported to increase yield in both oranges and lemons (Embleton et al.,1988).

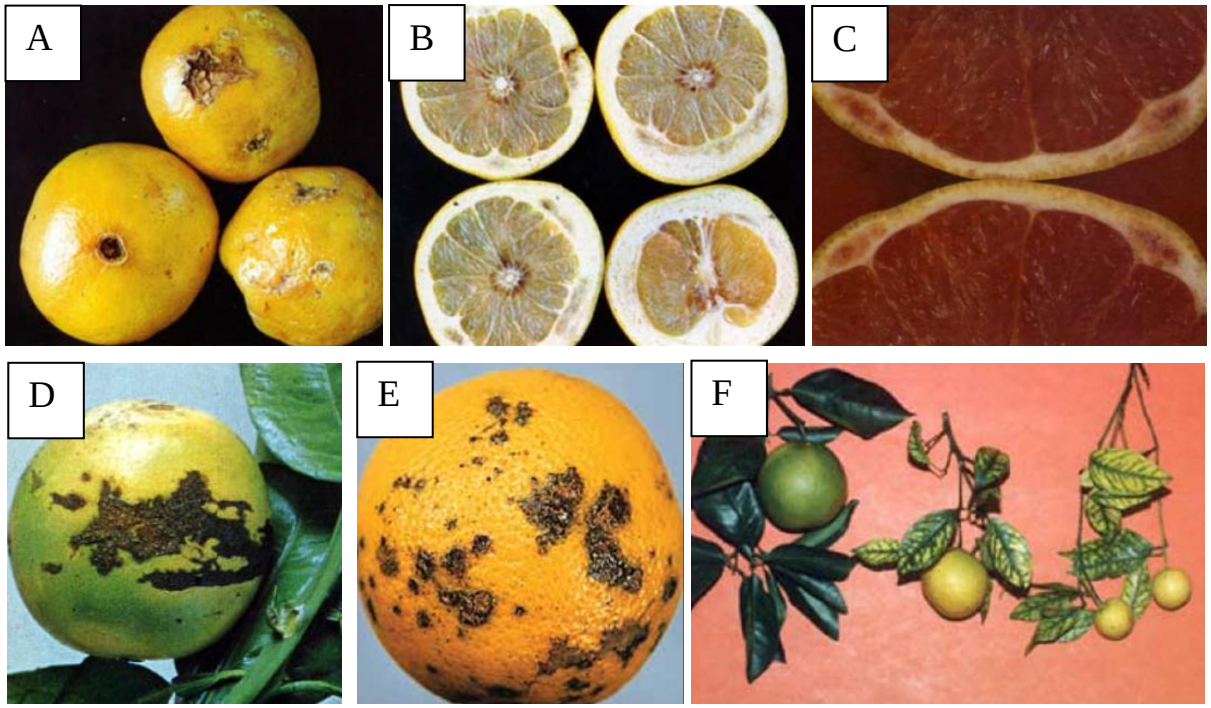

Figure 10. Micronutrient deficiencies. A, B and C - Boron deficiency symptoms on grapefruit: A – external symptoms, B – internal symptoms including gumming and a thick rind, C – Gum pockets formed in rind (Obreza and Morgan, 2008); D and E - Copper deficiency causes “ammoniation” of *Citrus* fruit (D - Zekri and Obreza, 2013; E - Obreza and Morgan, 2008); F - Interveinal chlorosis and small leaves are characteristic of Zn deficiency (Obreza and Morgan, 2008).

Nutrient uptake and fertilisation methods

Mineral nutrient uptake pathways

Soil uptake

Plants are well adapted for the uptake of water and nutrients from the soil due to the presence of an extensive root system (Briskin and Bloom, 2010). Roots are designed for the uptake of mineral nutrients and is much more effective in the absorption of mineral nutrients than leaves. Once transported across the root membrane, mineral nutrients are translocated to different plant organs primarily via the xylem (Marschner et al., 1996) with root uptake of mineral nutrients generally being an active process (Eichert, 2013). Soil temperature has a very large influence on root uptake, with low temperatures having a negative impact on uptake as soil temperature determines the hydraulic conductivity thereof (Kriedemann and Barrs, 1981).

Foliar uptake

The model by Thornley (1972) prescribes the roots to supply mineral nutrients to the shoots via the xylem, whilst the shoots supply carbon to the roots via the phloem. Mineral nutrients can, however, be remobilised and transported between plant organs and back to the roots via the phloem, provided the mineral nutrient in question is phloem mobile (Marschner et al., 1996). The foliar application of phloem mobile nutrients is possible due to this occurrence. However, in contrast to root uptake, foliar nutrient uptake is a passive process. Foliar uptake occurs through two pathways, stomatal penetration and the cuticular pathway (Eichert, 2013). Leaves are, however, not designed for the uptake of mineral nutrients with a lipophilic cuticle that repels water and stomata that are protected from water penetration (Schönherr and Bukovac, 1972).

Fertilisation methods

Soil application

Fertiliser formulation and form influences its effect on the tree and fruit as well as the efficacy thereof, as Berger et al. (1996) demonstrated in a study using a comparison of K sources. Efficacy of a fertiliser is defined by how much of the applied product is eventually absorbed by the plant to metabolize (Maynard and Lorenz, 1979). The N requirement of an orchard is subject to the uptake efficiency of the fertiliser used (Quiñones et al., 2004).

Most studies on the effect of different fertiliser forms have been done on N fertiliser. Nitrate (NO_3^-) and ammonium (NH_4^+) are the most important forms of N for nutrition (Von Wirén et al., 1997). Nitrate ions are not easily adsorbed to soil colloids and very mobile, ammonium ions are not as easily leached or volatilized (Barker and Mills, 1980). The N from fertiliser is affected by soil processes such as leaching, volatilisation, denitrification and immobilisation (Quiñones et al., 2004, 2007) which are the so-called indirect losses (Maynard and Lorenz, 1979). Direct losses are those happening due to the error on the producer's side, such as incorrect application timing, excessive application or use of the wrong fertiliser (Maynard and Lorenz, 1979). These processes make N unavailable to the tree and necessitates replacement. Soil characteristics and prevailing soil conditions have a large impact on the optimal fertiliser application timing and amount (Ting and Attaway, 1971), with certain soils being more prone to leaching and therefore being more sensitive to high N applications (Boman et al., 2008).

Both the source and amount of N can influence the efficacy of the fertiliser to promote fruit production**.** Some N fertilisation options include fertigation, dry granular broadcast treatments, controlled-release N, soluble source N, urea and organic N.

Alva et al. (1998) compared fertigation and dry granular broadcast N management treatments in 'Valencia' orange trees. Fertigation increased nutrient uptake, resulting in a greater yield, but no significant differences in leaf nutrient concentrations or fruit quality was reported. Koo (1988b) reported controlled-release N to be superior to soluble sources. Application frequency did not influence the effectiveness of controlled release sources, but did differ in the soluble sources with respect to leaf N content and fruit production. Quiñones et al. (2007) also found that fertigation is superior to granular fertiliser. He et al. (2008), however, did not find any differences between different N sources on grapefruit yield or quality.

Benefits of controlled-release fertilisers include that nutrients are available for an extended period and leaching is reduced (Maynard and Lorenz, 1979). Controlled-release sources can thus be applied less frequently (Jackson and Davies, 1984; Koo, 1988b).

Organic N is not commonly used in *Citrus* since the development of synthetic chemical fertilisers and only 0.1% of fertiliser applied to *Citrus* is of an organic nature (Obreza and Ozores-Hampton, 2000).

Foliar application

Foliar fertilisation is not the main method of supplying nutrients, but is rather a form of supplementation. Leaves are usually the target organ with foliar applications, but other organs, such as fruit, can also be targeted. The application of foliar nutrients should coincide with specific phenological times such as flowering and fruit set for the greatest effect, therefore it can be beneficial when nutrient requirements are high during these periods (Lovatt, 1999) and under conditions when the uptake of soil-applied nutrients are hampered (El-Otmani et al., 2000b). The uptake of soil applied nutrients can be hindered by unfavourable soil conditions brought about by conditions such as unfavourable soil temperatures, soil water content, pH or salt content (Lovatt, 2013). Furthermore, foliar nutrient applications can be seen as a more environmentally friendly method as there is less accumulation of nutrients in the soil and leaching thereof into the groundwater (Lovatt, 2009).

Nutrients applied on foliage should be phloem mobile for it to move to other plant parts and not just have a localized effect. The application of foliar nutrients can be done alone or in combination with other products such as pesticides (Ting and Attaway, 1971) or plant growth regulators (El-

Otmani et al., 2000a). Various mineral nutrients can be sprayed with effects thereof depending on the element in question, the phenological stage it was applied and the concentration. Applying low-biuret urea in winter, or at pre-bloom and spring increases flowering and yield with negligible effects on fruit quality (Ali and Lovatt, 1994; Lovatt, 1999). Nitrogen is usually applied as urea on foliage, but can be successfully replaced with potassium nitrate (KNO_3) which has the added beneficial effect of increasing both N and K contents of the leaf (Erner and Ya'acov, 2004). In grapefruit, KNO_3, mono-potassium phosphate (MKP) and di-potassium phosphate (DKP) applied at post bloom and/or summer increased fruit size (Boman and Hebb, 1998). Applying potassium phosphite at colour break on 'Clementine' mandarin advances fruit maturation (El-Otmani et al., 2000b). Mono-potassium phosphate and mono-ammonium phosphate (MAP) applied at six weeks after full bloom reduced the TA of 'Thoro Temple' tangor (Mudau et al., 2005). Rind texture was improved by the application of MKP, MAP and urea ammonium phosphate (UAP) applied at six weeks after full bloom in 'Nouvelle' and 'Thoro Temple' tangor as well (Mudau et al., 2005). Certain deficiencies such as Zn are corrected more easily with foliar sprays than soil applications (Swietlik, 2002).

Conclusion

The effect of mineral nutrients on *Citrus* fruit quality has been well documented over many decades, with much of what is known currently being grounded on classical, baseline studies. Yet, many discrepancies exist in literature, mainly as the impact of mineral nutrition on the phenology, as well as growth and development in *Citrus* are influenced by so many factors and may vary with the tree nutritional status, climatic and soil conditions, between cultivars and with the ratios and interactions that occur between the various elements. To date most mineral nutrition research regarding production and fruit quality has focused on N, followed closely by K as the other macro and micronutrients has been said to be of lesser importance for *Citrus* fruit quality and therefore not a serious consideration, unless severely deficient. Nevertheless, all aspects and principles of mineral nutrition should be considered when planning a fertilisation program. In addition to acknowledging that various climatic regions, cultivars and soil types can react differently to the availability of mineral elements, areas of current research also focus on ascertaining the proper timing of fertilisation practices within the tree phenology and fruit growth stage. These complex considerations when designing a fertilisation program are all required to improve production and quality, whilst minimizing fertiliser losses for both economic and environmental reasons.

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Chapter 3: Evaluation of the efficacy of early summer foliar application of MAP on 'Nadorcott' late mandarin for an extended harvest window

Abstract

Citric acid levels in *Citrus* fruit are influenced by prevailing climate during development. Fruit from production areas with cold winters take longer to decrease in citric acid levels, which in turn may result in a delay in harvest date. Such a delayed harvest can aggravate alternate bearing patterns due to inhibition of flower development and fruit set of the following season. 'Nadorcott' is a high-value, late mandarin cultivar that is particularly prone to alternate bearing. To evaluate the impact of phosphate containing products on acid content reduction, foliar applications of mono-ammonium phosphate (MAP) at different concentrations in addition to calcium arsenate (Ca-As) were applied over two consecutive seasons in a production area with a cold winter in the Western Cape. Potassium nitrate (KNO_3), known to increase the acid content of *Citrus* fruit, was used as a negative control treatment. The treatments were applied at stage II of fruit growth in Nov., at seven to eight weeks after full bloom (WAFB). An additional MAP treatment was applied later in the season at six weeks before harvest. In addition to quantifying the citric acid content, all other fruit quality parameters, for example soluble solids content (SSC/°Brix) and rind colour was measured. The Ca-As and MAP treatments decreased the citric acid content with up to 0.34% and 0.42%, respectively, depending on the method of determination, although not always statistically significantly at the 5% confidence level. MAP foliar sprays could be considered for application under commercial production conditions, especially in 'on' years when fruit tend to be smaller and more acidic. The effects on other fruit quality parameters were negligible, except for significant phytotoxicity due to the MAP foliar application sprayed six weeks before harvest.

Additional keywords: alternate bearing, arsenate, fertilisation, foliar fertilisation, potassium nitrate

Introduction

The internal quality and maturity of *Citrus* fruit are characterized by soluble solids content (SSC), titratable acidity (TA) and the ratio of SSC:TA. The TA refers to all the free organic acids present in *Citrus* juice, with citric acid as the main organic acid. For that reason, the citric acid content correlates well with the commercial TA measurements as determined through titration (Sadka et al., 2000a; Sinclair, 1984). All the organic acids and sugars present in the fruit mainly constitute the SSC (Albertini et al., 2006; Sinclair, 1984). *Citrus* fruit growth development has a sigmoidal pattern with

three distinct stages (Bain, 1958). During stage II, citric acid content increases until the fruit reaches approximately 50% of its final size (Erickson, 1968; Sinclair, 1984), where after it decreases gradually towards the end of the season until harvest (Iglesias et al., 2007; Sadka et al., 2000a, b). A high acid content can reduce *Citrus* fruit quality or delay the harvest (Sadka et al., 2000a).

The rate of citric acid breakdown is influenced by climate (Goodrich, 2000; Marsh et al., 2000; Mudau et al., 2005), with air temperature as the main factor influencing the time for *Citrus* fruit to reach maturity (Barry et al., 2000). In production areas with colder winters, acid content reduction takes longer, therefore delaying fruit maturity and harvest date. In contrast the acid content of fruit from warmer production areas is reduced at an earlier stage, due to the higher respiration rate experienced by the fruit. As the respiration process produces energy and carbon compounds needed for the ripening processes (Tucker, 1993), a higher respiration rate promotes ripening and advances maturity. Organic acids, which are products from the citric acid cycle during respiration, are metabolically oxidized within the mitochondria where these aliphatic organic acids are first altered and then exported to accumulate within the vacuole (Sinclair, 1984). As organic acids are used to sustain respiration, levels decrease towards ripening (Ulrich, 1970; Tucker, 1993). In contrast to the organic acids in the pulp, sugars increase throughout fruit development and maturation (Albertini et al., 2006; Iglesias et al., 2007; Richardson et al., 1997).

The acid content of *Citrus* fruit is reduced by phosphorus (P) applications (Anderson, 1966; Smith, 1966), as this molecule is critical for respiration and energy release via the phosphorylation process and ATP which is required for the normal functioning of cells (Smith, 1966). This role of phosphate in supplying energy through ATP within the mitochondria could be the mechanism reducing the acid content during maturation (Smith et al., 2002). Adequate P availability is thus of key importance during fruit ripening as it decreases acidity, increases the ratio of SSC to acidity in addition to decreasing rind thickness, however P is known to reduce the colour development of the rind (Alva et al., 2006; Koo, 1988).

Potassium (K) is strongly associated with increased acidity, size, mass, rind thickness, and the number of green *Citrus* fruit, while it decreases juice content and the SSC:TA ratio (Alva et al., 2006; Koo, 1988). Potassium's main impact is that of increased fruit size and acidity (Alva et al., 2006; Du Plessis and Koen, 1988; Erner et al., 2004), however it could also negatively influence rind quality, where too high levels thereof lead to a coarse, thick rind (Alva et al., 2006; Smith et al., 1963). Phosphorus and K have a contrasting effect on fruit acidity, however when a combined P- and K-containing foliar spray, for example mono-potassium phosphate (MKP) was applied to *Citrus* it decreased TA in 'Shamouti' orange and 'Star Ruby' grapefruit and 'Mihowase Satsuma' mandarin

(Lavon et al., 1996; Mudau et al., 2005) and thereby increased the SSC:TA ratio leading to an earlier harvest date.

The internal quality of *Citrus* fruit can effectively be altered and improved by the application of arsenic compounds during fruit development (Anderson, 1966; Deszyck and Sites, 1954; Reitz and Hunziker, 1961; Sadka et al., 2000a). However, the use of these products has been prohibited in most countries (Sadka et al., 2000a). These compounds, which can be added either as soil or foliar applications, may include lead arsenate ($PbHAsO_4$), calcium arsenate ($Ca_3(AsO_4)_2$ (Ca-As) and sodium arsenite ($NaAsO_2$). Arsenic compounds affect *Citrus* physiology by reducing the acidity (Deszyck and Sites, 1954; Miller et al., 1933; Reitz and Hunziker, 1961; Sadka et al., 2000a; Ting and Attaway, 1971; Wilson and Obreza, 1988; Yamaki, 1990ab), resulting in earlier harvest maturity as estimated by a lower SSC:TA ratio (Sadka et al., 2000a). In 1954, Deszyck and Sites stated the mechanism by which acid reduction through arsenic products occurs as unknown. In addition, it is possible that the mode of action of these arsenic compounds, for example arsenate and arsenite, could also differ distinctly (Sadka et al., 2000a). Subsequent studies into the mode of action indicated that after application, arsenite inhibits citrate synthase activity temporarily whereas arsenate is associated with the phosphate transport system where it impedes phosphorylation by competing for electrons with phosphate. Furthermore, arsenate reduces the activity of citrate synthase as well as the concentration of Acetyl-CoA (Sadka et al., 2000a; Vines and Oberbacher, 1965; Yamaki, 1990ab). Arsenite decreases total acidity by lowering citric acid content, whilst it has no effect on other organic acids. In addition, there seems to be an initial decrease, after application during early fruit development, after which the difference between the arsenite treatment and control remains constant (Sadka et al., 2000a). The timing of application of arsenic compounds is very important and foliar applications have been found to only elicit the desired response if applied early enough at post-bloom or before the fruit reaches a certain minimum size (Deszyck and Sites, 1954; Ting and Attaway, 1971).

Of paramount importance in the efficacy of any nutrient application is the correct timing during a specific fruit growth stage. For example, when using K foliar sprays, K has a certain window period in which it will increase fruit diameter, but where after it will no longer have an effect (Erner et al., 2004). The optimum application to increase fruit size through potassium nitrate (KNO_3) was between 40 - 60 days after full bloom (DAFB) in this study. Furthermore, certain phenological stages require a larger amount of specific nutrients compared to other stages (Lovatt, 2009).

Alternate bearing is the phenomenon where a large crop, the ‘on’ year, is followed by a small crop, the ‘off’ year, in a continuous cycle which can vary in intensity. A delay in harvest can aggravate

alternate bearing, or even be the main cause of it, through the effect of the current crop load on flowering and thus consequently the following season's possible yield (Monselise and Goldschmidt, 1982). Flowering is negatively affected by both a delay in harvest and the current fruit load, which collectively results in a possible depletion of reserves and hormonal imbalance (El-Otmani et al., 2004).

The 'Nadorcott' mandarin is a late maturing soft *Citrus* harvested in July or Aug. in the Western Cape Province, with the tendency of too high acid content, in this winter rainfall production area of South Africa, when other harvest indices such as SSC or rind colour are optimum. By decreasing the acid content fruit will mature sooner and be ready for harvest at an earlier date. Thereafter the tree will have more recovery time from the current year's crop, with an improved flowering and fruit set for the next season. The aim of this study is to determine whether foliar application of MAP directly after full bloom as well as before harvest could be used as a management strategy to reduce acid levels to enable earlier harvesting.

Materials and methods

Sites and plant material

The trial was conducted on 'Nadorcott' mandarin trees (*C. reticulata* Blanco) on Carrizo rootstock, planted in 2004 at a 5 m x 2 m spacing, in a North to South row direction. The trees were in a commercial orchard on the farm Kanetvlei, De Doorns, Western Cape Province, South Africa (33°30'47.6"S 19°31'12.1"E). The area has a mediterranean climate with hot summers and cold winters with rainfall and an annual precipitation of 585 mm. The trees included in the experiment were healthy and uniform, with buffer trees between treatment trees and buffer rows between rows with treated trees. The trial was repeated over two seasons (2015 and 2016).

Treatments

The treatments were applied to trees as foliar sprays with a motorised knapsack sprayer until runoff. Each tree received approximately 2.25 L of the mixture or was applied at 2250 $L.ha^{-1}$, which is comparable to commercial foliar applied mineral nutrients at a low volume coverage (1500 L – 3000 $L.ha^{-1}$). In both seasons, Ca-As, mono-ammonium phosphate (MAP) and KNO_3 was applied (Table 1).

Table 1. Mineral nutrient products applied [calcium arsenate (Ca-As), mono-ammonium phosphate (MAP) and potassium nitrate (KNO_3)], concentrations sprayed, with application times with respect to weeks after full bloom (WAFB), weeks before harvest (WBH), and fruit growth stages, together with the amount of product applied in gram per tree ($g.tree^{-1}$) and kilogram per hectare ($kg.ha^{-1}$) on 'Nadorcott' mandarin in De Doorns, in the 2015 and 2016 season, in a trial aimed to advance harvesting time.

Commercial product name	Product	Chemical formula	Concentration (%)		Application time		Fruit growth stage	$g.tree^{-1}$ and $kg.ha^{-1}$ (1000 trees per ha)							
								Ca-As		N		P		K	
			2015	2016	WAFB	WBH		2015	2016	2015	2016	2015	2016	2015	2016
Calcium arsenate, Plaaskem (Pty) Ltd	Calcium arsenate (Ca-As)	$(Ca_3(AsO_4)_2)$	0.05	0.1	7		Stage II	1.0	1.9						
Vitassol™ MAP, SQM VITAS	Mono-ammonium phosphate (MAP)	$(NH_4H_2PO_4)$	0.8	1.5	7		Stage II			2.1	4.1	4.5	8.8		
			1.5	2						4.1	5.4	9.0	11.7		
			0.8 x 2	1.5 x 2	7 & 8					8.3	8.1	17.9	17.6		
				2 x 2	7 & 8						10.8		23.4		
			1	1		6	Stage III			2.7	2.7	5.9	5.9		
Multi-K GG, Haifa Chemicals	Potassium nitrate	(KNO_3)	2	2	7		Stage II			6.2	12.2			17.6	34.6

Quality measurements

From each tree (replicate), ten fruit per tree were sampled for external and internal quality measurements at 20, 15, 9 and 4 weeks before harvest and at harvest, in the 2015 and 2016 season. Fruit were sampled from the window area of the tree approximately 20 cm into the canopy. A five-year historical harvest date was used to estimate pre-harvest sampling intervals with harvest at 17 August in 2015 and 04 August in 2016. The orchard has a history of alternate bearing delivering 60 ton in the 2015 season and 40 ton in the 2016 season.

Internal quality

Citric and Total Acid Content, Pulp Soluble Solids Content and Total Sugars

The internal quality was quantified as citric acid content (%), SSC which is measured and expressed as °Brix, the ratio of SSC:citric acid and juice content. The fruit were juiced with a mechanical citrus juicer (Sunkist®, Chicago, USA) and used for internal quality measurements. Citric acid was measured with an automatic titrator (Metrohm Titrando with an 815 Robotic USB Sample Processor and tiamo software, Metrohm, Switzerland). °Brix was determined on the same sample using an electronic refractometer (PR-32 Palette, Atago Co, Tokyo, Japan).

HPLC MEASUREMENTS. In addition to the automatic titrator and electronic refractometer, the glucose, fructose, sucrose, citric and total acid content of the juice sample was determined using high-performance liquid chromatography (HPLC) (Agilent 1100 Series, Agilent Technologies, Santa Clara, CA, United States). Citrus juice was frozen in 2 mL Eppendorf tubes prior to storage at -40°C for collective analysis to decrease any possible variability. For extraction, the samples were defrosted and the juice samples were mixed with a Vortex before centrifugation at 4000 rpm for 4 min (5417 R centrifuge, Eppendorf, Hamburg, Germany). After centrifugation, 100 µL of the clear supernatant was pipetted into 50 mL falcon tubes, followed by 9900 µL of distilled water to make a 100 times dilution of the extracted juice. The aqueous supernatant was again mixed with a vortex before being filtered through a 0.45 µm syringe filter into HPLC vials to remove solid particles. Separation was achieved with a Rezex ROA-Organic Acid H+ (8%) column (150×7.8 mm, 8 µm) [Phenomenex, Torrance, Calif., U.S.A.) which was thermostated at 30°C as the solid phase with 2 mM sulphuric acid (H_2SO_4) solution as the mobile phase at an isocratic flow rate of 0.3 $mL.min^{-1}$. A diode array detector (DAD) set at 210 nm and a refraction index detector (RID) was used to determine the acids and sugars. Standard solutions of glucose, fructose, sucrose and citric acid were used for peak identification and quantification.

External quality

The external quality was quantified by measuring rind colour with a colorimeter (Konica Minolta CR-400, Tokyo, Japan) on two sides of the fruit. Colour was expressed as hue angle (°). Fruit diameter and rind thickness was determined using an Electronic Fruit Size Measure (GÜSS Manufacturing (Pty) Ltd., Strand, South Africa) and an electronic caliper (CD-6"C, Mitutoyo Corp, Tokyo, Japan), respectively. The average fruit mass was determined using combined fruit weight.

Cold storage

The impact of treatments on the storage potential was assessed by storing 15 fruit sampled per replicate (n=10) after harvest at either 4°C or -0.6 °C for 30 days. The external and internal fruit quality parameters were determined after cold storage as described previously and the fruit were evaluated for physiological rind disorders after seven days of shelf life at room temperature.

Experimental design and statistical analysis

The experimental design was a randomised complete block with six treatments in the 2015 season and seven treatments in the 2016 season, replicated in ten blocks. Analysis of variance (ANOVA) was carried out, with means separated using Fisher's LSD ($p < 0.05$ and 0.1) and was carried out using StatSoft Statistica (version 13.0, StatSoft, Inc.).

Results

Internal quality

Citric and Total Acid Content

There was a significant reduction in the citric acid content, as measured with the automatic titrator, at harvest in the 2015 season but not at any other sampling date during the 2015 season or at harvest in the 2016 season (Tables 2 and 5). Furthermore, there were statistically significant differences between treatments in values obtained using HPLC analysis for citric and total acid content in both seasons (Tables 2 and 5). In the automatic titrator results, at harvest in the 2015 season, the citric acid content of the control was significantly higher than the Ca-As treatment and all the MAP treatments (Table 2). In the 2016 season, the control once again had the highest citric acid percentage, but did not differ significantly from the Ca-As and MAP 2% + 2% treatments (Table 5).

During the 2015 season, the citric and total acid content, as measured using HPLC analysis, produced no significant differences during fruit development, however at harvest significant

differences between treatments emerged (Table 2). In the HPLC analysis, in contrast to the citric acid values obtained using the automatic titrator, the MAP 0.8% + 0.8% treatment had the lowest value for citric acid content significantly compared to the control. The Ca-As treatment for the citric and total acid content was also significantly lower than values reported for the control (Table 2). The MAP 0.8% + 0.8% treated fruit had a significantly lower total acid content than all the treatments except for the Ca-As and KNO_3 treatments. For total acid content the Ca-As treatment did not differ significantly from the KNO_3 treatment.

In the 2016 season, significant differences in citric and total acid content as measured with HPLC between treatments were obtained (Table 5). The control had significantly higher citric and total acid content in comparison to the Ca-As treatment. For citric acid, none of the other treatments differed significantly from the control, except for the Ca-As treatment. Also, the MAP 1.5% or the MAP 2% + 2% treatments did not differ significantly from the Ca-As treatment in citric acid content (Table 5).

The Ca-As treatment was also the only treatment that had a significantly lower total acid content in comparison to the control, as none of the other treatments differed significantly from the control.

Pulp Soluble Solids Content and Total Sugars

During the 2015 season, no significant differences in sucrose, glucose, fructose or total sugar content at any sampling date was recorded, however at harvest all these parameters displayed significant differences (Table 3). For the sucrose content, fruit from all treatments produced significantly higher values than the MAP 0.8% + 0.8% treatment, except for the KNO_3 treatment. The other treatments did not differ significantly from each other, except for the MAP 1.5% treatment which was significantly higher than the KNO_3 treatment. A similar pattern was also observed with the glucose and fructose and total sugar values where the MAP 0.8% + 0.8% treatment recorded significantly lower values than all the other treatments, whilst the KNO_3 treatment scored significantly lower than both the MAP 0.8% and MAP 1.5% treatments (Table 3). Overall, the MAP 0.8% + 0.8% and KNO_3 treatments tended to have the lowest sugar values, whilst the MAP 0.8% and MAP 1.5% treatments were inclined to have the highest sugar values.

In 2016 at harvest there were no significant differences in the sucrose and total sugar content, however significant differences in the glucose and fructose values were recorded (Table 5). The glucose values obtained from the control and the Ca-As treated fruit were significantly lower than all the MAP treatments, except for the MAP 1.5% and the KNO_3 treatments. The fructose content of the

control was significantly lower than all the MAP treatments, but did not differ significantly from the Ca-As and KNO_3 treatment. The fructose value of the Ca-As treated fruit was significantly lower than recorded in all the MAP treatments except for the MAP 1.5% treatment. Alternatively, the MAP 2% + 2% fructose content was significantly higher than all the none-MAP treatments. There were no significant differences observed at any sampling date or season in the SSC (°Brix) measured (Tables 4 and 5).

Citric acid:SSC and Juice content

The ratio of SSC to citric acid was positively affected by the foliar treatments at harvest and significantly so in the 2015 season (Tables 4 and 5). In 2015, the control had the lowest ratio value whilst the Ca-As treatment recorded the highest ratio, reflecting the citric acid value differences reported for the respective treatments (Table 2). All treatments had significantly higher ratios compared to the control (Table 4). The values of the MAP-containing treatments and that from the KNO_3 treatments were comparable, with none of them differing significantly from one another. The ratio obtained from MAP 0.8% treated fruit was the only treatment where values did not differ significantly from that of the Ca-As treated fruit. In 2016, at harvest, the SSC to citric acid ratio did not differ significantly at the 5% confidence level ($p=0.0962$) (Table 5). The juice content was not significantly affected by any treatment in either season (Tables 4 and 5).

External quality

The influence of alternate bearing is evident in the fruit mass and diameter between seasons, where 2015 can be considered an ‘on’, with 2016 as an ‘off’ year (Tables 6 and 7). However, none of the foliar treatments affected the fruit, mass or rind colour (hue°) significantly over the two seasons, except for a slight hue° difference between the control and MAP 0.8% at 20 weeks before harvest (Table 6). This however appeared to be a random occurrence and not of commercial significance.

Cold Storage

Internal quality

For citric acid content, there were no significant differences, irrespective method of estimation, season or storage temperature (Table 8).

As for the citric acid, the total acid content did not differ significantly in any of the season or temperature storage regimes, except for the fruit storage at -0.6°C in 2016 where the control recorded the highest total acid content. This value was significantly higher than obtained in fruit from

the MAP 2% + 2%, MAP 1.5% and Ca-As treatments respectively. The MAP 2% + 2% treatment was also significantly lower than the value obtained from fruit of the MAP 2% treatment (Table 8).

In the 2015 season, there was no significant difference in the percentage sucrose, glucose, fructose and total sugar following storage at either -0.6°C and 4°C (Table 9). In contrast to this observation, there were significant differences in all these parameters after storage at both -0.6°C and 4°C in the 2016 season (Table 9). Even though no clear pattern could be determined, a trend was noted where fruit from the Ca-As treatment had the highest and fruit from KNO_3 treatment the second highest sucrose content, following storage at both temperatures in 2015 and at 4°C in 2016. The MAP 0.8% + 0.8% treated fruit had the second lowest sucrose content after storage at -0.6°C, and the lowest sucrose content following storage at 4°C in 2015. As before cold storage, there were no significant differences in SSC values of fruit from any of the treatments, following cold storage in either the 2015 and 2016 season (Table 10).

In 2015 the ratio of SSC to citric acid was only significantly affected by the treatments during cold storage at 4°C (Table 10), where the control fruit recorded the lowest ratio of all the treatments, lower than all the other treatments except for the KNO_3 treatment. In the 2016 season, no significant difference was recorded between any of the treatments, irrespective of the storage temperature (Table 8). No significant differences in juice content at either storage temperature nor in any season was reported (Table 10).

External quality

The external fruit quality parameter, for example diameter, mass, rind colour and rind defects were not significantly affected by any treatment after cold storage at 4°C or -0.6°C, irrespective of the season (Table 11).

MAP foliar application after colour break

External and internal quality

There was no significant difference between the citric or total acid content of fruit from the control and the MAP 1% treatment which was applied six weeks before harvest, as measured with either the automatic titrator or by means of HPLC analysis, in both seasons (Table 12).

For the 2015 season, SSC in the control and 1% MAP treatment did not differ significantly, however in the 2016 season the 1% MAP treated fruit recorded a significantly higher SSC value than the control fruit (Table 12). For percentage sugars (glucose, fructose, sucrose and total sugars) in the

2015 season, no significant difference between the control and 1% MAP treated fruit was recorded (Table 12), but in the 2016 season the 1% MAP treated fruit had significantly higher percentage sugar values than reported for control fruit (Table 12). There was no significant difference between the control and 1% MAP treated fruit in the ratio of SSC to citric acid or in juice content of either season (Table 12).

The 1% MAP treatment was applied after colour break or approximately six weeks before harvest. In both seasons, the rind was affected by the treatment; leading to a phytotoxic burn (Fig. 1) which affected 65-70% of all fruit on the 1% MAP treated trees.

Cold storage

In 2015, citric acid content as measured with the automatic titrator and HPLC analysis as well as total acid content was significantly lower in the MAP 1% treated fruit after storage at 4°C, thereby positively affecting the ratio of SSC:TA (Tables 13 and 14). Also, similar to the reports at harvest, there was no significant difference in SSC between the control and 1% MAP treated fruit, after storage at -0.6°C or 4°C in the 2015 season, although a significantly lower SSC value was reported in the 2016 season for the control fruit (Table 13). A significant difference in the ratio of citric acid to SSC between the control and 1% MAP treatment after storage at 4°C was recorded in the 2015 season (Table 13), whilst there was no significant difference at -0.6°C in the 2015 season or at either temperature in the 2016 season (Table 13). Similar to determinations at harvest, the control always scored a lower ratio than the MAP 1% treated fruit, due to the lower citric acid content and higher SSC thereof.

There was also no significant difference between the control and 1% MAP treated fruit in any of the sugars, following storage at -0.6°C and 4°C in the 2015 season (Table 14). After storage at 4°C in the 2016 season the 1% MAP treated fruit recorded significantly higher values for percentage sucrose, glucose, fructose and total sugar content compared to the control fruit. In the same season, storage at -0.6°C also produced a higher fructose content in the 1% MAP treated fruit compared to the control. There was no significant difference in the juice content between the control and 1% MAP treated fruit at either storage temperature, both in the 2015 or 2016 season (Table 13).

The external quality of 1% MAP treated fruit where MAP was applied after colour break was not determined after storage. The majority of the fruit was affected by a phytotoxic burn (Fig. 1), which interfered with the scoring for other blemishes. Furthermore, the MAP treatment damaged the fruit to such an extent that its application would not be recommended for commercial application, leaving the quantification of other quality parameters irrelevant.

Discussion

Calcium arsenate was effective and consistent in reducing the citric acid content of 'Nadorcott' mandarin fruit. Mono-ammonium phosphate treatments applied seven to eight weeks after full bloom (WAFB) also produced promising results, especially for the 2015 season where a clear trend in data was observed.

Citric acid values between treatments at harvest in the 2015 season were significantly different when obtained via the automatic titrator compared to the HPLC analysis. Results generated by means of the automatic titrator indicate values for the Ca-As and all the MAP treatments to be significantly lower than the control. However, in the HPLC analysis, only fruit harvested from the highest concentration MAP treatment (MAP 0.8% + 0.8%) was significantly lower than the control, along with the Ca-As treated fruit. The MAP and KNO_3 treatments did however achieve slightly lower citric and total acid content than the control, although not significant at the 5% confidence level. In the 2016 season, when citric acid was determined by means of the automatic titrator, Ca-As and MAP treatments produced lower values than the control, but not significantly, whereas the Ca-As treated fruit recorded significantly lower citric acid levels than the control when determined by HPLC. After storage, trends observed at harvest still existed, although the extent was less apparent. Similarly, differences in citric acid levels between treatments when sampling throughout the season were not significant. This was unexpected as the mechanism by which arsenic compounds decreases citric acid content has been reported to be most effective at an early fruit developmental stage after application (Sadka et al., 2000a).

The ratio of SSC to citric acid content in fruit was affected and reported to be more favourable, mostly because of the reduction of citric acid by Ca-As and MAP treatments. This ratio is important as it determines the palatability of the fruit. By reducing citric acid content, this ratio was possibly shifted earlier into a more desirable range. At harvest in the 2015 season, the ratio of all the treatments were higher and more desirable than that of the control, with the same trend visible in the 2016 season, although not significantly so. Similarly, after storage in both seasons, a pattern existed where the control produced the lowest and least desirable ratio, although only significantly so after storage at 4°C, in the 2015 season. The other internal quality parameters such as juice content, SSC, and percentage sugars as measured by HPLC, was minimally affected and if so, no clear pattern could be observed within and between the 2015 and 2016 seasons.

The KNO_3 foliar application tended to result in larger fruit, similar to a report by Boman (2002), although not significantly so in our study. Acid content was also not increased as expected

by the foliar application of KNO_3. A possible reason could be due to the timing of the spray, which resulted in a slightly larger fruit diameter and mass, which in turn caused a lower than expected acid and sugar content and not the increase as reported in literature. Ultimate fruit size is determined at the early stages of fruit growth (Rabe, 2000).

None of the external fruit quality parameters were affected by any of the foliar treatments applied seven to eight WAFB. Alternate bearing, which was prevalent in the orchard, had a much larger effect on fruit diameter and mass, where fruit with a much greater diameter and mass were reported in the 2016 season than for the 2015 season. However, the amount of fruit on the trees were also much less in the 2016 season, with the 2015 season being an 'on' year and the 2016 season an 'off' year. The 'on' years are characterised by small, acidic fruit and the 'off' years by large fruit with thick, coarse rinds (Monselise and Goldschmidt, 1982). Increasing concentrations of MKP has been found to be more effective to influence juice acid content than lower concentrations (Mudau et al., 2005). It is therefore possible that differences in the 2015 season at a higher concentration could have been more significant than the application thereof in the 2016 season, in the light of the alternate bearing 'on' year influencing the results. Fruit size differences between the two seasons could thus explain the effectiveness of the treatments to reduce acid content in the 2015 season, but not the 2016 season. In terms of providing guidelines that will take the alternate bearing habit into account, MAP should not be recommended in an 'off' year to reduce the juice acid content as the efficacy could be minimal.

The additional 1% MAP treatment sprayed at six weeks before harvest only decreased the citric acid content after storage at 4°C. The treatment had minimal effectiveness and furthermore led to phytotoxicity, affecting most of the treated fruit. It is however valuable to note that MAP negatively affected the fruit only after, but not before colour break.

Conclusion

Arsenical compounds are still more effective than MAP foliar sprays to reduce the organic acid content in *Citrus* fruit. The MAP treatments did, however, significantly reduce citric acid content in the 2015 season. This can potentially lead to an earlier harvest date that would be economically beneficial. In particular, the higher concentrations and double applications of MAP treatments, although still less effective than Ca-As, showed promising results in lowering the citric acid content and increasing the ratio of SSC to citric acid content. By using a double application after fruit set an earlier harvest can be achieved, without other fruit quality parameters being negatively affected. The timing of the sprays is of critical importance as it can determine whether the foliar application may

be effective. The fact that MAP foliar applications were more effective in an 'off' year at lower concentrations than an 'on' year can have implications for management decisions as to whether sprays should be applied in a certain year or not. Further experiments are required to determine the window of application after full bloom where foliar applications would remain most effective. Furthermore, the 1% MAP foliar application at six weeks before harvest is a good example of not only ineffectiveness, but also of possible phytotoxicity that may result from incorrect timing of application. To conclude, MAP treatments show potential as a management tool to advance the harvest date of 'Nadorcott' mandarin within 'on' years of alternate bearing cycles, when fruit are small and in general more acidic**.**

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Table 2. The influence of Ca-As, N-, P- and K-containing foliar sprays on the acid content (%) of 'Nadorcott' mandarin fruit pulp collected from De Doorns throughout the 2015 season.

Parameter	Treatment	20 WBH[z]	15 WBH	9 WBH	4 WBH	Harvest
Citric acid (%) (Automatic titrator)	Control	5.14[NS]	3.37[NS]	2.41[NS]	2.01[NS]	1.82 a*
	Ca-As	5.18	3.03	2.16	1.79	1.50 b
	MAP 0.8%	4.65	2.85	2.10	1.84	1.61 b
	MAP 1.5%	4.76	3.07	2.14	1.88	1.63 b
	MAP 0.8% x2	5.03	3.10	2.21	1.95	1.58 b
	KNO_3	4.98	3.15	2.28	1.95	1.64 ab
	p-value	*0.2659*	*0.1920*	*0.2260*	*0.5530*	*0.0292*
Citric acid (%) (HPLC)	Control	-[y]	3.59[NS]	2.34[NS]	2.30[NS]	2.01 a*
	Ca-As	-	3.12	2.10	2.05	1.67 bc
	MAP 0.8%	-	3.28	1.95	2.00	1.85 abc
	MAP 1.5%	-	3.24	2.07	2.09	1.86 ab
	MAP 0.8% x2	-	3.28	2.19	2.18	1.59 c
	KNO_3	-	3.06	2.17	2.13	1.79 abc
	p-value	-	*0.6674*	*0.1578*	*0.3718*	*0.0374*
Total acid (%) (HPLC)	Control	-	3.81[NS]	2.59[NS]	2.59[NS]	2.29 a
	Ca-As	-	3.29	2.36	2.34	1.95 bc
	MAP 0.8%	-	3.48	2.21	2.29	2.15 ab
	MAP 1.5%	-	3.42	2.34	2.37	2.15 ab
	MAP 0.8% x2	-	3.47	2.46	2.47	1.84 c
	KNO_3	-	3.24	2.43	2.41	2.07 abc
	p-value	-	*0.6428*	*0.1625*	*0.3333*	*0.0222*

[z]WBH = Weeks before harvest (Harvest: 17/08/2015 and 04/08/2016)
[NS]No significant differences
[y]Data not collected
*Means with a different letter within a column differ significantly at the 5% level (Fischer's LSD)

Table 3. The influence of Ca-As, N-, P- and K-containing foliar sprays on the sugar content (%) of 'Nadorcott' mandarin fruit pulp collected from De Doorns throughout the 2015 season.

Parameter	Treatment	15 WBH[z]	9 WBH	4 WBH	Harvest
Sucrose (%)	Control	3.01[NS]	6.87[NS]	6.79[NS]	7.19 ab*
	Ca-As	2.90	6.78	7.18	7.48 ab
	MAP 0.8%	2.57	6.22	6.54	7.43 ab
	MAP 1.5%	3.11	6.41	6.64	7.49 a
	MAP 0.8% x2	3.23	6.17	6.47	6.20 c
	KNO_3	3.48	6.28	6.48	6.81 bc
	p-value	*0.2606*	*0.0870*	*0.0837*	*0.0024*
Glucose (%)	Control	1.48[NS]	2.28[NS]	2.68[NS]	2.67 ab
	Ca-As	1.36	2.26	2.65	2.67 ab
	MAP 0.8%	1.40	2.19	2.55	2.83 a
	MAP 1.5%	1.44	2.26	2.59	2.80 a
	MAP 0.8% x2	1.49	2.19	2.51	2.29 c
	KNO_3	1.55	2.20	2.51	2.56 b
	p-value	*0.6689*	*0.6970*	*0.0884*	*0.0005*
Fructose (%)	Control	1.24[NS]	1.96[NS]	2.30[NS]	2.31 ab
	Ca-As	1.13	1.93	2.27	2.31 ab
	MAP 0.8%	1.16	1.90	2.22	2.49 a
	MAP 1.5%	1.22	1.95	2.24	2.45 a
	MAP 0.8% x2	1.24	1.89	2.19	1.97 c
	KNO_3	1.29	1.89	2.20	2.23 b
	p-value	*0.6644*	*0.7631*	*0.4265*	*0.0001*
Total sugar (%)	Control	5.73[NS]	11.11[NS]	11.77[NS]	12.17 ab
	Ca-As	5.39	10.97	12.11	12.46 ab
	MAP 0.8%	5.12	10.31	11.32	12.75 a
	MAP 1.5%	5.76	10.62	11.47	12.73 a
	MAP 0.8% x2	5.97	10.24	11.17	10.45 c
	KNO_3	6.31	10.37	11.19	11.61 b
	p-value	*0.3758*	*0.1143*	*0.0901*	*0.0009*

[z]WBH = Weeks before harvest (Harvest: 17/08/2015 and 04/08/2016)

[NS] No significant differences

*Means with a different letter within a column differ significantly at the 5% level (LSD)

Table 4. The influence of Ca-As, N-, P- and K-containing foliar sprays on internal fruit quality parameters of 'Nadorcott' mandarin fruit pulp collected from De Doorns throughout the 2015 season.

Parameter	Treatment	20 WBH[z]	15 WBH	9 WBH	4 WBH	Harvest
SSC (Brix°)	Control	10.2[NS]	11.0[NS]	13.4[NS]	14.2[NS]	14.8[NS]
	Ca-As	10.0	10.9	13.3	14.3	14.9
	MAP 0.8%	9.9	10.8	13.1	14.0	14.9
	MAP 1.5%	9.9	10.9	13.0	14.2	14.8
	MAP 0.8% x2	9.9	10.8	12.8	13.9	14.1
	KNO_3	9.9	10.8	13.4	14.1	14.9
	p-value	*0.7797*	*0.9637*	*0.3117*	*0.9079*	*0.4106*
Ratio (SSC/Citric acid)	Control	2.0[NS]	3.3[NS]	5.6[NS]	7.2[NS]	8.2 c*
	Ca-As	2.0	3.6	6.3	8.1	10.2 a
	MAP 0.8%	2.2	3.8	6.3	7.8	9.3 ab
	MAP 1.5%	2.1	3.6	6.1	7.7	9.2 b
	MAP 0.8% x2	2.0	3.6	6.0	7.4	9.1 b
	KNO_3	2.0	3.5	5.9	7.3	9.2 b
	p-value	*0.4210*	*0.2952*	*0.2146*	*0.3314*	*0.0032*
Juice content (%)	Control	41.3[NS]	36.3[NS]	56.3[NS]	52.1[NS]	51.2[NS]
	Ca-As	43.0	37.7	55.7	52.7	55.4
	MAP 0.8%	44.6	37.0	56.3	54.0	52.7
	MAP 1.5%	42.8	34.6	56.2	53.6	53.4
	MAP 0.8% x2	42.9	33.2	56.0	53.2	53.8
	KNO_3	42.3	36.0	54.8	54.3	52.5
	p-value	*0.4619*	*0.4560*	*0.5769*	*0.3722*	*0.7811*

[z]WBH = Weeks before harvest (Harvest: 17/08/2015 and 04/08/2016)

[NS]No significant differences

*Means with a different letter within a column differ significantly at the 5% level (LSD)

Table 5. The influence of Ca-As, N-, P- and K-containing foliar sprays on the internal fruit quality parameters of 'Nadorcott' mandarin fruit pulp in the 2016 season, harvested from De Doorns.

Treatment	Citric acid (%) (Automatic titrator)	SSC (Brix°)	Ratio (SSC/citric acid)	Juice content (%)	HLPC analysis					
					Citric acid (%)	Total acid (%)	Sucrose (%)	Glucose (%)	Fructose (%)	Total sugar (%)
Control	1.79^{NS}	14.1^{NS}	7.9^{NS}	54.1^{NS}	1.91 a*	2.22 a	6.63^{NS}	2.47 b	2.12 d	11.23^{NS}
Ca-As	1.60	14.3	9.1	54.5	1.56 b	1.84 b	6.09	2.46 b	2.14 cd	10.69
MAP 1.5%	1.66	14.1	8.7	55.2	1.74 ab	2.07 a	6.46	2.68 ab	2.34 abc	11.49
MAP 2%	1.72	14.0	8.2	55.9	1.87 a	2.19 a	6.56	2.75 a	2.39 ab	11.70
MAP 1.5% x2	1.67	13.9	8.4	55.4	1.80 a	2.14 a	6.63	2.74 a	2.39 ab	11.76
MAP 2% x2	1.60	13.9	8.8	54.9	1.76 ab	2.09 a	6.62	2.79 a	2.45 a	11.85
KNO_3	1.68	14.4	8.7	56.1	1.71 ab	2.00 ab	6.04	2.54 ab	2.23 bcd	10.82
p-value	*0.2552*	*0.2847*	*0.0962*	*0.2021*	*0.0455*	*0.0268*	*0.1557*	*0.0333*	*0.0144*	*0.1542*

^{NS}No significant differences (Harvest: 17/08/2015 and 04/08/2016)

*Means with a different letter within a column differ significantly at the 5% level (LSD)

Table 6. The influence of Ca-As, N-, P- and K-containing foliar sprays on the external fruit quality parameters of 'Nadorcott' mandarin fruit collected from De Doorns throughout the 2015 season.

Parameter	Treatment	20 WBH[z]	15 WBH	9 WBH	4 WBH	Harvest
Diam. (mm)	Control	43.9[NS]	55.1[NS]	59.5[NS]	59.6[NS]	60.1[NS]
	Ca-As	43.7	54.3	58.4	58.5	59.9
	MAP 0.8%	45.4	56.2	60.0	59.7	60.8
	MAP 1.5%	44.5	53.6	59.8	58.1	59.6
	MAP 0.8% x2	44.0	55.3	59.4	59.0	60.2
	KNO_3	44.9	56.0	59.8	59.8	62.0
	p-value	*0.2332*	*0.2104*	*0.6567*	*0.8516*	*0.2939*
Mass (g)	Control	42.4[NS]	49.2[NS]	80.7[NS]	82.3[NS]	83.8[NS]
	Ca-As	41.9	46.4	76.2	76.1	82.2
	MAP 0.8%	47.4	53.1	83.6	81.5	87.8
	MAP 1.5%	43.7	43.3	82.1	74.5	81.8
	MAP 0.8% x2	43.2	48.7	80.9	78.7	83.0
	KNO_3	45.2	50.9	82.2	81.3	91.5
	p-value	*0.1567*	*0.3242*	*0.5534*	*0.7794*	*0.2299*
Hue angle (°)	Control	121.5 a*	101.6[NS]	64.7[NS]	60.2[NS]	61.1[NS]
	Ca-As	121.7 ab	103.9	65.2	60.5	61.6
	MAP 0.8%	122.2 b	104.3	65.1	59.9	60.5
	MAP 1.5%	122.3 ab	105.0	65.4	60.0	60.8
	MAP 0.8% x2	122.5 ab	106.1	67.4	60.8	60.3
	KNO_3	121.7 ab	103.4	65.4	59.8	60.4
	p-value	*0.0488*	*0.1284*	*0.2205*	*0.3800*	*0.0591*

[z]WBH = Weeks before harvest (Harvest: 17/08/2015 and 04/08/2016)
[NS]No significant differences
*Means with a different letter within a column differ significantly at the 5% level (LSD)

Table 7. The influence Ca-As, N-, P- and K-containing foliar sprays on the external fruit quality parameters of 'Nadorcott' mandarin in 2016, harvested from De Doorns.

Treatment	Diam. (mm)	Mass (g)	Hue angle (°)	Rind thickness (mm)
Control	70.1[NS]	119.6[NS]	59.3[NS]	2.0[NS]
Ca-As	69.2	116.8	59.7	1.9
MAP 1.5%	69.1	117.3	59.1	1.9
MAP 2%	69.2	117.4	58.6	2.0
MAP 1.5% x2	68.6	114.6	58.7	2.1
MAP 2% x2	69.5	118.6	58.5	2.0
KNO_3	69.4	117.4	58.0	2.0
p-value	*0.4189*	*0.7911*	*0.2402*	*0.4835*

[NS]No significant differences

Table 8. The influence of Ca-As, N-, P- and K-containing foliar sprays on the acid content (%) of 'Nadorcott' mandarin fruit pulp following cold storage for 30 days at -0.6°C and 4°C respectively, after harvest from De Doorns in the 2015 and 2016 seasons.

Year	Treatment	Storage temperature (°C)	Citric acid (%) (Automatic titrator)	Citric acid (%) (HPLC)	Total acid (%) (HPLC)
2015	Control	-0.6	1.82^{NS}	1.98^{NS}	2.25^{NS}
	Ca-As		1.60	1.85	2.11
	MAP 0.8%		1.66	1.76	2.02
	MAP 1.5%		1.64	1.87	2.14
	MAP 0.8% x2		1.68	1.88	2.15
	KNO_3		1.72	2.00	2.27
	p-value		*0.4301*	*0.4829*	*0.4179*
2015	Control	4	1.80^{NS}	2.04^{NS}	2.31^{NS}
	Ca-As		1.48	1.68	1.96
	MAP 0.8%		1.61	1.81	2.11
	MAP 1.5%		1.60	1.83	2.11
	MAP 0.8% x2		1.59	1.82	2.10
	KNO_3		1.66	1.90	2.18
	p-value		*0.0538*	*0.0624*	*0.0672*
2016	Control	-0.6	1.65^{NS}	1.89^{NS}	2.20 a*
	Ca-As		1.52	1.64	1.94 bc
	MAP 1.5%		1.55	1.68	2.00 bc
	MAP 2%		1.62	1.79	2.10 ab
	MAP 1.5% x2		1.65	1.72	2.02 abc
	MAP 2% x2		1.52	1.56	1.87 c
	KNO_3		1.60	1.74	2.03 abc
	p-value		*0.5988*	*0.0525*	*0.0490*
2016	Control	4	1.62^{NS}	1.76^{NS}	2.06^{NS}
	Ca-As		1.48	1.71	2.04
	MAP 1.5%		1.53	1.71	2.05
	MAP 2%		1.63	1.78	2.10
	MAP 1.5% x2		1.64	1.82	2.16
	MAP 2% x2		1.53	1.56	1.87
	KNO_3		1.60	1.76	2.08
	p-value		*0.3940*	*0.0955*	*0.0525*

^{NS}No significant differences

*Means with a different letter within a column differ significantly at the 5% level (LSD)

Table 9. The influence of Ca-As, N-, P- and K-containing foliar sprays on the sugar content (%) of 'Nadorcott' mandarin fruit pulp following cold storage for 30 days at -0.6°C and 4°C respectively, after harvest from De Doorns in the 2015 and 2016 seasons.

Year	Treatment	Storage temperature (°C)	Sucrose (%)	Glucose (%)	Fructose (%)	Total sugar (%)
2015	Control	-0.6	7.56^{NS}	3.04^{NS}	2.67^{NS}	13.28^{NS}
	Ca-As		8.16	3.08	2.69	13.92
	MAP 0.8%		7.36	3.00	2.64	13.00
	MAP 1.5%		7.76	3.12	2.73	13.61
	MAP 0.8% x2		7.52	2.94	2.57	13.02
	KNO_3		7.98	3.20	2.83	14.01
	p-value		*0.0501*	*0.3442*	*0.2880*	*0.1898*
2015	Control	4	7.60^{NS}	3.30^{NS}	2.88^{NS}	13.78^{NS}
	Ca-As		7.92	3.28	2.85	14.05
	MAP 0.8%		7.63	3.37	2.96	13.96
	MAP 1.5%		7.64	3.27	2.87	13.79
	MAP 0.8% x2		7.42	3.12	2.74	13.27
	KNO_3		7.62	3.27	2.88	13.76
	p-value		*0.5198*	*0.4874*	*0.4516*	*0.6326*
2016	Control	-0.6	6.76 a*	2.98 a	2.57 a	12.30 a
	Ca-As		6.59 ab	2.84 abc	2.47 ab	11.91 abc
	MAP 1.5%		6.41 abc	2.90 ab	2.55 a	11.87 abc
	MAP 2%		6.59 ab	2.95 a	2.58 a	12.12 ab
	MAP 1.5% x2		6.10 cd	2.76 bc	2.42 ab	11.27 cd
	MAP 2% x2		5.90 d	2.67 c	2.34 b	10.91 d
	KNO_3		6.17 bcd	2.81 abc	2.47 ab	11.45 bcd
	p-value		*0.0044*	*0.0197*	*0.0475*	*0.0107*
2016	Control	4	5.99 b	2.90 bc	2.55 b	11.44 bc
	Ca-As		6.56 a	3.08 a	2.71 a	12.35 a
	MAP 1.5%		6.19 ab	3.06 ab	2.73 a	11.98 ab
	MAP 2%		6.17 ab	2.97 ac	2.63 ab	11.77 abc
	MAP 1.5% x2		6.27 ab	3.05 ab	2.70 a	12.02 ab
	MAP 2% x2		5.90 b	2.81 c	2.50 b	11.21 c
	KNO_3		6.47 a	3.06 ab	2.72 a	12.25 a
	p-value		*0.0206*	*0.0119*	*0.0146*	*0.0162*

^{NS}No significant differences

*Means with a different letter within a column differ significantly at the 5% level (LSD)

Table 10. The influence of Ca-As, N-, P- and K-containing foliar sprays on internal fruit quality parameters of 'Nadorcott' mandarin fruit pulp following cold storage for 30 days at -0.6°C and 4°C respectively, after harvest from De Doorns in the 2015 and 2016 seasons.

Year	Treatment	Storage temperature (°C)	SSC (Brix°)	Ratio (SSC/citric acid)	Juice content (%)
2015	Control	-0.6	15.9^{NS}	8.9^{NS}	57.4^{NS}
	Ca-As		16.0	10.1	56.4
	MAP 0.8%		15.8	9.7	57.6
	MAP 1.5%		15.7	9.7	54.6
	MAP 0.8% x2		15.3	9.3	55.6
	KNO_3		16.1	9.5	54.3
	p-value		*0.7541*	*0.2712*	*0.4845*
2015	Control	4	15.8^{NS}	8.9 c*	61.3^{NS}
	Ca-As		16.0	11.1 a	63.6
	MAP 0.8%		15.9	10.0 ab	62.2
	MAP 1.5%		15.8	10.0 ab	62.0
	MAP 0.8% x2		15.6	10.0 ab	58.7
	KNO_3		15.6	9.6 bc	59.5
	p-value		*0.9451*	*0.0069*	*0.0558*
2016	Control	-0.6	14.6^{NS}	9.0^{NS}	55.4^{NS}
	Ca-As		14.8	9.9	54.7
	MAP 1.5%		14.6	9.6	55.0
	MAP 2%		14.7	9.2	54.9
	MAP 1.5% x2		14.7	9.0	54.0
	MAP 2% x2		14.5	9.6	54.6
	KNO_3		14.5	9.2	55.5
	p-value		*0.7766*	*0.4358*	*0.9115*
2016	Control	4	14.8^{NS}	9.2^{NS}	44.4^{NS}
	Ca-As		15.0	10.3	42.9
	MAP 1.5%		14.7	9.8	47.4
	MAP 2%		14.9	9.2	46.0
	MAP 1.5% x2		14.9	9.2	43.3
	MAP 2% x2		14.8	9.8	43.1
	KNO_3		14.8	9.4	43.7
	p-value		*0.9373*	*0.1880*	*0.3366*

^{NS}No significant differences

*Means with a different letter within a column differ significantly at the 5% level (LSD)

Table 11. The influence of Ca-As, N-, P- and K-containing foliar sprays on the external quality parameters of 'Nadorcott' mandarin fruit pulp following cold storage for 30 days at -0.6°C and 4°C respectively, after harvest from De Doorns in the 2015 and 2016 seasons.

Year	Treatment	Storage temperature (°C)	Diam. (mm)	Mass (g)	Hue angle (°)	Rind Defect index	Rind thickness (mm)
2015	Control	-0.6	55.2[NS]	61.7[NS]	61.9[NS]	0.00[NS]	-[y]
	Ca-As		54.2	58.9	62.7	0.00	-
	MAP 0.8%		53.3	57.3	62.3	0.00	-
	MAP 1.5%		54.2	59.6	61.8	0.04	-
	MAP 0.8% x2		53.7	57.2	61.8	0.00	-
	KNO_3		54.8	61.3	61.7	0.02	-
	p-value		*0.4111*	*0.6353*	*0.2330*	*0.2330*	-
2015	Control	4	54.1[NS]	59.3[NS]	62.5[NS]	0.01[NS]	-
	Ca-As		53.8	58.2	62.9	0.03	-
	MAP 0.8%		53.8	59.1	62.9	0.02	-
	MAP 1.5%		54.0	59.3	62.6	0.04	-
	MAP 0.8% x2		53.1	56.4	62.4	0.00	-
	KNO_3		54.2	60.8	62.6	0.00	-
	p-value		*0.91420*	*0.8097*	*0.8577*	*0.5686*	-
2016	Control	-0.6	64.0[NS]	103.3[NS]	61.0[NS]	0.6[NS]	1.5[NS]
	Ca-As		62.6	97.7	61.4	0.6	1.4
	MAP 1.5%		64.1	104.5	61.2	0.6	1.6
	MAP 2%		63.2	100.3	60.6	0.7	1.4
	MAP 1.5% x2		63.3	101.4	61.0	0.7	1.5
	MAP 2% x2		64.2	105.3	60.8	0.6	1.6
	KNO_3		64.0	101.0	60.8	0.5	1.5
	p-value		*0.8085*	*0.8033*	*0.8090*	*0.2373*	*0.4682*
2016	Control	4	64.0[NS]	104.1[NS]	61.1[NS]	0.4[NS]	1.6[NS]
	Ca-As		62.2	94.5	61.9	0.4	1.4
	MAP 1.5%		63.4	102.2	61.3	0.4	1.7
	MAP 2%		62.8	98.9	61.1	0.4	1.6
	MAP 1.5% x2		62.5	98.4	60.7	0.3	1.6
	MAP 2% x2		62.8	99.8	61.2	0.3	1.5
	KNO_3		63.7	103.1	61.0	0.3	1.6
	p-value		*0.5570*	*0.4785*	*0.3950*	*0.3476*	*0.2717*

[NS] No significant differences

[y]Data not collected

Table 12. The influence of the foliar application of mono-ammonium phosphate (MAP) 1% at six weeks before harvest on the internal fruit quality parameters of 'Nadorcott' mandarin fruit pulp in the 2015 and 2016 seasons, harvested from De Doorns.

Year	Treatment	Citric acid (%) (Automatic titrator)	SSC (Brixº)	Juice content (%)	Ratio (Citric acid/SSC)	HPLC analysis					
						Citric acid HPLC (%)	Total acid HPLC (%)	Sucrose (%)	Glucose (%)	Fructose (%)	Total sugar (%)
2015	Control	1.82^{NS}	14.8^{NS}	51.2^{NS}	8.2^{NS}	2.01^{NS}	2.29^{NS}	7.19^{NS}	2.67^{NS}	2.31^{NS}	12.17^{NS}
	MAP 1%	1.62	14.5	52.9	9.1	1.87	2.16	7.20	2.63	2.29	12.12
	p-value	*0.0999*	*0.4942*	*0.2624*	*0.0816*	*0.3338*	*0.3914*	*0.9527*	*0.6975*	*0.7807*	*0.9189*
2016	Control	1.79 ^{NS}	14.1 b*	54.1 ^{NS}	7.9 ^{NS}	1.91 ^{NS}	2.22 ^{NS}	6.63 b	2.47 b	2.12 b	11.23 b
	MAP 1%	1.72	14.7 a	56.7	8.6	1.91	2.19	7.06 a	2.80 a	2.44 a	12.31 a
	p-value	*0.3467*	*0.0029*	*0.0684*	*0.0858*	*0.9414*	*0.7069*	*0.0417*	*0.0022*	*0.0012*	*0.0071*

^{NS}No significant differences

*Means with a different letter within a column differ significantly at the 5% level (LSD)

Table 13. The influence of the foliar application of mono-ammonium phosphate (MAP) 1% at six weeks before harvest on the internal fruit quality parameters of 'Nadorcott' mandarin fruit pulp following cold storage for 30 days at -0.6°C and 4°C respectively, after harvest from De Doorns in the 2015 and 2016 seasons.

Year	Treatment	Storage temperature (°C)	Citric acid (%)	SSC (Brix°)	Ratio (SSC/ Citric acid)	Juice content (%)
2015	Control	-0.6	1.82^{NS}	15.9^{NS}	8.9^{NS}	57.4^{NS}
	MAP 1%		1.63	15.4	9.5	55.1
	p-value		*0.1405*	*0.3982*	*0.1791*	*0.3141*
	Control	4	1.81 a*	15.8^{NS}	8.8 b	61.3^{NS}
	MAP 1%		1.54 b	15.7	10.3 a	60.1
	p-value		*0.0292*	*0.8033*	*0.0113*	*0.3718*
2016	Control	-0.6	1.64^{NS}	14.6 b	9.0^{NS}	55.3^{NS}
	MAP 1%		1.61	15.3 a	9.5	56.0
	p-value		*0.6634*	*0.0151*	*0.1598*	*0.3199*
	Control	4	1.62^{NS}	14.8 b	9.2^{NS}	43.9^{NS}
	MAP 1%		1.59	15.4 a	9.7	46.5
	p-value		*0.6114*	*0.0384*	*0.1710*	*0.3995*

^{NS}No significant differences

*Means with a different letter within a column differ significantly at the 5% level (LSD)

Table 14. The influence of the foliar application of mono-ammonium phosphate (MAP) 1% at six weeks before harvest on the internal quality parameters determined with HPLC of 'Nadorcott' mandarin fruit pulp following cold storage for 30 days at -0.6°C and 4°C respectively, after harvest from De Doorns in the 2015 and 2016 seasons.

Year	Treatment	Storage temperature (°C)	Citric acid (%)	Total acid (%)	Sucrose (%)	Glucose (%)	Fructose (%)	Total sugar (%)
2015	Control	-0.6	1.98^{NS}	2.25^{NS}	7.56^{NS}	3.04^{NS}	2.67^{NS}	13.28^{NS}
	MAP 1%		1.86	2.13	7.64	3.11	2.75	13.51
	p-value		*0.3817*	*0.4024*	*0.8133*	*0.6097*	*0.4899*	*0.7069*
	Control	4	2.05 a	2.31 a	7.58^{NS}	3.29^{NS}	2.88^{NS}	13.76^{NS}
	MAP 1%		1.70 b	1.97 b	7.21	3.11	2.73	13.06
	p-value		*0.0135*	*0.0120*	*0.3028*	*0.1813*	*0.1961*	*0.2380*
2016	Control	-0.6	1.89^{NS}	2.20 ^{NS}	6.75 ^{NS}	2.98 ^{NS}	2.57 b	12.28^{NS}
	MAP 1%		1.86	2.16	6.96	3.15	2.76 a	12.87
	p-value		*0.7068*	*0.5656*	*0.2589*	*0.0628*	*0.0360*	*0.1270*
	Control	4	1.76 ^{NS}	2.06 ^{NS}	5.98 b	2.90 b	2.55 b	11.42 b
	MAP 1%		1.81	2.12	6.92 a	3.22 a	2.86 a	12.99 a
	p-value		*0.5111*	*0.4114*	*0.0013*	*0.0083*	*0.0067*	*0.0035*

^{NS}No significant differences

*Means with a different letter within a column differ significantly at the 5% level (LSD)

Figure 1. Phytotoxicity on ‘Nadorcott’ mandarin fruit from De Doorns caused by a 1% mono-ammonium phosphate (MAP) sprayed approximately six weeks before harvest, after colour break.

Chapter 4: The effect of timing of nitrogen application in combination with different irrigation regimes on fruit quality of 'Nadorcott' mandarin

Abstract

Nitrogen (N) fertilisation and irrigation have an important influence on citrus fruit yield and quality as high N and water availability increases production. Excessive N and over-irrigation are, however, both detrimental to citrus fruit quality. Therefore, with both these factors there is a trade-off between quality and yield, which requires skilful management to achieve optimum economic benefit. It is widely accepted that N applied late in the fruit growth season will detrimentally affect citrus fruit quality, especially rind colour development. Due to the significant impact that both late N application and irrigation are likely to have on citrus fruit quality, it is hypothesised that the possible negative impact on fruit quality induced by late N applications could be indirectly reduced through the irrigation volume supplied. To test this hypothesis N was applied along with different irrigation volumes to 'Nadorcott' mandarin in Citrusdal over two seasons, 2015 and 2016. Nitrogen was applied in two split applications in both seasons, first at 50 $kg.ha^{-1}$ in 2015 and then at 100 $kg.ha^{-1}$ in 2016. Three irrigation regimes were used: standard (control), increased and decreased irrigation. Citrus fruit quality parameters were determined at harvest as well as after cold storage at -0.6°C and 4°C. The occurrence of postharvest rind disorders was also recorded in both seasons along with creasing, which was only recorded in the 2015 season. Additional N did not negatively impact on fruit quality, but the application amount can be reduced when in combination with high irrigation volumes. High and low irrigation regimes, in combination with late N application, had a more pronounced effect on fruit quality than N fertilisation alone. Higher irrigation volumes decreased the incidence of creasing and increased fruit diameter and mass. From literature, it is well-known, that over-irrigation can, however, have detrimental consequences for postharvest storage life. Therefore, the late application of N was found not to be detrimental to fruit quality, however this outcome is to an extent dependent on the irrigation regime, as the latter factor may be the main determinant in management strategies regarding N fertilisation practices.

Additional keywords: alternate bearing, citrus, fertilisation, flowering, late mandarin, pitting

Introduction

In citriculture optimum management of nutrition and irrigation is essential for the consistent production of high quality fruit (Koo, 1988). Nitrogen (N) along with potassium (K) are the macro-nutrients in a commercial fertilisation program that have the most significant influence on *Citrus* fruit quality. It is widely accepted that N applied late in the season will detrimentally affect *Citrus* fruit quality, especially fruit rind colour. Under the correct management, irrigation increases production by increasing yield and fruit size, whereas the impact of over-irrigation on *Citrus* fruit quality is known to negatively impact on fruit quality (Kriedemann and Barrs, 1981). However, the interaction between later N application during fruit development and over-irrigation on *Citrus* fruit quality has not been studied extensively. For instance, whether any detrimental effect on fruit quality imposed by N application late in the season can be mitigated by the amount of water received by the tree requires exploration.

Prior to and during flowering adequate N is critical as the number of flowers and fruit set is determined by the N level in the tree (Smith, 1966a, b). According to Smith (1966a) N application can be accessed by *Citrus* throughout the year, but N is more readily acquired and in higher amounts during warmer months as soil temperatures has a marked influence on the uptake rate of mineral nutrients. Also, the N use efficiency (NUE) in young *Citrus* trees typically increases in the warmer summer months (Martínez-Alcántara et al., 2012). The mobilization of N reserves is activated in spring to meet the high N requirement of the tree in this period when soil temperatures are low (Martínez-Alcántara et al., 2011; Martínez-Alcántara et al., 2015; Quiñones et al., 2004). Large percentages of total N in comparison to N tree reserves are used for flowering, when there is vigorous vegetative growth or when the tree is heavily bearing. Nitrogen reserves are firstly stored in leaves, followed by shoots and roots (Martínez-Alcántara et al., 2015). Changes in total N content of leaves can occur throughout the season. This is due to N being a highly mobile element, both in the soil and plant, thus causing soil N content to strongly influence leaf content (Smith, 1966b).

Fertilisation has a long-term effect on overall tree growth and development. Adequate nutrition is required throughout the production cycle to ensure that the orchard is managed optimally to deliver fruit of expected quantity and quality. Fruit production of the current season is therefore not the main drive for N fertilisation programmes, but an investment is made to ensure the appropriate and timely vegetative development of the tree along with the formation of strong bearing shoots for the following season (Menino et al., 2004a). Fruit quality of the current season can, however, still be affected by long-term N fertilisation practices and strategies and should not be comprised.

In general, the effect of N on *Citrus* fruit quality is perceived to be negative with increasing N fertilisation decreasing fruit diameter and mass (Chapman, 1968; Dasberg et al., 1983; Du Plessis and Koen, 1988; Koo, 1988; Quaggio et al., 2006; Reitz and Koo, 1959; Smith, 1966a) and increasing acid content (Koo, 1988; Reitz and Koo, 1959; Smith, 1966a) and rind thickness (Embleton et al., 1978; Dasberg, 1983; Koo, 1988; Smith, 1966a). Furthermore, it has been extensively documented to increase the amount of green fruit at harvest (Koo, 1988). However, different outcomes of N-based mineral nutrition studies exist as time of application seems to play an important role in addition to reports of N not affecting fruit quality and only resulting in changes in leaf and rind N content (Castel and Ginestar, 1996). Of interest is that Protopapadakis et al. (2004) reported K to have had a greater influence than N regarding tree collapse of 'Encore' mandarin where leaves of heavily bearing trees were reported to be severely deficient in K compared to normal bearing trees, but not differing significantly with respect to N content. This study emphasizes the importance of interpreting the individual level of other elements as well.

The influence of irrigation on *Citrus* phenology as well as on fruit quality and production has been comprehensively covered in older literature by authors like Kriedemann and Barrs (1981) and Doorenbos and Kassam (1979). Strategies such as deficit irrigation (DI) is used to manipulate fruit quality, tree physiology and flowering. Deficit irrigation (DI) is a method by which irrigation is managed to gain economic benefit by having predetermined periods in the season where less water is provided than is required to meet the current evapotranspiration (ET) (Behboudian and Mills, 1997). Regulated deficit irrigation (RDI) refers to DI applied before the rapid phase of rapid fruit growth, early in the season, whilst late-season RDI is when DI is applied immediate prior to harvest (Behboudian and Mills, 1997). Water stress, mostly manifested as suboptimal growth rate, is due to a low availability of water to the plant or when a level of water deficit occurs to such an extent that it can lead to mild or severe water stress. *Citrus* trees can be quite susceptible to water stress, particularly so as the tree structure exhibits a combination of large a canopy together with shallow root growth, creating an extensive evaporative area, but with a small surface potential for absorption (Kriedemann and Barrs, 1981). However, directly opposite to water deficit, excessive irrigation volumes also has detrimental consequences for fruit quality and production (Koo, 1988).

In *Citrus* phenology, reproductive development is promoted by moderate water stress, whilst fruit set and enlargement is dependent on the adequate availability of water (Kriedemann and Barrs, 1981). The effect of water stress on the tree depends on the stage of crop growth, with certain stages, such as cell division, being more critical for fruit growth and quality than others (Behboudian and Mills, 1997). Severe water stress at any growth stage reduces yield, however the stages of flowering

and fruit set is highly sensitive to water stress, to such an extent that Bower and Wolstenholme (1996) stated the total production to be determined by events occurring during these critical two months after bloom. During the second phase of fruit growth water stress is likely to decrease fruit size (Kriedemann and Barrs, 1981) but normally not fruit number. Therefore, both the timing and volume of the irrigation is important with respect to fruit production and quality (Kriedemann and Barrs, 1981).

Water stress such as deficit irrigation increases the soluble solids content (SSC) (Castel and Buj, 1990; Castel and Ginestar, 1996; Chartzoulakis et al., 1999; Peng and Rabe, 1998) as well as the acid content of *Citrus* fruit (Castel and Buj, 1990; Castel and Ginestar, 1996; Chartzoulakis et al., 1999). Differences exist between the effect various levels of water stress may have on fruit quality. Yakushiji et al. (1998) reported a higher sugar content in moderately drought-stressed trees compared to severely drought-stressed trees. Conflicting results regarding fruit size and mass have been listed as either unaffected by deficit irrigation or not decreased (Castel and Buj, 1990; Castel and Ginestar, 1996; Eliades, 1994; Peng and Rabe, 1998). During fruit maturation, water deficit can reduce fruit quality through an increase in rind thickness as well as acid content (Castel and Buj, 1990; Castel and Ginestar, 1996). Irrigation on the contrary has a diluting effect, leading to fruit with a higher juice content, but lower SSC and acid content. Furthermore, over-irrigation can increase the incidence of certain rind blemishes (Kriedemann and Barrs, 1981). Yet, no significant differences were reported in fruit quality or yield of grapefruit when irrigation was increased by 27% (Eliades, 1994). Thus, quality problems relating to inadequate irrigation, particularly the timing and severity thereof, is considered far more common than those linked to over-irrigation.

Water stress has been used successfully to influence flowering, as is common practice in the lemon production areas of Sicily, where water stress is used to promote flowering (Barbera et al., 1988). Yield can also be reduced by water stress during flowering and fruit set, due to a reduction in fruit number and/or fruit size. For example, in grapefruit, Eliades (1994) found that decreased irrigation throughout the year reduced production potential, both in terms of less and smaller fruit. In ‘Salustiana’ oranges, Castel and Buj (1990) found a decrease in fruit yield due to a reduction in fruit size, but not number of fruit. Alternatively, Chartzoulakis et al. (1999) also found a similar reduction in production with water stress in navel oranges, but in this study, it was mainly due to a lower amount of fruit being present throughout the year. Yet, in a study by Castel and Ginestar (1996) a lower irrigation volume on ‘Clementina de Nules’ mandarin did not significantly affect either yield or fruit number.

It can be concluded that when interpreting results from various studies that it is important to also consider the severity and timing of deficit irrigation, as this interaction may largely determine the impact this external stress will have on tree growth, fruit development and quality. The phenological stage when deficit irrigation is applied determines the effect it will have on the tree and fruit quality (Doorenbos and Kassam, 1979).

As both N and irrigation is known to have distinct effects on fruit quality, a combination of N with different irrigation regimes might inflict contrasting effects on *Citrus* fruit quality. For instance, the negative effects of N may become more apparent with increased irrigation, or the adverse effects of water stress may be enhanced under conditions of elevated N. Castel and Ginestar (1996) combined four irrigation regimes with two N rates [120 and 210 kg N per hectare ($kg.ha^{-1}$) respectively], but found no interaction between these main effects regarding the fruit quality parameters of juice percentage, SSC and titratable acidity (TA). An interaction between irrigation and N was however seen with respect to the extent of nitrate leaching, although the irrigation effect appeared to be far greater than the N effect. A similar study by Montaña et al. (2004) combined three irrigation- and two N rates (178.5 and 297.5 kg N ha^{-1}) and also found no interaction between these main effects for fruit quality parameters.

The aim of this study was to obtain a deeper understanding of the impact of the interaction between irrigation and N during phase II and III of fruit development on *Citrus* fruit quality, for example rind colour and susceptibility to physiological disorders, in 'Nadorcott' mandarin, grown in a mediterranean climate. Once established whether the additional application of N has a detrimental effect on fruit quality and whether the timing and volume of irrigation plays a more important role than N with regards to fruit quality, the possible beneficial effects of optimum irrigation and N fertilisation in terms of promoting flowering and fruit set on the tree can be further investigated. Results of this study may guide N fertilisation recommendations to also consider the interaction of both timing and amount of irrigation received.

Materials and methods

Site and plant material

The trial was conducted on 'Nadorcott' mandarin trees (*C. reticulata* Blanco) grafted on Carrizo rootstock, planted in 2005 at 5 m x 2 m in a North to South row direction, in a commercial orchard on the farm Boontjiesriver, Citrusdal, Western Cape Province, South Africa (32°34'56.2"S 19°01'45.6"E). The area has a mediterranean type climate with hot summers and winter rainfall.

Nutrition and irrigation was supplied according to commercial recommendations in a fertigation system, where trees received 184 kg N.ha^{-1} from Jul. 2015 until Jun. 2016 and 189 kg N.ha^{-1} from Jul. 2016 up to Jun. 2017 respectively. The soil is quite sandy with rocks present as well and a very low organic matter content. Mulches were not used in the orchard. All trees included in the experiment were healthy and uniform, with buffer trees between the treatment trees. The trial was repeated over two seasons (2015 and 2016).

Treatments

2015 season

The six treatments that were applied to the 'Nadorcott' trees consisted of three levels of irrigation [standard (X); increased (2X); decreased (½X)] where each level of irrigation was combined with two levels of N (with additional N as '+N' or without additional N). The allocated X, 2X and ½X levels of irrigation was design to be comparative values of sufficient, increased or decreased irrigation volumes and should not be considered equivalent to exactly double (2X) or half (½X) the volume of the standard irrigation (X) (Table 1; Appendix A). A single tree was considered as the experimental unit. Nitrogen in the form of limestone ammonium nitrate (LAN) fertiliser [LAN (28), ©Agricol] was spread evenly per tree, next to drippers.

Table 1. Irrigation treatments applied either as decreased (½X), standard (X) or increased irrigation (2X) in 2015 and 2016 in a trial where fruit quality of 'Nadorcott' mandarin in response to the timing of N and in combination with different irrigation regimes was studied.

Treatment	**2015**		**2016**	
	L.h^{-1} delivery	% of X	L.h^{-1} delivery	% of X
½X	2.3	50	3.2	50
X	4.6	100	6.4	100
2X	6.2	135	9.6	150

The single-line irrigation (X) with two drippers per tree at a delivery rate of 2.3 L.h^{-1} was altered early in March, 25 weeks after full bloom and 16 weeks before harvest (stage II of fruit growth) by removing a micro dripper for the decreased irrigation (½X) and by adding a 1.6 L.h^{-1} micro dripper for the increased irrigation (2X). By mid-August, shortly after harvest, the orchard's irrigation was converted from a single-line irrigation to double-line irrigation system, with four 1.6 L.h^{-1} micro drippers per tree.

Additional nitrogen equivalent to 50 kg.ha^{-1} was applied to treatments as LAN. To reduce N leaching, fertilizer application was split into two applications of 25kg of N.ha^{-1} on 16 Mar. and 20 Apr. respectively. Relevant calculations are provided in Appendix A.

2016 season

The differentiated ½X, X and 2X water delivery treatment ratios were applied 29 weeks after full bloom (8 Apr.) and 12 weeks before harvest using microtube adapters (Netafim, Cape Town; Table 1). The same trees as in 2015 were used, however, the additional N application was adjusted to 100 kg N.ha^{-1}, based on the 2015 results, which indicated no treatment effect (Appendix A). The fertilizer was applied as previously, in split applications, on 11 Mar. and 19 Apr. respectively.

Quality measurements

All sampling and analysis procedures to determine fruit quality were performed as described in Chapter 3 (pg. 47-48). To summarize: ten fruit were sampled as described in Chapter 3 at harvest per tree for external and internal quality evaluations in both the 2015 and 2016 season, where after internal fruit quality parameters [SSC, citric acid content (automatic titrator) and juice percentage] including HPLC analysis of the citric and total acid and sugars as well as external fruit quality parameters (colour and rind disorder incidence) were determined.

In addition, the rind pigments, chlorophyll and carotenoids, were determined as present at harvest of the 2016 season, using the flavedo of four fruit. The flavedo was immediately frozen in liquid N (N_2) and stored in a -80°C freezer until freeze drying in a Christ BETA 1-8 LO plus freeze dryer (Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany). Immediately following freeze-drying the flavedo was milled with an analytical grinder (Yellow line, A10, IKA-Werke, Staufen, Germany) to a fine powder. All subsequent steps were performed in a semi-shaded environment to prevent any light from detrimentally affecting the pigments. In a Kimax tube, 0.05 g of dried, milled flavedo was weighed off and 2 mL ethanol was added. The tube was vortexed and then transferred to a mechanical shaker (IKA KS 500, IKA-Werke, Staufen, Germany) for 30 min at a speed of 265 rpm. Next, the mixture was centrifuged (5810 R centrifuge, Eppendorf, Hamburg, Germany) for 5 min at 4000 rpm and 4°C. The alcoholic supernatant was transferred to a savant tube, where after the tube closed to prevent evaporation. The ethanol extraction steps were repeated to ensure complete extraction. Thereafter, 2 mL hexane and butylated hydroxytoluene (BHT: 2,6-Di-t-butyl-p-cresol) (No. B-1378; Lot 62F-0573, Sigma Chemical Company, St Louis, USA) solution, consisting of 0.01 g of BHT and 100 mL hexane, was added to the tube. The tube was vortexed and

put on the mechanical shaker for 15 min at a speed of 265 rpm. The tube was then centrifuged for 5 min at 4000 rpm and 4°C. The hexane phase was subsequently transferred to the savant tube with the ethanol phase. The steps from addition of hexane and BHT solution was repeated twice more. The extracts were finally dried in the savant tubes under vacuum using a Savant SC210A SpeedVac Concentrator (Thermo Scientific Inc., Waltham, MA, United States) at a medium temperature. The dried extract was reconstituted with 8 mL acetone. The tubes were vortexed and the mixture transferred to Eppendorf plastic tubes before centrifuging for 7 min at 4000 rpm. The mixture was then transferred to cuvettes and spectrophotometer readings were done at wavelengths of 450, 644, 662 and 664 nm on a Cary 60 UV-Vis Spectrophotometer (Agilent Technologies, Santa Clara, CA, United States).

Nitrogen uptake

To determine the N content of the soil, roots, leaves and fruit, samples were collected from six blocks per treatment in 2016. The root samples were taken on 01 Apr., 11 May and 17 Jun. respectively underneath the drippers, in the location where the N fertilizer was placed, with care being taken not to sample regrowth. Leaf samples, collected approximately 3-4 cm behind the fruit, together with fruit were sampled on 01 Apr. (end stage II; before winter), 11 May (stage III; winter) and 27 Jun. (at harvest) respectively. Samples were analysed for total N by a commercial analytical laboratory (Bemlab (Pty) Ltd., Strand, South Africa), with the results expressed as $mg.100g^{-1}$ dry weight (DW) of N. The standard preparation and analysis of samples was followed using the ICP-OES (Inductively Coupled Plasma – Optical Emission spectrometer) (Varian PRX-OEX, Varian, Inc. Corporate, Palo Alto, CA, USA) procedure and a N analyser (LECO FP528 Nitrogen analyser, LECO cooperation, St. Joseph, Michigan, USA).

Flowering

In the 2016 season, flowering was determined at full bloom (30 Sept.) on six pre-tagged shoots per tree which also included the previous season's vegetative flush. Shoot length (cm) was measured and total flowers per shoot was counted and expressed as flowers per cm ($flowers.cm^{-1}$) in addition to recordings of the total number of vegetative nodes, leafy inflorescences and leafless inflorescences per shoot.

Cold Storage

The impact of the various treatments on the storage potential of the 'Nadorcott' fruit was ascertained by storing 15 commercial mature fruit per replicate (n=10) directly after harvest at either

4°C and -0.6°C for 30 days. To induce any latent rind disorders, the fruit were dehydrated for four days at 30°C, followed by rehydration for 24h in at 100% humidity, in plastic bags prior cold storage in the 2016 season after the previous season had no serious rind disorders (Alférez et al., 2003). The external and internal fruit quality parameters were determined after cold storage as described in Chapter 3 (pg. 47-48) and the fruit were evaluated for physiological rind disorders after seven days of shelf life at room temperature. Fruit were also scored for green rind colour using a scorecard from 0 to 4 for increasing green colouration in for fruit harvested in 2015, but not in 2016 as no green fruit were present.

Experimental design and statistical analysis

The experimental design was a randomised complete block design, of ten blocks in the 2015 season and eight blocks in the 2016 season. Data was analysed as a factorial design with irrigation and N as main effects. Analysis of variance (ANOVA) was carried out using StatSoft Statistica (version 13.0, StatSoft, Inc.) where means were separated using Fisher's LSD ($p < 0.05$ and 0.1) at the 5% confidence level.

Results

Internal fruit quality

Citric and Total Acid Content

AT HARVEST. A significant interaction between irrigation and N emerged for citric acid content, as measured with the automatic titrator and by means of HPLC, but also for the total acid content of fruit at harvest in both seasons (Tables 2, 3 and 4). At harvest in 2015, citric acid recorded in control fruit and in fruit from treatments X+N and ½X+N were significantly higher than that of fruit which received increased irrigation in combination with N (2X+N) (Tables 2 and 3). The automatic titrator generated citric acid values from fruit obtained from the other treatments did not differ significantly from each other (Table 2). For citric acid determined by means of HPLC analysis, fruit that were obtained from the ½X+N treatment had significantly higher citric acid values than recorded for fruit from the 2X and ½X treatments respectively. In addition, fruit from the ½X treatment also had significantly higher citric acid content than fruit that were produced under the 2X+N treatment regime (Table 3).

At harvest in 2016, a significantly higher citric acid content, irrespectively of method of determination, was measured in fruit that was obtained from the 2X and ½X treatments compared to

that recorded in fruit harvested from the 2X+N treatment (Tables 2 and 3). The ½X+N treatment fruit also had a significantly higher citric acid content compared to the 2X+N treatment fruit when determined by means of the automatic titrator (Table 2).

AFTER COLD STORAGE. Data analysis for citric acid content as measured with the automatic titrator after storage did not produce a significant interaction between irrigation and N at either cold-storage temperatures in 2015 or at -0.6°C in 2016 (Table 2). Following storage at 4°C, fruit harvested in 2016 from the ½X+N treatment had a significantly higher citric acid content than that of fruit obtained from the ½X and 2X+N treatments, whereas fruit from the other treatments did not differ significantly from each other with respect to citric acid content (Table 2). Following cold storage irrespective of temperatures or season, a general trend was noted that the citric acid content obtained by means of automatic titrator on fruit harvested from the ½X treatment was higher than that of fruit obtained from the 2X treatment (Table 2).

For citric and total acid content as measured through HPLC analysis a significant interaction was shown after storage at -0.6°C in the 2015 season, but not after storage at 4°C in 2015 or at either temperature in 2016 (Tables 3 and 4). Citric acid content was significantly higher for fruit of the control, 2X and ½X+N treatments compared to fruit harvested from the 2X+N treatment, whereas the other treatments did not differ significantly from any of these above-mentioned treatments (Table 3). Total acid content was not influenced by irrigation as main effect (Table 4).

AUTOMATIC TITRATOR VS HPLC DETERMINATIONS OF CITRIC ACID AND TOTAL ACID. A trend emerged where the citric acid content as measured by means of the automatic titrator indicated fruit from the 2X+N treatment to consistently have the lowest values compared to fruit harvested from ½X+N having the highest or one of the highest values (Table 2). The same trend was also observed in 2015 for citric acid determined through HPLC analysis, but this trend was not consistent for 2016 (Table 3). Similarly, in 2015, the total acid content again reflected the trend observed with the citric acid content as was reported in 2015 for automatic titrator determined values where fruit harvested from the ½X+N had generally significantly higher total acid content than fruit obtained from the 2X+N treatment. Nitrogen as a main effect did not significantly influence the citric or total acid content, at harvest or after storage, as measured with the automatic titrator or HPLC analysis (Tables 2, 3 and 4).

Pulp Soluble Solids Content and Total Sugars

SOLUBLE SOLIDS CONTENT (SSC). At harvest in 2016, but not in 2015, a significant interaction was shown between the main effects of irrigation and N for soluble solids content (SSC)

(Table 5). In 2016, fruit harvested from the 2X+N treatment recorded significantly lower SSC levels than fruit from all the other treatments, except for fruit obtained from the control and X+N treatments. However, following storage at -0.6°C and 4°C in the 2015 season and at 4°C in 2016 there was a significant interaction between irrigation and N for SSC, following similar trends (Table 5). In the 2015 season, a significantly higher SSC was measured in fruit that resulted from the ½X+N treatment compared to fruit obtained from the X+N and 2X treatments, after storage at -0.6°C or from 2X+N treatment fruit, after storage at 4°C.

In 2016 at harvest, the SSC value of the ½X+N and 2X treated fruit was significantly higher than that of the 2X+N treatment fruit, but SSC values for fruit from all treatments did not differ significantly following storage at -0.6°C for 30 days. A trend emerged where the ½X+N treatment fruit consistently had the highest SSC, whilst the 2X+N treatment fruit consistently had the lowest SSC value. These values mostly differed significantly from each other, irrespective of the season, or whether the data was collected at harvest or a following cold-storage period of 30 days. Soluble solids content was significantly influenced by irrigation in one of the two instances, however this effect was not carried forward during cold storage as the data showed no significant interaction after storage at -0.6°C in 2016 (Table 5). When considering irrigation, a general trend was observed where fruit obtained from the ½X treatment had a significantly higher SSC compared to those harvested from the control or 2X treatment. The main effect of N did not influence the SSC significantly at any sampling date (Table 5).

SOLUBLE AND TOTAL SUGARS. At harvest in the 2015 season, a significant interaction was shown between irrigation and N for the soluble sugars, namely glucose, fructose and sucrose as well as for total sugars as was determined by means of HPLC (Tables 6, 7, 8 and 9). A significantly higher sucrose and glucose was measured in the 2015 season from fruit harvested from the ½X+N treatment compared to fruit collected from the other treatments (Tables 6 and 7). The fructose and total sugar content measured in fruit from the ½X+N treatment, as was observed for sucrose and glucose, were also significantly higher than that of fruit from all the other treatments. Fructose content recorded in fruit from ½X and X treatments were also significantly higher than that recorded in 2X+N treatment fruit (Table 8). When total sugar content was calculated, a similar pattern that was described for the fructose content was again evident, however the values obtained from ½X and 2X+N treatment fruits did not differ significantly from each other (Table 9).

For fruit, subjected to cold storage, there was a significant interaction between irrigation and N in glucose and fructose content following storage at -0.6°C, in both seasons (Tables 7 and 8). This interaction was also present for total sugar content after storage at -0.6°C (Table 9) and for fructose

content after storage at 4°C in the 2016 season (Table 8). Regarding the glucose and fructose content values after storage at -0.6°C in the 2015 season, both the ½X+N and 2X treatments were significantly higher than that recorded in fruit from the 2X+N treatment, whilst the other treatments did not differ significantly from each other (Table 7 and 8). In 2016, after storage at -0.6°C, fruit collected from the 2X treatment was significantly higher in percentage glucose, fructose and total sugar content than fruit harvested from the ½X, X+N and 2X+N treatments (Tables 7, 8 and 9).

After storage at 4°C in 2016, the percentage fructose content recorded for the ½X+N treatment and control fruit was significantly higher than that measured in the 2X treatment fruit (Table 8). Of the all HPLC determined soluble sugars, only fructose was significantly influenced by irrigation in one instance, which was at harvest in 2016 (Tables 6, 7, 8, and 9) when the fructose content of fruit from the ½X treatment was significantly higher than that of the other two irrigation treatments. No significant differences at the 5% confidence level for any sugar parameter with irrigation as main effect was observed after storage, at either temperature, in both seasons (Tables 5, 6, 7, 8 and 9). Nitrogen did, however, influence glucose, fructose, sucrose and total sugar significantly at harvest in 2016, in that treatments which did not receive additional N, generally had significantly higher sugar values (Tables 6, 7, 8, and 9). Although the other sampling dates produced non-significant results, a similar trend was noted, except after storage at 4°C in 2016.

Citric acid:SSC and Juice content

Despite having significant differences in citric acid and SSC, the ratio of these parameters did not differ at the 5% significance level, neither at harvest nor after storage, irrespective of storage temperature or season (data not shown). For juice content a significant interaction was calculated between irrigation and N after storage at 4°C in 2016. Furthermore, juice content was significantly influenced by N as main effect at harvest in 2015, where fruit exposed to late N application treatments, produced a higher juice content compared to fruit that receive no late N application (data not shown). However, both these incidents appeared unrelated, with no clear explanation available.

External quality

Rind colour and carotenoids

AT HARVEST. In 2015, hue° as an indicator of rind colour was significantly influenced by irrigation as main effect (Table 10). Fruit harvested from the ½X treatment produced the highest hue° value and differed significantly from fruit delivered by the 2X treatment, but not from the X treatment fruit, which also did not differ significantly from the 2X treatment fruit. Increased irrigation therefore

led to more orange coloured fruit. Even though being significantly different, the difference would unlikely be detected, nor influence fruit preference by the consumer. Although not significant, the pattern was the opposite in the 2016 season. In 2016 no significant interaction was obtained between irrigation and N application for any of the other external fruit quality parameters such as carotenoid content in the flavedo (Table 10) or chlorophyll content (data not shown).

AFTER COLD STORAGE. No significant interaction between irrigation and late N applications for rind quality parameters could be detected in either seasons (Table 11). In the 2015 season, however, irrigation did have a significant influence on all the rind quality parameters, following storage (Table 11). Unexpectedly fruit harvested from the 2X treatment had the least green colouration and differed significantly from the ½X treatment after storage, at both temperatures. The incidence of creasing also differed significantly with the 2X treatment fruit having significantly less creasing compared to the other two treatments at -0.6°C and for the ½X treatment following storage at 4°C. Rind staining, although differing significantly, had no discernible pattern and was most likely brought about by storage at the higher temperature (4°C), as no damage was observed following storage at the lower temperature of -0.6°C. Additional N only had a significant effect on the amount of green colour present following storage at 4°C, where the additional N application increased green colouration in 2015. That the sampled fruit used for storage at -0.6°C showed no significant differences is evidence of the small differences in green colouration between fruit. This observed difference was however isolated and of such a small extent that it would not be of commercial significance (Table 11).

Fruit diameter and mass

Fruit diameter and mass as measured at harvest was only influenced by irrigation in 2015 (Table 10). The 2X treatment fruit had significantly larger diameters and mass compared to the other irrigations treatment which received less water. Nitrogen as main effect did not influence fruit diameter or mass, irrespective of season (Table 10).

Vegetative growth and flowering

In the 2016 season, a significant interaction between N and irrigation for the number of vegetative buds was calculated (Table 12). The only difference that was however recorded was a significantly higher amount of vegetative buds in trees which received 2X+N compared to trees with had been exposed to the 2X treatment. Irrigation and N as main effects had no significant impact on either vegetative growth or flowering expression (Table 12).

Nitrogen uptake

The additional N treatments did not result in any significant differences in the N content of either the soil, roots, leaves, fruit pulp or rind at any sampling date (Table 13).

Discussion

Rind colour was generally not influenced by the additional application of N later during fruit development (end stage II), but was however negatively impacted by higher irrigation volumes. The finding in this study that N did not influence the rind colour conflicts with literature where both an increase of N and the late application thereof has been reported to increase the number of green fruit at harvest (Koo, 1988; Dasberg, 1983; Reitz and Koo, 1959; Smith, 1966b). Most studies with respect to the effect of N fertilisation on rind colour was focused on orange cultivars in Florida, USA, where cultivar differences and a different prevailing climate (humid subtropical) could have led to the alternative response to N application and timing that was documented in their reports. It is important to note that in our study neither chlorophyll nor carotenoid content was affected by the additional N application treatments, which again is a contrary finding to literature where high N-fertilisation has been reported to decrease the carotenoid content of *Citrus* as well as inhibit the breakdown of chlorophyll (Huff, 1983; 1984). During the current study on 'Nadorcott' mandarin irrigation indicated a significant effect on rind colour and fruit quality than late N application, along with other possible factors such as the macro- and micro-climate of the orchard. The significantly lower hue° value measured in fruit harvested in 2015 from the 2X treatments implicated less green and more orange coloured fruit at higher irrigation than was harvested from the ½X treatment. This contrasts with literature which states that higher irrigation volumes increase the number of green fruit at harvest (Koo, 1988). Yet, there was no difference in carotenoid content recorded between fruit from the various irrigation treatments. Visually these treatments did not greatly affect rind colour (Fig. 1), although a decrease in irrigation again resulted in more green rind surface, which appeared to be a random occurrence. The lack of the expected colour – reduced carotenoid development – response could possibly be attributed to other internal factors having a larger impact such as a lack of strong competition due to the low fruit load as is relevant in the 'off' year of 2016. In the 2015 season ('on' year) most of the fruit had green rind colouration present, whilst most fruit had no green colour in the 2016 season ('off' year).

During the 2015 season, a high incidence of creasing, a highly complex and problematic disorder of *Citrus* fruit, was recorded in the orchard. Various cultivars and areas in South Africa are known to be affected by creasing (Du Plessis and Maritz, 2004), but the occurrence in 'Nadorcott'

mandarin has not been reported previously (Fig. 2). Smaller fruit are more susceptible to creasing (Du Plessis and Maritz, 2004), which along with other factors, would have caused the higher incidence thereof in the 2015 season. Our results indicated that the incidence of creasing was decreased with increasing irrigation. Raciti and Scuderi (1970) also reported that trees experiencing water stress had more severe creasing compared to trees exposed to optimum irrigation levels. It is possible that the availability of a higher volume of water would result in improved rind conditions, as for example, the uptake of calcium (Ca), a macro-element known to be critical in creasing (Storey et al., 2002), would be promoted under conditions with sufficient water availability. Rind staining was observed at the higher storage temperature (4°C) and has also to our knowledge never been documented for *Citrus* fruit. However, it did not manifest at the lower temperature (-0.6°C).

As expected, increased irrigation significantly promoted fruit diameter and mass. However, the application of N late within the growing season could possibly explain the non-responsiveness in terms of decreasing fruit size, as this fruit quality parameter is mostly determined in the cell division stage (Rabe, 2000). It is possible that the additional N did not influence the N:K ratio or stimulated vegetative growth, due to the late timing of the applications. If the N:K ratio was disturbed due to a much higher contribution N in relation to the K content it would have resulted in smaller fruit as well as stimulated vegetative growth, which in turn would have increased the production of gibberellic acid, which would have interfered with flower bud initiation (Grierson, 2002) and rind colour development. However, these effects where generally not evident in our study.

In terms of internal quality, it appeared that irrigation was the major factor affecting both acid and sugar levels. Previous studies on the combination of irrigation and N have not seen an effect on *Citrus* fruit quality due to an interaction (Castel and Ginestar, 1996; Montaña et al., 2004). Interaction was found in this study, but patterns were mostly unclear. Irrigation had a much larger influence than N, whilst the combination of irrigation and N seemed to influence the fruit quality, depending on other factors such as fruit load, with differences more distinct at a higher crop load. The acid content of fruit from the ½X+N treatment were generally recorded to be the highest, whereas fruit from the 2X+N treatment had the lowest acid content. Yet, these treatments rarely differed significantly from their counterpart treatments without additional N. To argue that the effect was purely due to the interaction would be ambitious; therefore, future research should be extended to different cultivars and other orchards in this area. Crop load undoubtedly had a major impact on fruit quality parameters, also acid content in particularly. In the soluble and total sugar content, similar trends were observed as with the acid content, although less pronounced. The lack of a clear pattern

in the 2016 season could once again be attributed to the reduced fruit load. Furthermore, N content varied immensely.

The bloom and vegetative growth of trees in 2016, following two consecutive seasons of additional irrigation and late N applications, did not seemed to be affected by these treatments. This result was unexpected as a comparative study on 'Lane Late' oranges reported that increasing N fertilisation stimulated vegetative development (Menino et al., 2004b). The significantly increased number of vegetative buds in trees which received the 2X+N treatment compared to the 2X treatment could be ascribed to that any N that was removed by leaching with increased irrigation was possibly replaced by the additional N application. A more feasible explanation is that an adequate status of availability of N in the soil and tree reserves existed, therefore the possible effect additional N could have exerted on the tree phenology was minimized. Furthermore, the second season was an 'off' year, which implicate that more than adequate reserves would have been available for flowering. Flowering was specifically only determined in the second season, to allow for a longer time span over which the tree could assimilate the additional N, which would increase the opportunity for the expression of any effect that irrigation or additional late N applications may have had on the flowering and vegetative phenology. Still, it is possible that the lack of difference in flowering expression could be ascribed to insufficient time from the first application of treatment to the second season's bloom, to observe a direct impact on flowering. The lack of a response could also be due to characteristic low soil temperatures in autumn in Citrusdal. In the management of N in an orchard, the current N status of the tree is of critical importance. The N content of the leaves that were analysed fell within the normal range of N values required for *Citrus*. The N treatments did slightly increase N content, but not significantly so to exceed the commercial range. According to Bemlab (Bemlab (Pty) Ltd., Strand, South Africa) the N content range for *Citrus* leaves in general is between 2.20-2.60%. For 'Valencia' orange cultivated in the Citrusdal area Du Plessis and Koen (1996) reported a N range in leaves of 2.0-2.4%, however, they may differ between areas. In conflict our findings from this study, Menino et al. (2004b) found that increased N fertilisation significantly influenced the N content of leaves, even though the N content of leaves were already in the high to excessive range.

The timing of N application however also determines the distribution thereof within the tree, with autumn applications leading to typically higher N accumulation in the roots (Quiñones et al., 2004). Soil temperatures can have a significant impact on N uptake at different stages. Late application of N (end stage II) does, however, have the ability to increase leaf N content. In the current study the control trees had a higher N content before N application, but by mid-season the root N content of the treated trees was 0.44% higher than the control or 118% of the control value, yet these

values did not differ significantly from each other. Similarly at harvest, even though values were not significantly different, the leaves of treated trees recorded an 0.43% increase in N content compared to the control, whilst the pulp showed a 0.31% increase in N content of N treated trees compared to control trees which did not receive any additional N applications. The soil N content determination, however, did not reveal any possible leaching of fertiliser nor provided any information on when fertiliser was removed from the soil as it is too broad of a determination and soil nitrate content should rather have been determined.

Other factors that could have significantly influenced the experimental results of this study could be the complicating alternate bearing habit that is so pronounced for 'Nadorcott' mandarin and the irrigation alteration. In an 'on' year, a large crop will impose water stress under conditions of competition between the various plant organs for photosynthates to meet demands for vegetative development (Lenz, 1967), whilst starch content in roots is reduced, and root activity affecting water absorption is altered (Kriedemann and Barrs, 1981). Apart from the phenomenon of alternate bearing, the season's rainfall experienced will also interact with and influence the possible of effect of irrigation treatments on tree phenology (Castel and Buj, 1990).

Conclusion

The purpose of this study was to determine the interaction and effect of late N applications and irrigation volumes on *Citrus* fruit quality along with any the possible beneficial effects that may result in the following season. It can be concluded that additional N applied late during fruit development did not detrimentally influence *Citrus* fruit quality. It is proposed that the expected effect was reduced to some extent by the combination of high irrigation volumes. High and low irrigation treatments in combination with late N application has been noted to have a larger impact on fruit quality than N itself as a main effect. Internal quality was affected to a much larger extent than external quality (colour and disorders) or flowering. The negative impact of over-irrigation on rind quality is of high importance and should always be a consideration as it can seriously influence the postharvest life of fruit, although not seen in this study. The late timing of N application on fruit quality was negligible in this case. In our study with 'Nadorcott' mandarin, the late application of N was not detrimental to fruit quality, however positive results will depend largely on individual situations in question where irrigation regimes should be considered when determining N fertilisation practices.

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Table 2. Citric acid content (%) of 'Nadorcott' mandarin fruit pulp as determined by automatic titrator at harvest and after cold storage at -0.6°C and 4° for 30 days for fruit harvested from Citrusdal in the 2015 and 2016 season. 'Nadorcott' trees were subjected to three irrigation regimes (½ X, X and 2X), in combination with either no additional N or additional soil nitrogen (N) applied late in the season as limestone ammonium nitrate (LAN) at 50 kg N.ha^{-1} in 2015 or 100 kg N.ha^{-1} in 2016.

Treatment[z]	2015			2016		
	Harvest	-0.6°C	4°C	Harvest	-0.6°C	4°C
Irrigation						
½X		1.71 a	1.72 a		1.37[NS]	
X		1.64 ab	1.62 ab		1.27	
2X		1.51 b	1.51 b		1.34	
Irrigation x Nitrogen						
½X	1.67 ab*	1.63[NS]	1.62[NS]	1.48 a	1.35[NS]	1.21 b
½X+N	1.86 a	1.79	1.82	1.48 a	1.40	1.42 a
X (Control)	1.70 a	1.67	1.61	1.38 ab	1.26	1.29 ab
X+N	1.75 a	1.62	1.64	1.37 ab	1.29	1.28 ab
2X	1.66 ab	1.60	1.58	1.50 a	1.44	1.38 ab
2X+N	1.48 b	1.43	1.43	1.28 b	1.25	1.18 b
p-value						
Irrigation	*0.0270*	***0.0324***	***0.0205***	*0.3202*	*0.4443*	*0.8635*
Nitrogen	*0.7079*	*0.7339*	*0.4905*	*0.0807*	*0.6375*	*0.9660*
Irrigation x Nitrogen	***0.0486***	*0.0722*	*0.0603*	***0.0422***	*0.0651*	***0.0286***

[z]X = standard irrigation; 2X = increased irrigation; ½X = decreased irrigation
[NS]No significant differences
*Means with a different letter within a column differ significantly at the 5% level (Fischer's LSD)

Table 3. Citric acid content (%) of 'Nadorcott' mandarin fruit pulp as determined by HPLC at harvest and after cold storage at -0.6°C and 4° for 30 days for fruit harvested from Citrusdal in the 2015 and 2016 season. 'Nadorcott' trees were subjected to three irrigation regimes (½ X, X and 2X), in combination with either no additional N or additional soil nitrogen (N) applied late in the season as limestone ammonium nitrate (LAN) at 50 kg N.ha^{-1} in 2015 or 100 kg N.ha^{-1} in 2016.

Treatment[z]	2015			2016		
	Harvest	-0.6°C	4°C	Harvest	-0.6°C	4°C
Irrigation x Nitrogen						
½X	1.77 b*	1.41 ab	1.50[NS]	1.67 ab	1.53[NS]	1.53[NS]
½X+N	2.00 a	1.61 a	1.65	1.51 bc	1.58	1.64
X (Control)	1.77 ab	1.52 a	1.54	1.50 abc	1.54	1.48
X+N	1.81 ab	1.49 ab	1.49	1.49 abc	1.48	1.54
2X	1.71 bc	1.52 a	1.55	1.68 ab	1.74	1.38
2X+N	1.49 c	1.27 b	1.30	1.39 c	1.52	1.43
p-value						
Irrigation	*0.0052*	*0.3070*	*0.1695*	*0.5663*	*0.3408*	*0.2015*
Nitrogen	*0.8034*	*0.6843*	*0.5186*	*0.0153*	*0.1987*	*0.2923*
Irrigation x Nitrogen	***0.0264***	***0.0452***	*0.0621*	***0.0302***	*0.0747*	*0.8510*

[z]X = standard irrigation; 2X = increased irrigation; ½X = decreased irrigation

[NS]No significant differences

*Means with a different letter within a column differ significantly at the 5% level (Fischer's LSD)

Table 4. Total acid content (%) of 'Nadorcott' mandarin fruit pulp as determined by HPLC at harvest and after cold storage at -0.6°C and 4° for 30 days for fruit harvested from Citrusdal in the 2015 and 2016 season. 'Nadorcott' trees were subjected to three irrigation regimes (½ X, X and 2X), in combination with either no additional N or additional soil nitrogen (N) applied late in the season as limestone ammonium nitrate (LAN) at 50 kg N.ha^{-1} in 2015 or 100 kg N.ha^{-1} in 2016.

Treatment[z]	2015			2016		
	Harvest	-0.6°C	4°C	Harvest	-0.6°C	4°C
Irrigation x Nitrogen						
½X	2.00 b*	1.60 ab	1.73[NS]	1.94 a	1.78[NS]	1.79[NS]
½X+N	2.24 a	1.80 a	1.87	1.76 bc	1.84	1.92
X (Control)	2.00 b	1.70 ab	1.75	1.78 abc	1.83	1.77
X+N	2.04 ab	1.67 ab	1.70	1.75 abc	1.74	1.82
2X	1.92 bc	1.71 a	1.76	1.94 ab	1.99	1.60
2X+N	1.70 c	1.45 b	1.49	1.66 c	1.80	1.72
p-value						
Irrigation	*0.0030*	*0.3722*	*0.1475*	*0.4918*	*0.3038*	*0.1227*
Nitrogen	*0.7580*	*0.6992*	*0.4775*	*0.0114*	*0.1699*	*0.1556*
Irrigation x Nitrogen	***0.0307***	***0.0464***	*0.0736*	***0.0300***	*0.0847*	*0.7960*

[z]X = standard irrigation; 2X = increased irrigation; ½X = decreased irrigation

[NS]No significant differences

*Means with a different letter within a column differ significantly at the 5% level (Fischer's LSD)

Table 5. Soluble solids content (SSC) of 'Nadorcott' mandarin fruit pulp at harvest and after cold storage at -0.6°C and 4° for 30 days for fruit harvested from Citrusdal in the 2015 and 2016 season. 'Nadorcott' trees were subjected to three irrigation regimes (½ X, X and 2X), in combination with either no additional N or additional soil nitrogen (N) applied late in the season as limestone ammonium nitrate (LAN) at 50 kg N.ha^{-1} in 2015 or 100 kg N.ha^{-1} in 2016.

Treatment[z]	2015			2016		
	Harvest	-0.6°C	4°C	Harvest	-0.6°C	4°C
Irrigation						
½X	16.0[NS]				15.8 a	
X	15.8				14.8 b	
2X	15.2				15.1 b	
Irrigation x Nitrogen						
½X	15.6[NS]	16.0 ab*	16.2 bc	14.4 ab	15.6[NS]	15.7 ab
½X+N	16.5	16.8 a	17.0 a	14.8 a	16.1	16.2 a
X (Control)	15.6	16.2 ab	16.5 ab	13.7 bcd	14.9	15.3 ab
X+N	16.0	15.7 bc	16.0 bc	13.5 cd	14.8	15.1 ab
2X	15.4	15.8 bc	15.9 bc	14.4 ab	15.6	15.8 a
2X+N	15.1	15.1 c	15.5 c	13.3 d	14.6	14.6 b
p-value						
Irrigation	*0.0667*	*0.0095*	*0.0218*	*0.0195*	***0.0154***	*0.1256*
Nitrogen	*0.2759*	*0.5625*	*0.9602*	*0.2000*	*0.3924*	*0.3742*
Irrigation x Nitrogen	*0.1540*	***0.0291***	***0.0309***	***0.0329***	*0.0815*	***0.0497***

[z]X = standard irrigation; 2X = increased irrigation; ½X = decreased irrigation

[NS]No significant differences

*Means with a different letter within a column differ significantly at the 5% level (Fischer's LSD)

Table 6. Sucrose (%) of 'Nadorcott' mandarin fruit pulp at harvest and after cold storage at -0.6°C and 4° for 30 days for fruit harvested from Citrusdal in the 2015 and 2016 season. 'Nadorcott' trees were subjected to three irrigation regimes (½ X, X and 2X), in combination with either no additional N or additional soil nitrogen (N) applied late in the season as limestone ammonium nitrate (LAN) at 50 kg N.ha^{-1} in 2015 or 100 kg N.ha^{-1} in 2016.

Treatment[z]	2015			2016		
	Harvest	-0.6°C	4°C	Harvest	-0.6°C	4°C
Nitrogen						
No N		5.39[NS]	5.77[NS]	6.76 a	6.50[NS]	5.61[NS]
N		5.31	5.40	6.18 b	6.40	5.96
Irrigation x Nitrogen						
½X	6.20 b*	5.18 [NS]	5.68 [NS]	6.94 [NS]	6.17 [NS]	5.85 [NS]
½X+N	6.66 a	5.51	5.60	6.09	6.52	6.28
X (Control)	6.24 b	5.36	5.78	6.40	6.50	5.78
X+N	6.08 b	5.29	5.38	6.23	6.24	5.87
2X	5.98 b	5.64	5.86	6.95	6.84	5.21
2X+N	5.93 b	5.13	5.24	6.23	6.43	5.72
p-value						
Irrigation	*0.0046*	*0.9613*	*0.9264*	*0.4091*	*0.2921*	*0.2223*
Nitrogen	*0.4433*	*0.6800*	*0.1075*	***0.0070***	*0.4799*	*0.1360*
Irrigation x Nitrogen	***0.0489***	*0.1976*	*0.5040*	*0.2005*	*0.1069*	*0.6780*

[z]X = standard irrigation; 2X = increased irrigation; ½X = decreased irrigation

[NS]No significant differences

*Means with a different letter within a column differ significantly at the 5% level (Fischer's LSD)

Table 7. Glucose (%) of 'Nadorcott' mandarin fruit pulp at harvest and after cold storage at -0.6°C and 4° for 30 days for fruit harvested from Citrusdal in the 2015 and 2016 season. 'Nadorcott' trees were subjected to three irrigation regimes (½ X, X and 2X), in combination with either no additional N or additional soil nitrogen (N) applied late in the season as limestone ammonium nitrate (LAN) at 50 kg N.ha^{-1} in 2015 or 100 kg N.ha^{-1} in 2016.

Treatment[z]	2015			2016		
	Harvest	-0.6°C	4°C	Harvest	-0.6°C	4°C
Nitrogen						
No N			2.66[NS]	2.78 a		2.99[NS]
N			2.50	2.52 b		3.10
Irrigation x Nitrogen						
½X	2.77 b*	2.33 ab	2.60[NS]	2.83[NS]	2.93 b	3.04[NS]
½X+N	3.17 a	2.58 a	2.79	2.62	3.11 ab	3.23
X (Control)	2.80 b	2.37 ab	2.74	2.69	3.08 ab	3.17
X+N	2.81 b	2.36 ab	2.43	2.49	2.87 b	3.03
2X	2.60 b	2.48 a	2.63	2.82	3.27 a	2.75
2X+N	2.54 b	2.11 b	2.28	2.43	2.94 b	3.04
p-value						
Irrigation	*0.0021*	*0.4801*	*0.1544*	*0.2574*	*0.4072*	*0.1416*
Nitrogen	*0.1116*	*0.7056*	*0.1438*	***0.0044***	*0.1468*	*0.3980*
Irrigation x Nitrogen	***0.0252***	***0.0278***	*0.0639*	*0.3944*	***0.0193***	*0.2080*

[z]X = standard irrigation; 2X = increased irrigation; ½X = decreased irrigation

[NS]No significant differences

*Means with a different letter within a column differ significantly at the 5% level (Fischer's LSD)

Table 8. Fructose (%) of 'Nadorcott' mandarin fruit pulp at harvest and after cold storage at -0.6°C and 4° for 30 days for fruit harvested from Citrusdal in the 2015 and 2016 season. 'Nadorcott' trees were subjected to three irrigation regimes (½ X, X and 2X), in combination with either no additional N or additional soil nitrogen (N) applied late in the season as limestone ammonium nitrate (LAN) at 50 kg N.ha^{-1} in 2015 or 100 kg N.ha^{-1} in 2016.

Treatment[z]	2015			2016		
	Harvest	-0.6°C	4°C	Harvest	-0.6°C	4°C
Irrigation						
½X			2.43[NS]	2.58 a		
X			2.27	2.29 b		
2X			2.22	2.30 b		
Nitrogen						
No N			2.36[NS]	2.51 a		
N			2.25	2.27 b		
Irrigation x Nitrogen						
½X	2.48 b*	2.06 ab	2.35[NS]	2.67[NS]	2.74 b	2.78 ab
½X+N	2.80 a	2.31 a	2.51	2.48	2.90 ab	2.98 a
X (Control)	2.49 b	2.10 ab	2.38	2.38	2.91 ab	3.01 a
X+N	2.43 bc	2.08 ab	2.17	2.19	2.72 b	2.79 ab
2X	2.33 bc	2.22 a	2.36	2.47	3.06 a	2.51 b
2X+N	2.24 c	1.89 b	2.08	2.13	2.76 b	2.76 ab
p-value						
Irrigation	*0.0012*	*0.5111*	*0.1185*	***0.0015***	*0.4727*	*0.0208*
Nitrogen	*0.3953*	*0.7285*	*0.2421*	***0.0042***	*0.1400*	*0.3788*
Irrigation x Nitrogen	***0.0319***	***0.0143***	*0.0871*	*0.4603*	***0.0309***	***0.0495***

[z]X = standard irrigation; 2X = increased irrigation; ½X = decreased irrigation

[NS]No significant differences

*Means with a different letter within a column differ significantly at the 5% level (Fischer's LSD)

Table 9. Total sugar (%) of 'Nadorcott' mandarin fruit pulp at harvest and after cold storage at -0.6°C and 4° for 30 days for fruit harvested from Citrusdal in the 2015 and 2016 season. 'Nadorcott' trees were subjected to three irrigation regimes (½ X, X and 2X), in combination with either no additional N or additional soil nitrogen (N) applied late in the season as limestone ammonium nitrate (LAN) at 50 kg N.ha^{-1} in 2015 or 100 kg N.ha^{-1} in 2016.

Treatment[z]	2015			2016		
	Harvest	-0.6°C	4°C	Harvest	-0.6°C	4°C
Nitrogen						
No N		9.9[NS]	10.79[NS]	12.05 a		11.36[NS]
N		9.75	10.16	10.97 b		11.90
Irrigation x Nitrogen						
½X	11.44 bc*	9.56[NS]	10.63[NS]	12.44[NS]	11.83 b	11.66[NS]
½X+N	12.63 a	10.40	10.90	11.20	12.52 ab	12.49
X (Control)	11.53 b	9.82	10.90	11.48	12.50 ab	11.96
X+N	11.32 bc	9.72	9.98	10.92	11.82 b	11.70
2X	10.90 bc	10.35	10.85	12.24	13.17 a	10.47
2X+N	10.71 c	9.14	9.60	10.79	12.13 b	11.52
p-value						
Irrigation	*0.0011*	*0.8526*	*0.4559*	*0.2266*	*0.3305*	*0.0888*
Nitrogen	*0.2344*	*0.6929*	*0.1190*	***0.0046***	*0.2405*	*0.1566*
Irrigation x Nitrogen	***0.0158***	*0.0598*	*0.1967*	*0.3787*	***0.0449***	*0.2709*

[z]X = standard irrigation; 2X = increased irrigation; ½X = decreased irrigation

[NS]No significant differences

*Means with a different letter within a column differ significantly at the 5% level (Fischer's LSD)

Table 10. External fruit quality parameters and carotenoid content of 'Nadorcott' mandarin fruit pulp at harvest and after cold storage at -0.6°C and 4° for 30 days for fruit harvested from Citrusdal in the 2015 and 2016 season. 'Nadorcott' trees were subjected to three irrigation regimes (½ X, X and 2X), in combination with either no additional N or additional soil nitrogen (N) applied late in the season as limestone ammonium nitrate (LAN) at 50 kg $N.ha^{-1}$ in 2015 or 100 kg $N.ha^{-1}$ in 2016.

Treatment[z]	2015			2016			
	Diam. (mm)	Mass (g)	Hue angle (°)	Diam. (mm)	Mass (g)	Hue angle (°)	Carotenoid content ($\mu g.g^{-1}$)
Irrigation							
½X	58.9 b*	74.4 b	64.7 a	73.4[NS]	129.1[NS]	59.7[NS]	1535.64[NS]
X	59.1 b	74.1 b	63.2 ab	73.5	129.6	60.1	1478.35
2X	61.1 a	81.3 a	62.3 b	73.0	127.3	60.4	1467.08
p-value							
Irrigation	***0.0237***	***0.0416***	***0.0240***	*0.8446*	*0.8608*	*0.8505*	*0.5794*
Nitrogen	*0.9622*	*0.9543*	*0.3553*	*0.3433*	*0.3992*	*0.3290*	*0.0744*
Irrigation x Nitrogen	*0.1032*	*0.1392*	*0.6655*	*0.1272*	*0.1568*	*0.5959*	*0.0828*

[z]X = standard irrigation; 2X = increased irrigation; ½X = decreased irrigation

[NS]No significant differences

*Means with a different letter within a column differ significantly at the 5% level (Fischer's LSD)

Table 11. Rind quality parameters of 'Nadorcott' mandarin fruit pulp after cold storage at -0.6°C and 4° for 30 days for fruit harvested from Citrusdal in the 2015 and 2016 season. 'Nadorcott' trees were subjected to three irrigation regimes (½ X, X and 2X), in combination with either no additional N or additional soil nitrogen (N) applied late in the season as limestone ammonium nitrate (LAN) at 50 kg N.ha^{-1} in 2015 or 100 kg N.ha^{-1} in 2016.

Treatment[z]	2015					2016	
	Green colour		Creasing		Staining	Rind defect index	
	-0.6°C	4°C	-0.6°C	4°C	4°C	-0.6°C	4°C
Irrigation							
½X	2.0 a*	1.7 a	1.0 a	0.9 a	0.2 b	1.0 [NS]	1.5 [NS]
X	1.7 ab	1.4 ab	0.8 a	0.7 ab	0.3 a	1.0	1.4
2X	1.5 b	1.0 b	0.4 b	0.4 b	0.2 b	1.3	1.6
Nitrogen							
No N	1.6[NS]	1.3 b	0.8[NS]	0.7[NS]	0.2[NS]	1.0[NS]	1.5[NS]
N	1.8	1.5 a	0.7	0.6	0.2	1.1	1.5
p-value							
Irrigation	***0.0145***	***0.0013***	***0.0090***	***0.0234***	***0.0368***	*0.3069*	*0.6331*
Nitrogen	*0.0882*	***0.0492***	*0.4624*	*0.8557*	*0.3071*	*0.4596*	*0.5559*
Irrigation x Nitrogen	*0.4192*	*0.8075*	*0.4680*	*0.4507*	*0.3517*	*0.3138*	*0.0723*

[z]X = standard irrigation; 2X = increased irrigation; ½X = decreased irrigation

[NS]No significant differences

*Means with a different letter within a column differ significantly at the 5% level (Fischer's LSD)

Table 12. Vegetative and reproductive characteristics of 'Nadorcott' mandarin trees in Citrusdal at full bloom (30 Sept.) in the 2016 season. 'Nadorcott' trees were subjected to three irrigation regimes (½ X, X and 2X), in combination with either no additional N or additional soil nitrogen (N) applied late in the season as limestone ammonium nitrate (LAN) at 50 kg N.ha^{-1} in 2015 or 100 kg N.ha^{-1} in 2016.

Treatment[z]	Shoot length (cm)	Bud type			Total flowers per shoot	Flowers per cm shoot
		Leafless	Leafy	Vegetative		
Irrigation						
½X	19.6[NS]	6.1[NS]	2.3[NS]		23.9[NS]	1.3[NS]
X (Control)	19.8	4.1	2.5		18.2	1.0
2X	20.0	4.3	2.7		18.2	1.0
Nitrogen						
No N	20.2[NS]	5.1[NS]	2.6[NS]		21.5[NS]	1.1[NS]
N	19.4	4.6	2.4		18.7	1.0
Irrigation x Nitrogen						
½X	19.8[NS]	6.0[NS]	2.6[NS]	0.1 ab*	26.8[NS]	1.4[NS]
½X+N	19.4	6.3	2.0	0.3 ab	21.1	1.1
X (Control)	20.7	3.6	2.7	0.4 ab	15.2	0.8
X+N	18.9	4.7	2.3	0.1 ab	21.2	1.1
2X	20.1	5.8	2.5	0.1 b	22.6	1.2
2X+N	19.9	2.9	3.0	0.4 a	13.8	0.8
N	19.4	4.6	2.4		18.7	1.0
p-value						
Irrigation	*0.9441*	*0.1438*	*0.6734*	*0.9336*	*0.1059*	*0.1447*
Nitrogen	*0.4323*	*0.4299*	*0.6875*	*0.3124*	*0.2650*	*0.4974*
Irrigation x Nitrogen	*0.7566*	*0.0509*	*0.5004*	***0.0133***	*0.0544*	*0.0714*

[z]X = standard irrigation; 2X = increased irrigation; ½X = decreased irrigation

[NS]No significant differences

*Means with a different letter within a column differ significantly at the 5% level (Fischer's LSD)

Table 13. Total nitrogen (N) content of soil, roots, leaves and fruit pulp and rind of 'Nadorcott' mandarin trees in Citrusdal at different sampling dates in the 2016 season. 'Nadorcott' trees were subjected to three irrigation regimes (½ X, X and 2X), in combination with either no additional N or additional soil nitrogen (N) applied late in the season as limestone ammonium nitrate (LAN) at 50 kg N.ha^{-1} in 2015 or 100 kg N.ha^{-1} in 2016.

Treatment	Soil N content (%)	Root N content (%)	Leaf N content (%)	Fruit N content	
				Pulp N content (%)	Rind N content (%)
Beginning of season (01/04/2016)					
No N	0.05^{NS}	2.72^{NS}	2.41^{NS}	1.94^{NS}	1.88^{NS}
N	0.04	2.36	2.38	1.96	1.89
p-value	*0.2897*	*0.7959*	*0.6325*	*0.0509*	*0.6711*
Mid-season (11/05/2016)					
No N		2.39^{NS}	2.57^{NS}	1.33^{NS}	1.44^{NS}
N		2.83	2.64	1.30	1.46
p-value		*0.7712*	*0.8576*	*0.3224*	*0.5330*
Harvest (27/06/2016)					
No N	0.06^{NS}	2.09^{NS}	2.89^{NS}	0.96^{NS}	1.33^{NS}
N	0.06	2.38	3.32	1.27	1.39
p-value	*0.6279*	*0.1183*	*0.6303*	*0.0536*	*0.7245*

^{NS}No significant differences

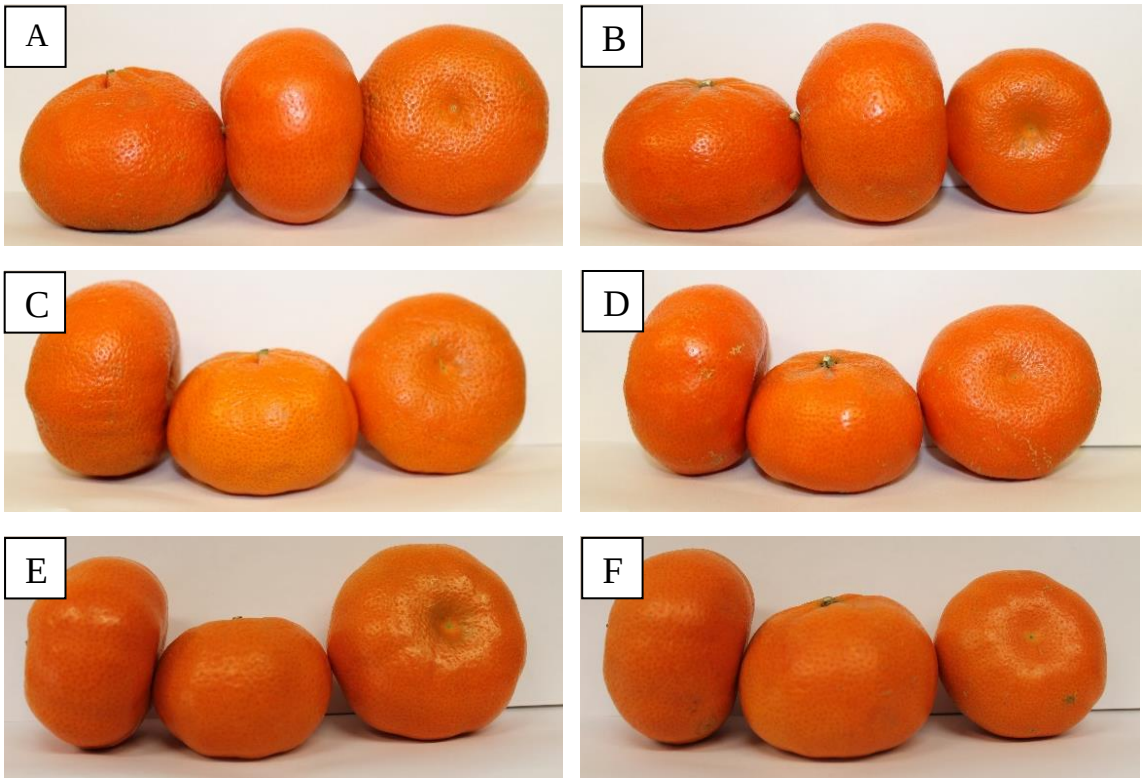

Figure 1. Representative fruit of 'Nadorcott' mandarin at harvest of each treatment taken in the 2016 season: A = standard irrigation without additional nitrogen (N), B = standard irrigation with additional N, C = increased irrigation without additional N, D = increased irrigation with additional N, E = decreased irrigation without additional N and F = decreased irrigation with additional N.

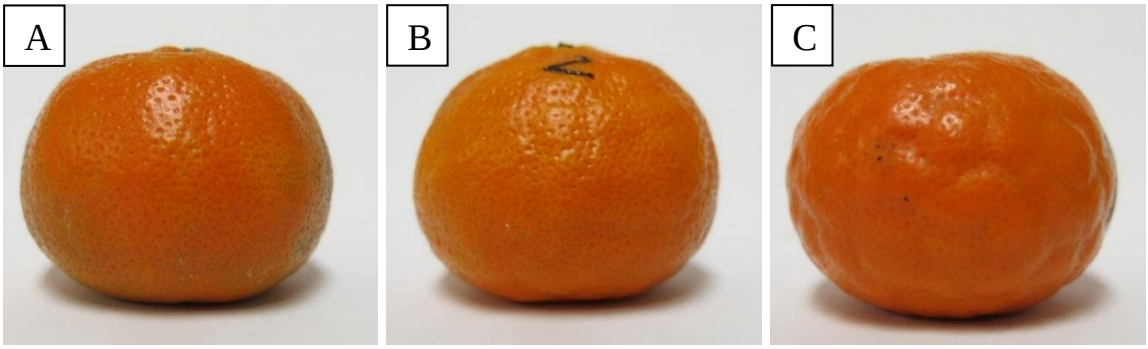

Figure 2. Creasing occurring in 'Nadorcott' fruit in the 2015 season at harvest which was scored on a severity index from 0 to 2 with A = 0 (no creasing), B = 1 (intermediate creasing/acceptable) and C = 2 (severe creasing/unacceptable).

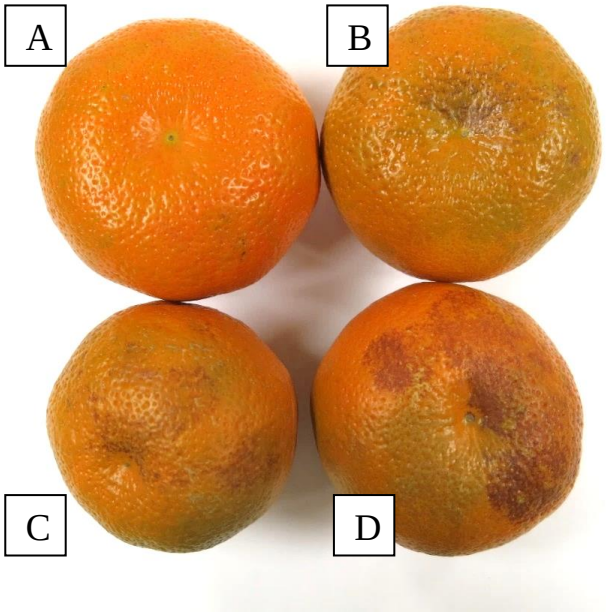

Figure 3. Rind staining in ‘Nadorcott’ mandarin as observed after storage at 4°C in the 2015 season scored from 0 to 4 with A = 0, B = 1, C = 2 and D = 4.

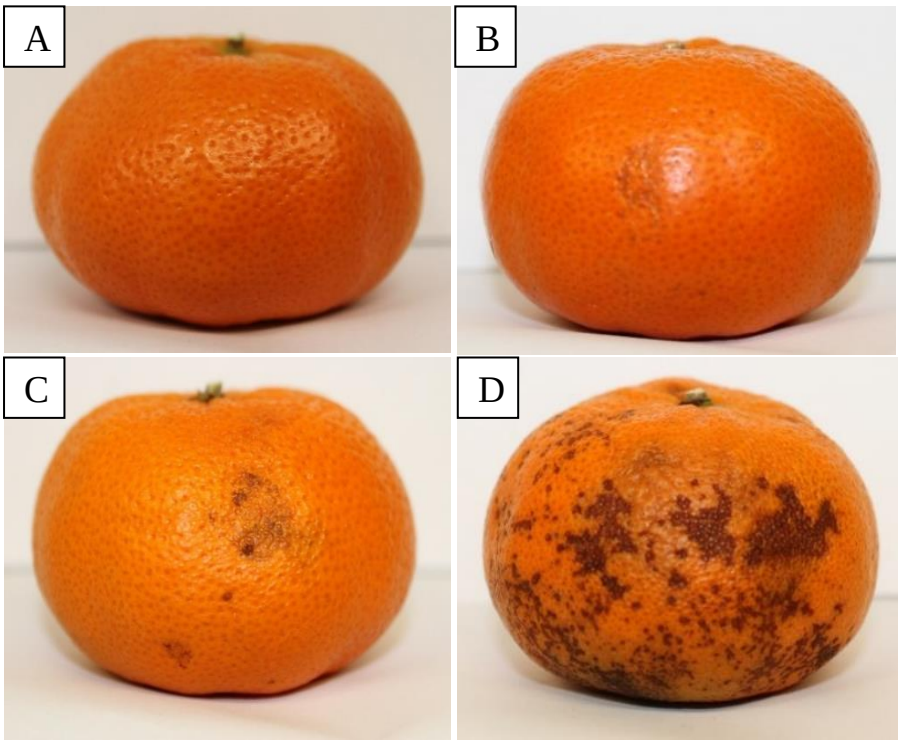

Figure 4. Rind staining and rind defects in ‘Nadorcott’ mandarin observed after storage at 4°C in the 2016 season scored from 0 to 4 with A = 0, B = 1, C = 2 and D = 4 (a similar scale was used after storage at -0.6°C but without the incidence of staining).

Chapter 5: The effect of additional late nitrogen in the form of foliar or soil applications on 'Nadorcott' mandarin fruit quality

Abstract

Nitrogen (N) is critical in *Citrus* mineral nutrition as it has the largest effect on both fruit quality and production factors of all the mineral elements. Increasing N fertilising rates are known to decrease *Citrus* fruit quality; however, yield is dependent on N, thus leading to a trade-off between fruit quality and yield. Certain phenological stages such as flowering and fruit set are periods of high nutrient requirement. Flowering occurs in spring when soil temperatures are still cold and mineral uptake by the roots is low. Therefore, the tree is largely dependent on N reserves, which should be adequate to support the flowering process. The aim of this study was (i) to determine whether additional N, either as limestone ammonium nitrate (LAN) or a foliar urea spray, applied late during Stage II of fruit growth, would detrimentally affect 'Nadorcott' mandarin fruit quality as well as (ii) establish any possible effect on vegetative growth and flowering of the next season. In order to accomplish this, N as LAN, was applied in two equal split applications of 50 and a 100kg N.ha^{-1} respectively at 26 and 30 weeks after full bloom, additional to the standard soil recommendation, and as a 1% foliar urea spray at 26 weeks after full bloom, in two production areas, Citrusdal and Nelspruit, in the 2016 season. Standard fruit quality parameters were determined during the season, at harvest and after cold storage at -0.6°C and 4°C for fruit collected from Citrusdal, but only at harvest for fruit obtained from Nelspruit. The occurrence of postharvest rind disorders after storage was recorded for both areas. The additional N applied late during Stage II of fruit growth had insignificant effects on 'Nadorcott' mandarin fruit quality in both areas. The effect on tree growth and development was, however, also negligible. It is concluded that in an orchard with adequate N levels, additional N will not improve the N reserve status of tree nor affect the expression of flowering of the next season. It can, however, be deduced that in an orchard with a known N deficiency occurring late in Stage II of fruit growth, the application of N would be unlikely to detrimentally impact on the current season's fruit quality. However, an improvement in the following season's bloom may be expected if soil temperatures allow uptake.

Additional keywords: alternate bearing, *Citrus*, fertilisation, flowering, foliar fertilisation, pitting

Introduction

Management of nitrogen (N) is considered of critical importance in *Citrus* mineral nutrition, as it is required in highest amounts and is known to have a major influence on *Citrus* fruit quality and production (Chapman, 1968; Smith, 1966). Elevated levels of N fertilisation rates are generally considered to negatively affect *Citrus* fruit quality, however suboptimum levels of N fertilisation have an adverse impact on yield, leading to a trade-off between quality and yield.

With respect to *Citrus* fruit quality, increasing N fertilisation rates decrease both fruit mass and size (Dasberg et al., 1984; Koo, 1988; Smith, 1966). Furthermore, high N levels in the leaves can suppress the promoting role of potassium (K) in fruit size (Du Plessis and Koen, 1988), whilst also negatively affecting rind colour by delaying the degreening of green fruit, consequently increasing the number of green fruit present at harvest (Koo, 1988; Reitz and Koo, 1959; Smith, 1966). Undesirable rind colour, for example green or yellow, caused by too high N fertilisation, can be induced by the inhibition of the conversion of chloroplasts to chromoplasts or by promoting re-greening which is the reversion of chromoplasts to chloroplasts. Carotenoid content is decreased by high N fertilisation along with the suppression of chlorophyll disappearance (Huff, 1983; 1984). Furthermore, increasing N fertilisation has been found to increase juice content (Embleton et al., 1978; He et al., 2008; Koo, 1988; Quaggio et al., 2006; Smith, 1966), soluble solids content (SSC) (He et al., 2008; Koo, 1988; Quaggio et al., 2006), acidity (Koo, 1988; Reitz and Koo, 1959; Smith, 1966) and rind thickness (Dasberg, 1983; Embleton et al., 1978; Koo, 1988; Smith, 1966).

Certain phenological stages such as flowering and fruit set have higher nutrient demands than other periods, but these critical stages occur in spring when soil temperatures are low and unfavourable soil conditions decrease N uptake compared to that of the warmer summer months (Lovatt, 1999; 2013). Ensuring adequate N reserves within the tree is of paramount important at these stages when soil uptake is low and considerable remobilisation of N within the tree is required. Supplementation of N as a foliar application can be used under these conditions when nutrient requirements are high (Lovatt, 1999). Earlier studies have shown that winter, pre-bloom and spring foliar application of low-biuret urea increased flowering and yield, but with negligible effects on fruit quality (Ali and Lovatt, 1994; Lovatt, 1999). The effect of urea on flowering is however, not purely through the increase in N, but more by enhancing the endogenous ammonia levels (Lovatt et al., 1988). Nitrogen-containing compounds have been reported to accumulate in stressed plants (Rabe, 1990). Many stress conditions, for example water stress, salinity stress, that increases flowering has been reported to correlate with an increase in endogenous ammonia. However, results from several

studies on N application and its effect on *Citrus* tree growth and development, are often varied and conflicting, mainly as a range of factors may impact on the efficacy of N applications.

'Nadorcott' mandarin is a late maturing type soft *Citrus* cultivar and therefore has a harvest date close to flowering, especially within winter rainfall production areas where climate has an overriding influence on the ripening process (Tucker, 1993). A late harvest may affect the flowering of the next season's crop detrimentally, aggravating a condition known as alternate bearing, which is essentially an unbalance between vegetative and reproductive growth (Lenz, 1967). Alternate bearing in mandarin cultivars leads to a reduction in fruit quality and creates management problems at both the tree and orchard level. Adequate N reserves is therefore of high importance to ensure the successful expression of flowering, especially in a cultivar prone to harvest late in the season.

The study was conducted in two areas to determine whether trees will react differently to the same treatments. Areas in South Africa can be classified as 'small fruit' or 'large fruit' areas with Citrusdal being the first and Nelspruit the latter (Du Plessis, 1980). The 'small fruit' areas (for example Citrusdal) are hotter with a mediterranean climate and the 'large fruit' areas (for example Nelspruit) are cooler receiving summer rainfall, referring to summer conditions. Fertiliser recommendations should differ between these two areas as N and K both have a significant effect on fruit size (Du Plessis and Koen, 1988; Du Plessis, 1996). Therefore, optimal leaf analysis norms for N and K between these two areas also differ with 'small fruit' areas requiring lower N and higher K levels compared to the 'large fruit' areas (Du Plessis, 1996). The aim of this study was thus to determine whether additional N foliar application can be used in 'Nadorcott' mandarin to positively influence overall tree condition, particularly regarding the promotion of flowering, without exerting a negative impact on fruit quality.

Materials and methods

Sites and plant material

Citrusdal

The trial was conducted on 'Nadorcott' mandarin trees (*C. reticulata* Blanco) on Carrizo rootstock, planted in 2007 at a 5 m x 2 m spacing, in a North to South row direction. The trees were based in a commercial orchard on the farm Tienrivieren, Citrusdal, Western Cape province, South Africa (32°47'46.1"S 19°04'30.5"E). The trees included in the experiment were healthy and uniform, with buffer trees between the treatment trees. The site has a typical mediterranean type climate with hot summers and winter rainfall, and sandy soils, characteristic of the Citrusdal area. According to

the producer, the experimental orchard had a known history of being prone to post-harvest disorders such as pitting.

Nelspruit

The trial was conducted on 'Nadorcott' mandarin trees (*C. reticulata* Blanco) on Carrizo rootstock planted in 2008 at a 5 m x 2 m spacing, in a North-West to South-East row direction. The trees were cultivated in a commercial orchard on Indigo farm, Nelspruit, Mpumalanga, South Africa (25°25'33.2"S 31°06'23.4"E). The trees included in the experiment were healthy and uniform, with buffer trees between the treatment trees. The area has a typical subtropical climate with summer rainfall.

Treatments

Four treatments were applied in both orchards: control, additional N of 50 kg $N.ha^{-1}$ (50 g N per tree), additional N of 100 kg $N.ha^{-1}$ (100 g N per tree) and a 1% urea foliar spray. Soil applications were made as limestone ammonium nitrate (LAN) fertiliser [LAN(28), ©Agricol] which was applied in split applications on 16 Mar. (approximately 26 weeks after full bloom) and 19 Apr. (approximately 30 weeks after full bloom) in Nelspruit and on 1 Apr. (approximately 26 weeks after full bloom) and 3 May (approximately 30 weeks after full bloom) in Citrusdal, respectively. The foliar application of 1% urea [Urea LB (Spray grade) (460 $g.kg^{-1}$) Nitrophoska (Pty) Ltd] was sprayed until drip off using a motorised knapsack sprayer, on 16 Mar. and 1 Apr. in Nelspruit and Citrusdal, respectively. These applications amounted to a total of 3.6 litres or 17 g N per tree in Nelspruit and 4.8 litres or 22 g N per tree in Citrusdal, adjusting for differing tree sizes. Relevant calculations are provided in Appendix A.

Quality measurements

As previously described in Chapters 3 (pg. 47-48) and 4 (pg. 78), ten fruit were sampled per tree for external and internal quality determinations at nine weeks before and at harvest (12 Jul. 2016) in Citrusdal, together with a sample for rind pigment analysis at harvest [Chapter 4 (pg. 78-79)]. Fruit from Nelspruit was only analysed at harvest (30 May 2016) for titratable acidity (TA) by the titration of 20 mL juice with 0.1 N sodium hydroxide, using phenolphthalein as an indicator. No HPLC and rind pigment analysis was performed for the Nelspruit produced fruit due to practical considerations.

Fruit growth and colour throughout season and flowering

The fruit diameter was determined by repeated measurements at 14, 10 and 2 weeks before harvest in Citrusdal as well as 11 weeks before and at harvest in Nelspruit with an electronic Fruit Size Measure and DataLogger (GÜSS Manufacturing (Pty) Ltd., Strand, South Africa). Fruit used for sampling were positioned in the 1 to 1.5 m from ground level and not deeper than 20 cm into the canopy ensuring adequate direct sunlight. The rind colour of the tagged fruit used for fruit diameter measurements was measured on the tree as the season progressed at 14 (pre-treatment), ten and two weeks before harvest in Citrusdal and at 11 weeks before harvest and at harvest in Nelspruit, using a colorimeter (Konica Minolta CR-400, Tokyo, Japan). A permanent marker was used to indicate the area on the rind for repetitive measurements. The impact of the various treatments on return bloom (flowering) was recorded on 29 Sept. 2016, as described in Chapter 4 (pg. 79).

Nitrogen uptake

Citrusdal

Soil, roots, leaves and fruit were sampled from the control, the 100 kg $N.ha^{-1}$ and the urea treatments prior to the treatment application, at the end of fruit stage II on 1 Apr. 2016. At stage III on 11 May 2016 which was estimated to be at nine weeks before harvest, root, leaf and fruit samples were again collected from the control and the 100 kg $N.ha^{-1}$ treatment, whereas only leaf and fruit samples were collected from the 1% urea foliar treatment. At harvest on 12 Jul. 2016, samples of soil, roots, leaves and fruit were collected from the control and soil treatment as well as fruit and leaf samples from the foliar treatment. See Chapter 4 (pg. 79).

Cold Storage and Postharvest Disorders

Citrusdal

Fifteen fruit of each treated tree was stored at -0.6°C or 4°C for 32 days. After harvest, but before cold storage, the fruit were subjected to de- and rehydration to induce rind pitting [Chapter 4 (pg. 79-80)]. The external and internal fruit quality parameters were determined after the storage period, as described previously [Chapter 3 (pg. 47-48)], where after the fruit were evaluated for physiological rind disorders according to a rating scale, following a shelf life period of seven days at room temperature (Fig. 1). Green colour was similarly scored according to a rating scale as described in Chapter 4 (pg. 80). In addition to the general fruit quality parameters measured, the rind thickness was also determined as described in Chapter 3 (pg. 48).

Nelspruit

Fifteen fruit of each treated tree was stored at -0.6°C and 4°C for 32 days. Before storage, fruit were de- and rehydrated [Chapter 4 (pg. 79-80)] to exacerbate the incidence of any possible rind defects. The fruit were evaluated for physiological rind disorders after seven days' shelf life on completion of the cold storage period.

Experimental design and statistical analysis

The experimental design was a randomised complete block with four treatments, replicated in ten blocks. Analysis of variance (ANOVA) was carried out, with means separated using Fisher's LSD ($p < 0.05$ and 0.1). Statistical analysis was carried out using StatSoft Statistica (version 13.0, StatSoft, Inc.).

Results

Citrusdal

There were no significant differences or any recognisable pattern of an increase or decrease in any of the internal or external *Citrus* fruit quality parameters either nine weeks before or at harvest (Table 1). In addition, there were no significant differences in any of the fruit quality parameters after storage at 4°C (Table 2). After storage at -0.6°C significant differences in the juice content between treatments emerged (Table 2). This increase in juice content with the 100kg $N.ha^{-1}$ and the 1% urea treatment noted with storage at -0.6°C appears to be isolated and not repeated at harvest (Table 1) or after storage at 4°C (Table 2). The urea spray might have slightly increased citric acid content at harvest and after storage, but was not significantly affected at the 5% confidence level (Tables 1 and 2).

No significant differences in hue° or fruit diameter as measured in the field was found between treatments at any sampling time during the season (Table 3). In the rind, the carotenoid content did not differ significantly between treatments, but chlorophyll content was affected (Table 4). There was a large variation in green colour of the fruit in all treatments (Fig. 2) but rind colour differences between treatments could not be detected (Fig. 3). Pigment analysis indicate that the chlorophyll b and combined chlorophyll content was significantly higher in the 1% urea foliar treatment compared to the other treatments. The chlorophyll a content of the rind from fruit that were harvested from the 1% urea spray treatment was also significantly higher than that of the soil-applied N treatments, but did not differ significantly from that of the control (Table 4).

None of the flowering parameters were affected by any of the treatments (Table 5). With respect to the total N content recorded from the soil, roots, leaves and fruit respectively, there were no significant differences at any sampling date, except for percentage root N content mid-season where the root N content of the additional 100 kg $N.ha^{-1}$ treatment was significantly higher than that of the control (Table 6).

Nelspruit

The fruit from Nelspruit showed significant differences between treatments for citric acid and SSC at harvest (Table 1). However, no distinct pattern emerged as none of the other *Citrus* fruit quality parameters differed significantly. No significant differences in hue° (Table 3) and fruit diameter in the orchard throughout the season were recorded between treatments prior to treatment application or at harvest (Table 3). There were no differences in rind colour (Fig. 4).

Discussion

Rind colour of 'Nadorcott' mandarin was not affected by the application of additional N during fruit development Stage II, 26 to 30 weeks after full bloom. This contrasts with literature, which states that both an increase in N fertilisation and a delay in the application thereof would postpone the degreening process and increase the amount of green fruit at harvest (Koo, 1988; Dasberg, 1983; Reitz and Koo, 1959; Quiñones et al., 2004). A possible explanation would be that the extent of the N influencing the rind colour might vary between cultivars as most studies cited above were conducted on oranges and not mandarin fruit.

As rind colour is determined by the chlorophyll and carotenoid composition and ratios, the impact of additional N on rind colour would be closely associated with a change in pigment composition. High N fertilisation is known to decrease carotenoid content in *Citrus* and inhibits the degradation or conversion of chlorophyll whilst low N fertilisation increases carotenoid content (Huff, 1983; 1984). In our study carotenoid content was not influenced by the various N treatments. Chlorophyll content, on the other hand was affected with chlorophyll b and combined chlorophyll content significantly higher in the 1% urea spray treatment compared to the control and soil-applied N. With respect to the chlorophyll a content, the 1% urea spray also had a significantly higher content compared to the soil-applied N treatments, but not to the control. A possible explanation for the response of chlorophyll to the urea treatment is that the latter is sprayed directly into the canopy and on the fruit surface. Nitrogen is therefore directly supplied to the flavedo as well as the leaves which are in close proximity to the fruit. Still, the elevated level of combined chlorophyll in the rind did not

translate into a visible rind colour effect. Furthermore, other factors such as the prevailing climate (low night temperatures) and endogenous hormones (auxin and ethylene levels) together with the interaction between these factors during fruit development and at maturity may have a larger impact on rind colour than nutrition. Areas where the night temperatures are typical lower, fruit release more ethylene due to temperature stress and consequently the development of rind colour is promoted through the breakdown of chlorophyll and production of carotenoids (Grierson, 2002a). Auxin, in turn, can also stimulate ethylene synthesis (Iglesias et al., 2007).

As with rind colour, fruit size was also not influenced by N fertilisation. The lack of a response to N in terms of fruit size could possibly be due to the late application in the season, as the final fruit size is known to be determined during the cell division stage of fruit growth (Rabe, 2000). The application time used in this study was thus not able to directly affect the N:K ratio which is considered important in controlling fruit size. Furthermore, the timing of the application was most likely also too late in the season to stimulate vegetative growth which is likely to detrimentally impact on the carbohydrate reserves allocated for fruit growth as well as stimulate the production of gibberellic acid. Gibberellins can prevent flower bud formation, if applied after flower bud initiation (Grierson, 2002b).

Overall, the application of additional N in the form of LAN or urea had a negligible effect on the external and internal fruit quality parameters in both production areas. In addition, and contrary to the hypothesis, where late N application would have transferred a positive impact on the following season's bloom and vegetative growth, no such response was recorded. Various reasons to explain the non-responsiveness of the phenology to additional N application, either as LAN or urea, may lie in the availability of adequate N within the soil and tree reserves.

The urea application as was used in this study, cannot be compared to a winter pre-bloom or spring application, as the objectives were distinctly different. The use of urea in this study aimed to improve N availability, whilst the conventional use of urea in spring is to boost endogenous ammonia levels for the stimulation and promotion of flowering. A possible explanation for the lack of response to the additional N application in Citrusdal area could possibly be associated with the microclimate in the orchard. This site was in a particularly cool part of the valley, where root activity required for uptake could already have been too inactive due to low autumn soil temperatures to take advantage of the additional nutrition provided. In the more humid Nelspruit area, the additional N could have leached out at an earlier stage, due to the nature of the intense summer precipitation typical for this region. The Citrusdal area can accumulate more reserves compared to the Nelspruit area due to higher temperatures in summer and lower temperatures in winter (Du Plessis, 1996). Therefore, it can be

speculated that with additional N application the problem is soil temperature for Citrusdal and rainfall for Nelspruit.

Flowering expression was determined to establish whether any positive short-term effects in the following season could be recorded. The absence of any direct response to the late N application might be ascribed to either an adequate N status of the tree where supplying N above adequate will not result in an effect. The short-term nature of the study in addition to the fact that the study was done in an 'off' production year (Citrusdal area) could have resulted in trees which had adequate reserves for flowering and fruit set for the following season's crop, therefore reducing the chance of a beneficial effect being recorded. Bemlab (Bemlab (Pty) Ltd., Strand, South Africa) states adequate leaf norms for *Citrus* in general as between 2.2-2.6%. Du Plessis (1996) in studies on 'Valencia' orange makes a distinction between 'small fruit' and 'large fruit' areas for leaf analysis norms. Indicating that leaf N content of seven-month-old leaves on fruiting terminals should be 2.0-2.4% in 'small fruit' areas and 2.1-2.7% in 'large fruit' areas. The Citrusdal area requiring a lower leaf N content was in both these ranges at all sampling dates and thus had an adequate to high N content.

As results obtained were somewhat unexpected, data from an additional season using orchards of more varied climates and microclimates, would be required before the use of late application N can be recommended as a practice that would not be a detriment to fruit quality and beneficial to tree reserve status to promote flowering. However, results from this study suggest that N can be applied over a longer time span and into the growing season than is currently the practice. Also, urea sprays did not detrimentally affect colour in the 2016 season compared to the control and other N treatments, in both production areas evaluated. Thus, once the risk of late N application in 'Nadorcott' mandarin to optimum rind colouration and any contribution to susceptibility to rind disorders have been established, the potential long-term benefits to promote tree reserve status and flowering, together with other management problems can be explored.

Conclusion

In this study, the application of additional late N, both as soil-applied and as a 1% urea foliar spray did not detrimentally affect 'Nadorcott' mandarin fruit quality. The absence of any negative effect of late N application on this alternate-bearing cultivar cannot be attributed to the late harvesting date, which contributed to any possible effect being less evident, as the early sampling dates similarly did not produced any significant differences between treatments and the control either. Future studies, to further explore possible beneficial effects is recommended, as orchards may need several seasons to show a response to differential N management practices. Influences such as the cultivar in

question, along with various environmental factors and the N available within the environment and tree can have important effects by itself or through interactions with each other. Furthermore, it may be that in a well-managed orchard, the application of additional nutrients, such as N, will not result in an immediate effect. From the current study, there is evidence that a 'Nadorcott' mandarin orchard known to have insufficient N in autumn may be fertilised with additional N, either through soil or as a urea foliar application, as it is unlikely to have a detrimental effect on fruit quality.

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Table 1. The internal and external fruit quality parameters of 'Nadorcott' mandarin fruit collected from Citrusdal at nine weeks before and at harvest and from Nelspruit at harvest in the 2016 season as influenced by the application of additional nitrogen (N) as limestone ammonium nitrate (LAN) at 50 kg N.ha^{-1} and 100 kg N.ha^{-1}applied in two split applications (26 and 30 weeks after full bloom) as well as a 1% urea spray (26 weeks after full bloom).

Area	Sampling date	Treatment	Internal fruit quality					External fruit quality		
			Citric acid (%)	Titratable acidity (TA)	SSC (Brix°)	Ratio[x]	Juice content (%)	Hue angle (°)	Mass (g)	Diam. (mm)
Citrusdal	9 WBH[z]	Control	1.52^{NS}	-[y]	11.0^{NS}	7.3^{NS}	51.0^{NS}	96.3^{NS}	88.1^{NS}	62.5^{NS}
		50 kg N.ha^{-1}	1.60	-	11.3	7.2	52.9	94.5	84.1	61.7
		100 kg N.ha^{-1}	1.55	-	11.0	7.2	54.0	95.7	88.6	62.5
		1% Urea spray	1.58	-	11.0	7.1	54.5	97.3	81.8	61.0
		p-value	*0.7069*	-	*0.6747*	*0.7472*	*0.1872*	*0.7425*	*0.2395*	*0.3052*
	Harvest	Control	1.05^{NS}	-	12.2^{NS}	11.7^{NS}	52.7^{NS}	60.0^{NS}	109.6^{NS}	68.9^{NS}
		50 kg N.ha^{-1}	1.04	-	12.6	12.1	51.1	58.3	116.0	70.5
		100 kg N.ha^{-1}	1.07	-	12.5	11.9	52.0	59.3	101.9	67.7
		1% Urea spray	1.12	-	12.5	11.5	51.8	59.0	105.5	68.7
		p-value	*0.6421*	-	*0.8264*	*0.6418*	*0.4566*	*0.5159*	*0.0683*	*0.2155*
Nelspruit	Harvest	Control	-	1.33 a*	13.4 a	10.2^{NS}	62.2^{NS}	56.3^{NS}	109.3^{NS}	63.7^{NS}
		50 kg N.ha^{-1}	-	1.08 b	12.0 b	11.2	62.5	54.3	121.0	66.7
		100 kg N.ha^{-1}	-	1.34 a	13.1 a	9.9	62.2	57.6	99.2	61.9
		1% Urea spray	-	1.28 ab	13.1 a	10.3	62.8	55.0	106.8	64.1
		p-value	-	*0.0200*	*0.0422*	*0.2585*	*0.8464*	*0.6268*	*0.3338*	*0.2968*

Harvest: Citrusdal - 12/07/2016 and Nelspruit - 30/05/2016

[NS]No significant differences

*Means with a different letter within a column differ significantly at the 5% level (Fischer's LSD)

[z]WBH = Weeks before harvest

[x]Citrusdal = TSC/Citric acid; Nelspruit = SSC/TA

[y]Data not collected

Table 2. The internal and external fruit quality parameters of 'Nadorcott' mandarin fruit collected from Citrusdal after cold storage for 32 days at -0.6°C and 4°C in the 2016 season as influenced by the application of additional nitrogen (N) as limestone ammonium nitrate (LAN) at 50 kg N.ha^{-1} and 100 kg N.ha^{-1} applied in two split applications (26 and 30 weeks after full bloom) as well as a 1% urea spray (26 weeks after full bloom).

		Internal fruit quality				External fruit quality			
Treatment	Storage temperature (°C)	Citric acid (%)	SSC (Brix°)	Ratio (SSC/ citric acid)	Juice content (%)	Hue angle (°)	Rind Thickness (mm)	Green colour	Rind defects
Control	-0.6	0.92^{NS}	12.2^{NS}	13.4^{NS}	35.0 b*	59.6^{NS}	2.2^{NS}	0.55^{NS}	0.6^{NS}
50 kg N.ha^{-1}		0.90	12.5	14.0	37.3 b	58.3	2.2	0.48	0.4
100 kg N.ha^{-1}		0.93	12.8	14.1	49.1 a	58.9	2.1	0.62	0.4
1% Urea spray		0.97	12.8	13.5	49.1 a	58.5	2.1	0.62	0.5
p-value		*0.6036*	*0.2547*	*0.5182*	*0.0043*	*0.3114*	*0.6836*	*0.8277*	*0.4217*
Control	4	0.87^{NS}	12.4^{NS}	14.3^{NS}	54.5^{NS}	59.8^{NS}	2.1^{NS}	0.64^{NS}	0.6^{NS}
50 kg N.ha^{-1}		0.90	13.1	14.7	55.0	57.8	2.0	0.37	0.4
100 kg N.ha^{-1}		0.91	13.1	14.6	54.8	58.5	2.0	0.57	0.5
1% Urea spray		0.98	12.9	13.6	56.7	57.9	2.0	0.54	0.5
p-value		*0.3838*	*0.2040*	*0.2924*	*0.0665*	*0.1115*	*0.7376*	*0.5421*	*0.5550*

^{NS}No significant differences

*Means with a different letter within a column differ significantly at the 5% level (Fischer's LSD)

Table 3. Rind colour (hue°) and fruit diameter (mm) throughout the season as influenced by the application of additional nitrogen (N) as limestone ammonium nitrate (LAN) at 50 kg N.ha^{-1} and 100 kg N.ha^{-1} applied in two split applications (26 and 30 weeks after full bloom) as well as a 1% urea spray (26 weeks after full bloom).

Area	Sampling date	Treatment	Hue angle (°)	Diam. (mm)
Citrusdal	14 WBH[z]	Control	122.2[NS]	51.5[NS]
		50 kg N.ha^{-1}	122.1	52.3
		100 kg N.ha^{-1}	122.6	51.8
		1% Urea spray	122.1	50.9
		p-value	*0.7731*	*0.7277*
	10 WBH	Control	106.2[NS]	59.0[NS]
		50 kg N.ha^{-1}	105.3	59.9
		100 kg N.ha^{-1}	107.2	59.4
		1% Urea spray	107.9	58.9
		p-value	*0.7081*	*0.8798*
	Harvest	Control	67.7[NS]	63.9[NS]
		50 kg N.ha^{-1}	66.5	65.2
		100 kg N.ha^{-1}	67.6	65.0
		1% Urea spray	66.4	64.6
		p-value	*0.9287*	*0.8426*
Nelspruit	11 WBH	Control	115.9[NS]	58.1[NS]
		50 kg N.ha^{-1}	117.6	57.3
		100 kg N.ha^{-1}	116.6	55.2
		1% Urea spray	116.0	56.6
		p-value	*0.0700*	*0.4931*
	Harvest	Control	81.4[NS]	65.9[NS]
		50 kg N.ha^{-1}	81.2	64.4
		100 kg N.ha^{-1}	84.7	61.4
		1% Urea spray	80.0	62.9
		p-value	*0.5028*	*0.4766*

Harvest: Citrusdal - 12/07/2016 and Nelspruit - 30/05/2016

[NS]No significant differences

*Means with a different letter within a column differ significantly at the 5% level (Fischer's LSD)

[z]WBH = Weeks before harvest

Table 4. The chlorophyll and carotenoid content (µg.g^{-1}) of 'Nadorcott' mandarin fruit rind flavedo collected from the Citrusdal production area at harvest as influenced by additional nitrogen (N) as limestone ammonium nitrate (LAN) at 50 kg N.ha^{-1} and 100 kg N.ha^{-1} applied in two split applications (26 and 30 weeks after full bloom) as well as a 1% urea spray (26 weeks after full bloom).

Treatment	Total Chlorophyll a (µg.g^{-1})	Total Chlorophyll b (µg.g^{-1})	Carotenoid content (µg.g^{-1})	Chlorophyll a and b (µg.g^{-1})
Control	4.80 ab*	11.57 b	1497.46^{NS}	16.37 b
50 kg N.ha^{-1}	1.60 b	0.00 b	1630.52	1.60 b
100 kg N.ha^{-1}	1.72 b	0.39 b	1519.27	2.11 b
1% Urea spray	9.18 a	47.89 a	1605.60	57.07 a
p-value	*0.0047*	*0.0059*	*0.1421*	*0.0038*

Harvest: 12/07/2016

^{NS}No significant differences

*Means with a different letter within a column differ significantly at the 5% level (Fischer's LSD)

Table 5. Flowering (29 Sept. = full bloom), including various vegetative and reproductive parameters on the shoots of 'Nadorcott' mandarin trees from Citrusdal as influenced by additional nitrogen (N) as limestone ammonium nitrate (LAN) at 50 kg N.ha^{-1} and 100 kg N.ha^{-1} applied in two split applications (26 and 30 weeks after full bloom) as well as a 1% urea spray (26 weeks after full bloom).

Treatment	Shoot length (cm)	Bud type			Total flowers per shoot	Flowers per cm
		Leafless	Leafy	Vegetative		
Control	20.24^{NS}	1.31^{NS}	1.70^{NS}	2.33^{NS}	5.56^{NS}	0.31^{NS}
50 kg N.ha^{-1}	21.40	1.94	1.78	1.36	6.19	0.32
100 kg N.ha^{-1}	19.90	1.11	2.97	0.75	7.33	0.36
1% Urea spray	22.50	3.14	2.17	0.72	10.56	0.43
p-value	*0.3703*	*0.2969*	*0.3134*	*0.2199*	*0.6297*	*0.9183*

^{NS}No significant differences

Table 6. The total nitrogen (N) content of soil, roots, leaves and fruit pulp and rind of 'Nadorcott' mandarin trees from the Citrusdal production area at different sampling dates as influenced by additional nitrogen as limestone ammonium nitrate (LAN) at 50 kg N.ha^{-1} and 100 kg N.ha^{-1} applied in two split applications (26 and 30 weeks after full bloom) as well as a 1% urea spray (26 weeks after full bloom).

Treatment	Soil N content (%)	Root N content (%)	Leaf N content (%)	Fruit N content	
				Pulp N content (%)	Rind N content (%)
Before application (01/04/2016)					
Control	0.05^{NS}	2.08^{NS}	2.86^{NS}	1.28^{NS}	1.36^{NS}
100 kg N.ha^{-1}	0.06	2.21	2.66	1.12	1.19
1% Urea spray	0.06	2.11	2.67	1.20	1.31
p-value	*0.1780*	*0.5514*	*0.3074*	*0.1397*	*0.1878*
Mid-season (11/05/2016)					
Control	-^{y}	2.02 b*	2.43^{NS}	1.27^{NS}	1.44^{NS}
100 kg N.ha^{-1}	-	2.52 a	2.35	1.17	1.25
1% Urea spray	-	-	2.44	1.22	1.41
p-value	-	*0.0279*	*0.6951*	*0.4504*	*0.0955*
Harvest (12/07/2016)					
Control	0.07^{NS}	2.71^{NS}	2.58^{NS}	1.13^{NS}	1.21^{NS}
100 kg N.ha^{-1}	0.06	2.56	2.42	1.11	1.06
1% Urea spray			3.08	1.20	1.12
p-value	*0.1019*	*0.4415*	*0.0119*	*0.6394*	*0.1966*

^{NS}No significant differences

*Means with a different letter within a column differ significantly at the 5% level (Fischer's LSD)

^{y}Data not collected

Figure 1. Rating index used to classify rind defects in 'Nadorcott' mandarin after storage at -0.6°C and 4°C for 32 days where 0 on the far left indicate no damage and 3 on the far right indicated severe damage.

Figure 2. Examples of green colour variation occurring between fruit of all treatments from the Citrusdal trial site – before storage.

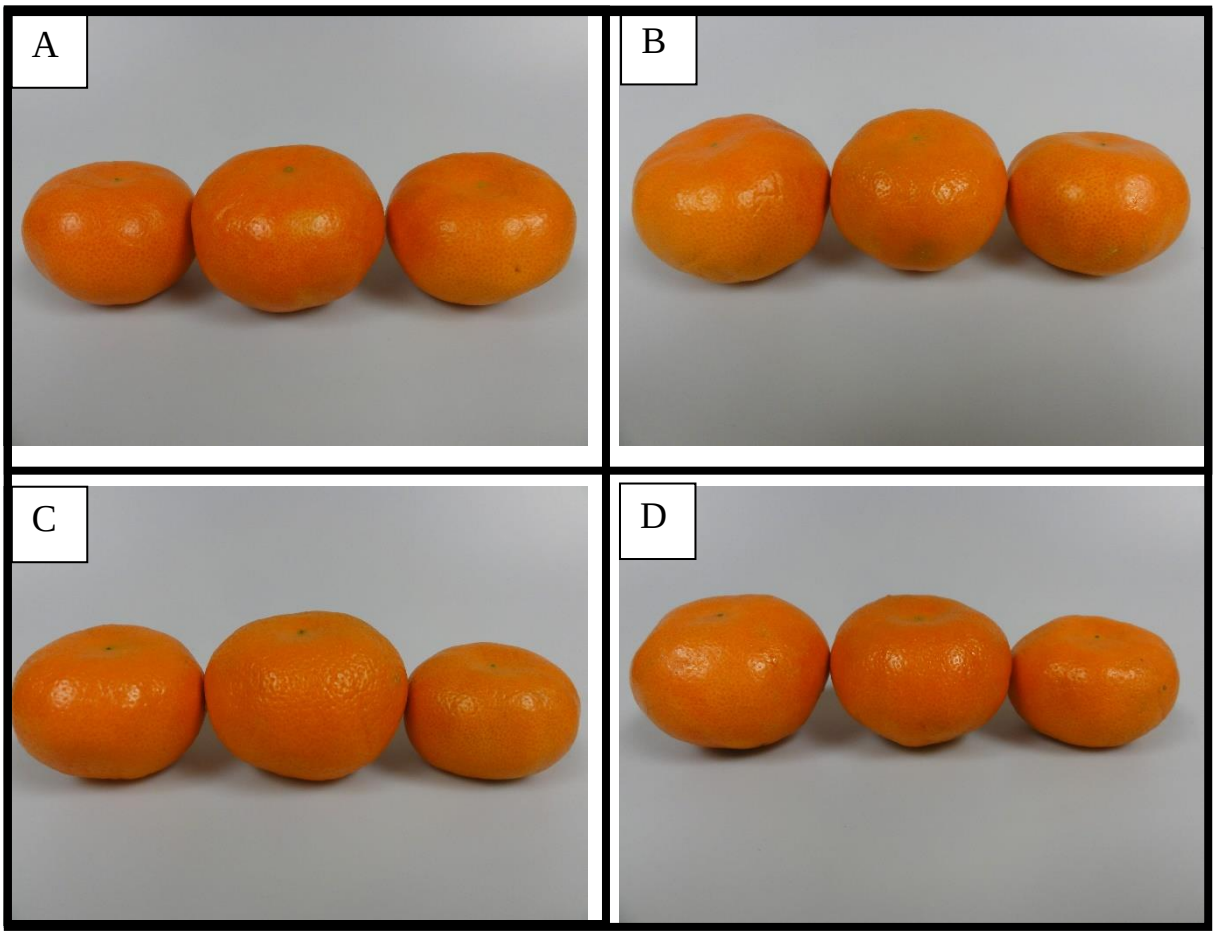

Figure 3. A comparison between external fruit quality of 'Nadorcott' mandarin fruit from the Citrusdal trial site of the different treatments at harvest: A – control, B – 50 kg $N.ha^{-1}$, C – 100 kg $N.ha^{-1}$ and D – 1% urea spray. No differences in external fruit quality was detected between treatments.

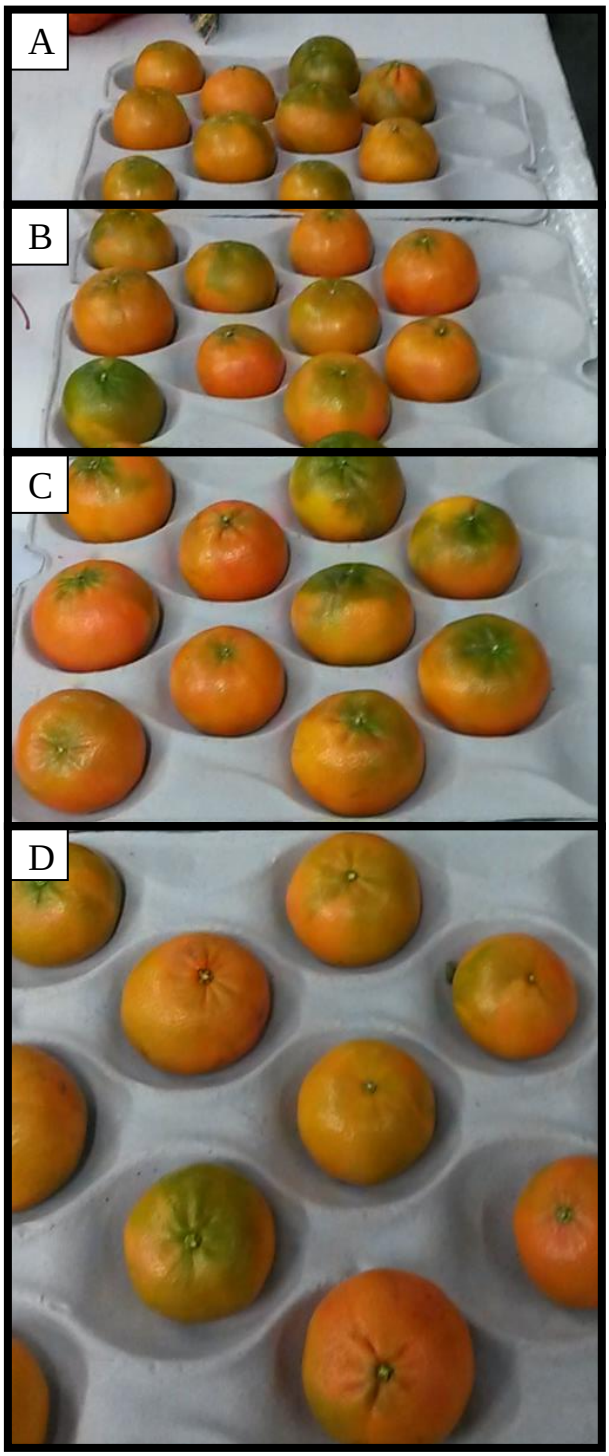

Figure 4. A comparison between external fruit quality of 'Nadorcott' mandarin fruit from the Nelspruit trial site of the different treatments at harvest: A – control, B – 50 kg $N.ha^{-1}$, C – 100 kg $N.ha^{-1}$ and D – 1% urea spray. No differences in external fruit quality was detected between treatments.

Chapter 6: The use of scanning electron microscopy (SEM) and fluorescence microscopy to study magnesium and calcium concentration and distribution in *Citrus* rind

Abstract

Rind colour-change in *Citrus* fruit from green to orange is indicative of the external ripening process. During ripening chloroplasts are converted to chromoplasts, which implicate that chlorophylls are degraded, whilst carotenoid formation occurs. The magnesium ion (Mg^{2+}) is an important constituent of the chlorophyll ring structure and is therefore also thought to be central to colour development and rind quality. During the season, Mg either can accumulate in the flavedo or be transported from the flavedo following colour development. In an attempt to determine how Mg-content changes throughout the season a Zeiss EVO MA15 Scanning Electron Microscope chamber was used to determine the Mg-content of *Citrus* rind. Furthermore, a Carl Zeiss LSM 780 confocal microscope and an Olympus IX81 widefield microscope was used to determine the occurrence of auto-fluorescence within *Citrus* rind, whereas Fura 2 dyes was used to map the distribution of calcium (Ca) and Mg within green and orange rind, respectively. Scanning electron microscopy was found to produce large variation when used for the quantification of Mg in the rind, although it does provide an indication of the presence and concentration ranges thereof. The Fura 2 probes and protocol evaluated was found ineffective to determine the distribution of Mg within *Citrus* rind. It is suggested that future research combines the use of these two technologies once a successful protocol for the use of Fura 2 dyes has been established.

Additional keywords: calcium, chloroplasts, chromoplasts, fura-2 (pentapotassium salt), mag-fura-2 (tetrapotassium salt), structural analysis

Introduction

External ripening of *Citrus* fruit refers to the visible rind colour-change from green to orange. During this process, chloroplasts are converted to chromoplasts, along with the breakdown of chlorophylls and synthesis of carotenoids (Huff, 1983; 1984; Iglesias et al., 2007). In *Citrus*, magnesium (Mg) is considered central to colour development as well as a critical factor to ensure rind quality as it can either accumulate in the flavedo throughout the season or be translocated from the flavedo, following colour development of the rind. Magnesium content has been reported to

decrease during the season with fruit development, for example in 'Nules Clementine' mandarin, the decrease in Mg coincides with chlorophyll degradation (Cronjé et al., 2011).

Magnesium has a fundamental biological role in photosynthetic carbon fixation (Lilley et al., 1974) and is the central ion of the chlorophyll ring structure (Gebert et al., 2009; Maguire and Cowan, 2002). The Mg cation differs largely in chemical properties from other biological relevant cations in that it has the smallest ionic radius, but with a very large hydration shell (Gebert et al., 2009; Maguire and Cowan, 2002). The fate of the Mg-ion following chlorophyll degradation in fruit is not well understood.

Microscopy techniques have been effectively used to study structural changes as observed with rind disorders in *Citrus*. Relevant research includes studies on the development of oleocellosis in 'Washington Navel' orange (Knight et al., 2002), observations on cell wall deterioration with exposure to ethylene in 'Navelate' oranges (*C. sinensis*, L. Osbeck) (Cajuste et al., 2011), examinations of chilling injury on grapefruit rind (Cohen et al., 1994) as well as investigation into the influence of hot water brushing treatments on the epicuticular waxes of *Citrus* fruit surface (Porat et al., 2000).

Developments in scanning electron microscopy (SEM) technology, in addition to structural analysis, can also provide an estimation of the mineral content of the tissue studied. The value of this localized mineral determination technique stems from the fact that the absolute amount of an element within a plant sample as would be obtained with conventional mineral analysis, is not necessarily a useful parameter (Maguire and Cowan, 2002). Similarly, methods to determine the distribution of calcium (Ca) within plant tissues and not only an estimation of total Ca provide more information on the functioning of Ca in biological systems with respect to cell wall integrity (Bush and McColl, 1987; Gilroy et al., 1993) and cell membrane stability (Saure, 2005) as implicated in physiological disorders. To this purpose Bonomelli et al. (2010) designed a protocol using Fura 2 dyes in fluorescence microscopy to visualise the distribution of Ca in vegetable tissues as Ca is integral for quality during and after storage. However, even though protocols developed for determining Ca in plant tissue should have the possibility to be adapted for the determination of Mg, fewer options are available for Mg determination (Maguire and Cowan, 2002).

In fluorescence microscopy, both ratio and single-wavelength dyes can be considered (Gilroy et al., 1993). Ratio dyes, with the Fura 2 dyes as examples, is dependent on the affinity of the cation for the dye and require emission at two wavelengths to determine the free concentration of the specific ion (Maguire and Cowan, 2002). The initial dyes developed for the measurement of Ca^{2+}

had a poor affinity for Ca^{2+} and was limited in terms of cell types as possible targets for the dye. However, the more advanced Fura 2 and other new generation dyes for measuring Ca^{2+} are much more effective and has application in almost all cell types. Regrettably dyes currently available for Mg^{2+} are still comparable in effectiveness to that of the earlier dyes cited for Ca^{2+} detection and quantification. Furthermore, the affinity of the Mg^{2+} to the dye is not the major problem but rather the affinity for Ca^{2+} that influences measurements (Maguire and Cowan, 2002). As a result of significant technical challenges with regard to Mg^{2+} determination through fluorescent microscopy, no comparative studies on the presence and distribution of Mg within *Citrus* rind through the use of Fura 2 probes could be traced in the literature to date.

The aim of this study was to firstly determine the amount of Mg and Ca within the rind using SEM and to secondly obtain an understanding of the distribution of these elements within *Citrus* rind using Fura 2 dyes with Fluorescent microscopy. Cellular localization of Mg and Ca would provide insight into the differences in flavedo Mg-content and distribution throughout the rind of green and orange fruit, with regard to changes during the season, particularly so at the rapid changes occurring at colour break.

Materials and methods

Scanning electron microscopy (SEM)

Citrus fruit, externally ripe and fully coloured, were sampled at harvest (Aug.) from a 'Nadorcott' mandarin orchard in the 2015 season. The fruit rind was cut into strips approximately 1 cm in length with a sharp blade. The cut rind was subsequently fixed in FAA (formaldehyde – acetic acid – alcohol) for 12 h. After fixation, the rind was washed for 2 h with each solution within an ethanol dilution series [50, 60, 70, 80, 90, 100%] respectively. In a next step, the samples were then washed with 100% acetone before critical point drying (CPD) in liquid carbon dioxide (CO_2) for 1 h 30 min with a Quorum Critical Point Dryer at the Central Analytical Facilities (CAF), Stellenbosch University, South Africa. After freeze-drying, the samples were positioned on SEM stubs using double sided carbon tape, where after samples were thinly gold-coated (15 nm) for six min in an Edwards S150A Gold Sputter Coater to increase the electrical conductivity of the sample surface and to avoid electron charge during analysis. Samples were loaded into a Zeiss EVO MA15 Scanning Electron Microscope chamber at the Electron Microbeam Unit of CAF. Samples were analysed by quantitative energy-dispersive X-ray spectroscopy (EDS) and wavelength-dispersive X-ray spectroscopy (WDS) using a Zeiss EVO MA 15 Scanning Electron Microscope (SEM). Major

elements were determined via EDS using an Oxford Instruments® XMax 20 mm^2 detector and Oxford INCA software. The element concentration in the samples was selectively quantified via WDS analysis using Oxford Instrument ®Wave Dispersive X-ray Spectrometer and Oxford INCA software. Beam conditions during the quantitative analyses on the EVO SEM were 20 kV and approximately 1.0A, with a working distance of 8.5 mm and a specimen beam current of −20.00 nA. Internal Astimex Scientific mineral standards were used for standardization and verification of the analyses. Pure Co were used periodically to correct for detector drift. The system was designed to perform high-resolution imaging concurrently with quantitative analysis, with detection limits ranging from ±0.05 to 0.1 dry weight percentage on the major elements using EDS, and 10 to 100ppm on trace elements using WDS. Semi-quantitative analysis of phase compositions of the SEM samples and their backscatter images require 15 µm thickness of carbon coating, a flat and polished surface.

Fluorescence microscopy

Citrus fruit, either fully coloured or green, were sampled from a 'Nadorcott' mandarin orchard throughout the 2016 season in order to develop an appropriate protocol for detection of Mg in *Citrus* rind. The protocol used was adapted from Bonomelli et al. (2010) as was developed for the detection of Ca in vegetable tissues. Rind sections approximately 8 mm thick and 16 mm wide were cut from the fruit and placed in tissue freezing media for 5 min prior to freezing in isopentane, which was pre-cooled with liquid N. The samples were then placed, in the correct orientation, on a small cork tile and covered with cold tissue freezing media. The cork tile was held in the cooled isopentane with tweezers until it was properly frozen. After freezing, the samples were stored at -80°C. The frozen rind samples sectioned into 15 µm thin segments at -22 °C using a Leica CM1860 UV Cryostat subsequently mounted on microscope slides at the CAF, Stellenbosch University.

The sections were circled with a wax pen. From this point, work was conducted in a semi-shaded environment. The sections were covered with 30 µL of 50 mM MES buffer that had been adjusted to pH 4.5 using sodium hydroxide (NaOH). The slides were placed in a humidified chamber for ten min. The Fura 2 probe, Mag-fura-2 tetrapotassium salt (fluorescent Mg indicator) and fura-2 pentapotassium (fluorescent Ca indicator), dimethyl-sulfoxide (DMSO) stock solutions were prepared. In a tube, 1 mg of the probe was dissolved in 1.2 mL of DMSO. In another tube, 3 µL of the probe solution, 300 µL ethyl acetate and 500 µL MES buffer were added together and mixed. Each rind section was loaded with 30 µL of the prepared aliquot. The control rind sections were loaded with a solution of ethyl acetate and MES buffer (300 µL ethyl acetate and 500 µL MES buffer).

The rind sections were then placed in a humidified chamber and incubated between 27-35°C (±33°C) for 60 min.

After incubation, rind sections were washed once with distilled water. MES buffer was added and the slides were covered before analysis with a Carl Zeiss LSM 780 confocal microscope for initial testing and later with an Olympus IX81 widefield microscope at CAF. The LSM 780 confocal microscope was only used to determine the presence or absence of sample auto-fluorescence. The closest wavelength to the Fura 2 probes' excitation peak at 350 nm within the Carl Zeiss LSM 780 confocal microscope range is 405 nm. This range is not adequate, therefore the IX81 widefield microscope with a Xenon burner for excitation, allowing the measuring of excitation at the 350 nm peak, was considered as an alternative. All stock solutions were protected from light and stored at -25°C when not in use.

Rind studies using the Olympus IX81 widefield microscope required a further adaptation of the protocol designed for usage with the Carl Zeiss LSM 780 confocal microscope and included an adjustment of the loading pH and increasing probe concentrations. All the possible combinations of pH 4.5 or 7.0 (MES buffer) and concentration of 6 µL or 20 µL of probe stock solutions were tested.

In further adjustments, fresh samples were used and the rind was hand-sectioned using a sharp blade into approximately 0.5 mm thick and 16 mm long sections. Subsequently samples were not placed in cooled isopentane. Half of these sections were fixed in FAA for 24 h prior to staining and the other half were kept fresh. The staining protocol was standardized at 6 µl probe stock solution at pH 4.5 (MES buffer).

Statistical analysis

Analysis of variance (ANOVA) was carried out with means separated by using Fisher's LSD ($p < 0.05$ and 0.1) for Mg- and Ca-content, comparing the content of each mineral in the different sections of the rind. The above-mentioned analysis was carried out using StatSoft Statistica (version 13.0, StatSoft, Inc.).

Results and discussion

Magnesium content within the rind of mandarin fruit exhibited a large variation between samples from the flavedo, mid-rind and albedo (Table 1). The Mg-content differences between rind sections were not significant. SEM mineral estimations are very accurate, however the large variance obtained in this study within the rind does not provide reasonable confidence for comparing concentration between treatments for example the colour development stage. The minimum Mg-

content measurement in the flavedo was lower than both mid-rind and in the albedo. To reduce the variability and deviations many samples is required which is both costly and time consuming, resulting in that SEM technology for the localized estimation of Mg being an ineffective method.

The Ca-content determined by means of SEM produced a much clearer trend than was observed with the Mg-content (Table 2). The flavedo had a significant larger Ca-content compared to the mid-rind and albedo. Overall, Ca-content was also much higher than the measured Mg-content of the rind, and values with less variation and an acceptable standard deviation was obtained. A concentration gradient of Ca was detected in the rind and SEM is recommended for the determination of Ca and Mg.

The Carl Zeiss LSM 780 confocal microscope was only used to detect sample auto-fluorescence (Fig. 1). A next protocol, using the Olympus IX81 widefield microscope Fura340 with a CFP filter (Fig. 2 and 3), was attempted to visualize the distribution and location of Mg and Ca within the rind. However, when contrasting samples were examined with this technology such as stained compared to unstained rind, or green versus fully developed rind (Fig. 4 and 5) no clear differences could be distinguished. Auto-fluorescence was more evident in the unstained than the stained sample (Fig. 4). Establishing pH range between 4.0 and 5.0 is a known technique to introduce the dye probes into the cells (Bonomelli et al., 2010). Method development was thus attempted to improve loading by means of a pH adjustment between 4.0 and 5.0 which is considered best for loading, while a pH 7.0 which was reported as optimum in other tissue types was also evaluated for rind sections. Further adjustments to probe concentrations were made to improve the chances of success (Bonomelli et al., 2010; Rudd and Franklin-Tong, 2001), despite the report by Bonomelli et al. (2010) where no signal differences between different probe concentrations were detected. Both fresh and FAA fixed sections were exposed to higher concentrations of the probe, but did not results in any improvement in staining. Thus, all protocols tested effectively failed to show that these dyes could be used effective for determination of Mg and Ca in *Citrus* rind tissue.

Various reasons could exist for the ineffectiveness of the probes. As optimum conditions for pH, probe concentrations and the use of fresh and FAA fixed tissues were explored, these can be excluded as possible causes for failure, except that the probes' effectiveness decreased over time. This could be due to possible decomposition with multiple freezing and thawing of the DMSO stock solutions, which then diminishes the ability of the dye to load cells (Molecular Probes™). As experiments were conducted over the season storage of stock solution and a possible reduction in its effectiveness was unavoidable. Furthermore, the extended thawing time that was required could have been a contributing factor to possible loss of probe action.

Working with live cells when doing auto-fluorescence, especially those containing chlorophyll can be problematic as the pigment has been known to mask the probe's signal (Bonomelli et al., 2010). This problem was not encountered. However, it is speculated that the light levels in the work-area may have been exceeded the optimum levels and thus reduced the effectiveness of the probe.

Alternatively, better results may have been obtained with the sections had been submerged in the dye, rather than being just bathed in a droplet, before being covered with microscope cover slide. However, due to the limited dye available this was not a possibility to explore. Overall, it must be taken into account that each cell type should be optimized for loading conditions, such pH and temperature separately as for example higher temperature will promote faster loading of the dye (Molecular Probes™). Different loading temperatures were not tested in this study. Bonomelli et al. (2010) established an effective protocol for the use of a Fura 2 probe (without esters), but did not find an appropriate protocol for the use of another probe, Fura 2AM probe, in vegetable tissues. In their study, it was unclear whether failure of the ineffective Fura 2AM probe was able to penetrate the tissue or whether the significant auto-fluorescence present prevented the detection of the signal. The probe has however been effectively introduced into FAA fixed vegetable tissues using MES buffer as a solvent at an acidic pH (pH 4.5). Therefore, it is possible that the probes evaluated in this study was not suitable for detection of mineral elements *Citrus* rind cells.

Magnesium concentration is known to vary between seasons in *Citrus* rind (Cronjé et al., 2011). It is therefore recommended that in a follow up study, the same orchard in the same season and from similar trees in the same canopy position should be compared. As the Fura 2 probes have been used successfully in other studies, it is proposed that results from this study should be used as a basis for developing a successful protocol for the use of Fura 2 probes on *Citrus* rind.

It can be concluded that SEM technology is not ideal in measuring the content of mineral elements present in low amounts such as Mg in *Citrus* rind. It does however provide value ranges of the respective tissues in the rind, which is useful for future studies. Calcium content, which is overall present at higher concentrations presented with less variation in the rind. A protocol for the use of Fura 2 probes was not established. To improve in future on the use Fura 2 probes for the location and quantification of Mg in *Citrus* rind, it is recommended to use corresponding samples for both fluorescence microscopy and SEM, where coordinates could ensure that the exact same area that is observed in fluorescence imaging can be studied under the SEM for mineral quantification.

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Table 1. The magnesium (Mg) content (percentage dry weight) of fully coloured 'Nadorcott' mandarin fruit rind (n=6) as determined with scanning electron microscopy (SEM).

Rind section	Mean	Minimum	Maximum	Std. Dev.
Flavedo	0.31[NS]	0.02	0.44	0.162
Mid-rind	0.27	0.17	0.32	0.060
Albedo	0.20	0.05	0.27	0.080

[NS] No significant differences

Table 2. The calcium (Ca) content (percentage dry weight) of fully coloured 'Nadorcott' mandarin fruit rind (n=6) as determined with scanning electron microscopy (SEM).

Rind section	Mean	Minimum	Maximum	Std. Dev.
Flavedo	1.34 a*	0.75	2.17	0.49
Mid-rind	0.63 b	0.49	0.79	0.11
Albedo	0.47 b	0.37	0.55	0.07

*Means with a different letter within a column differ significantly at the 5% level (LSD)

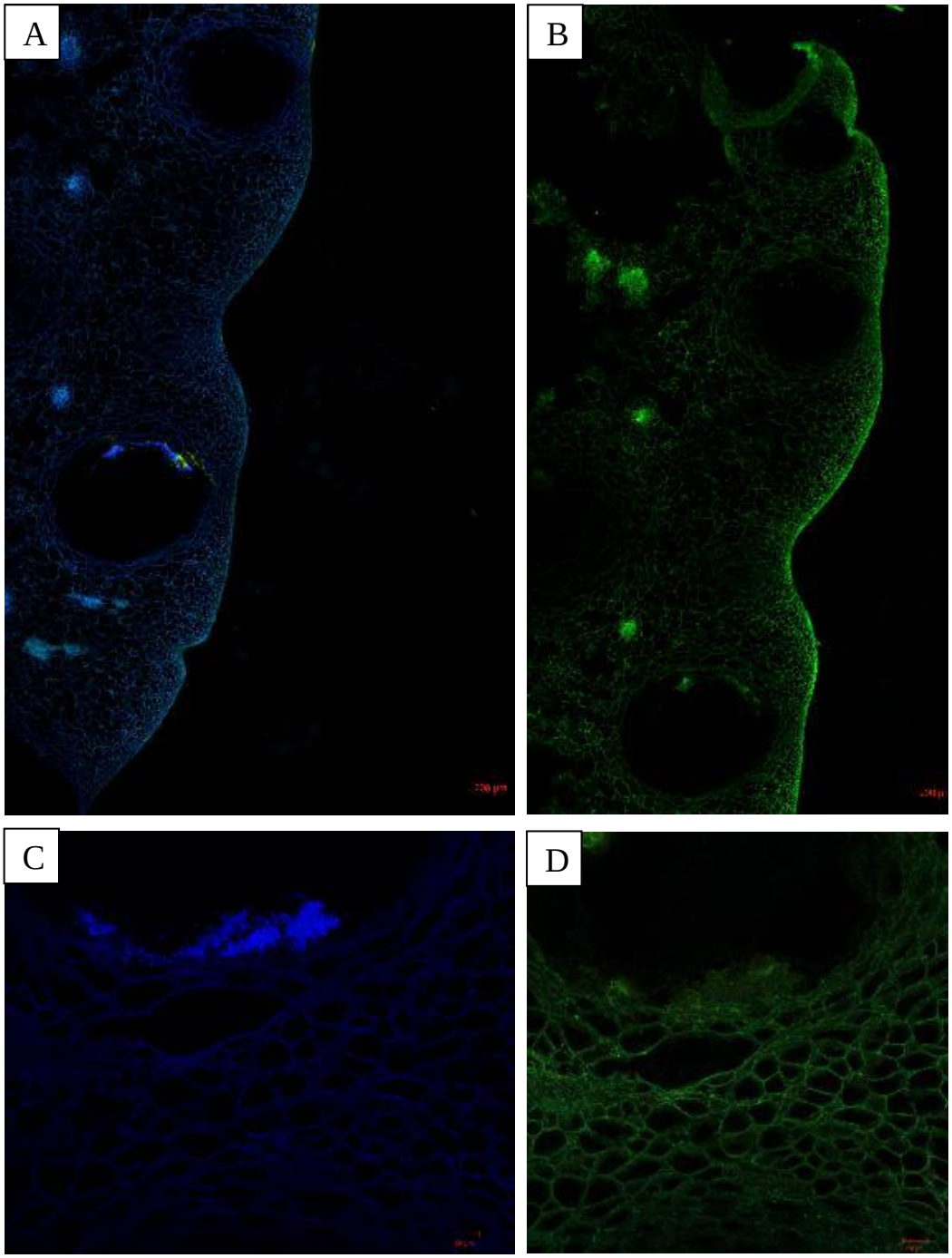

Figure 1. Images of 'Nadorcott' mandarin rind as viewed for the detection of auto-fluorescence, taken with the Carl Zeiss LSM 780 confocal microscope. Images A and C were taken with the same filter at different magnifications as was images B and D. The brighter the blue (A and C) or green (B and D) in the image, the higher the level of auto-fluorscence. As expected auto-fluorescence was seen in the flavedo. Unexpectedly, localised auto-fluorscence was seen within the rind.

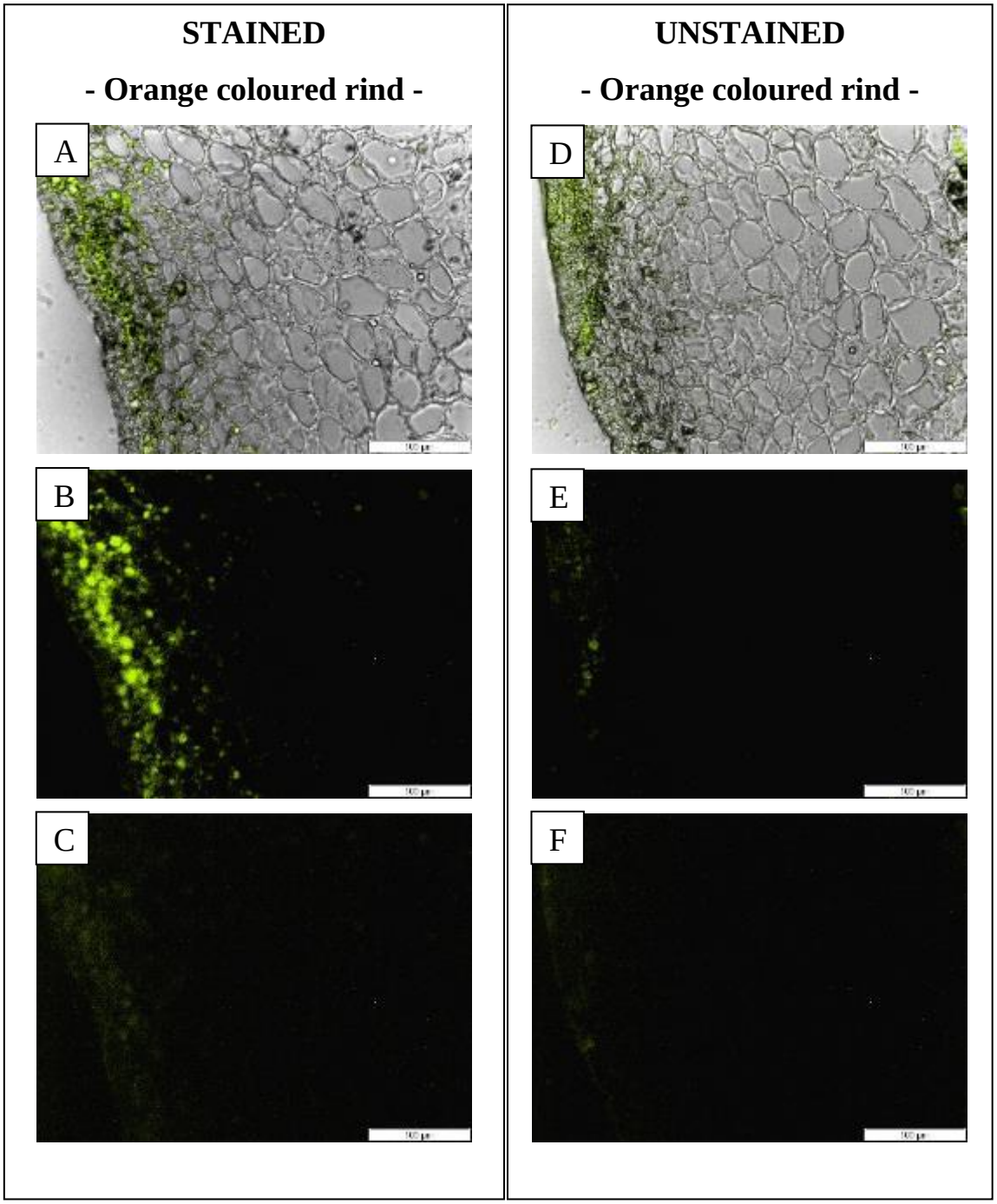

Figure 2. The Ca fluorescent dye stained (fura-2, pentapotassium salt) 'Nadorcott' mandarin rind sections: Fura340 with CFP filter (A and B) and Fura340 with UBG filter (C) using the Olympus IX81 widefield microscope. The unstained control 'Nadorcott' mandarin rind sections: Fura340 with CFP filter (D and E) and Fura340 with UBG filter (F) using the Olympus IX81 widefield microscope. A comparison of images A and B with images D and E indicate some degree of successful staining ability of the Fura 2 dyes. Especially when B (stained) is compared with E (unstained) the bright areas indicate where fluorescence was detected successfully.

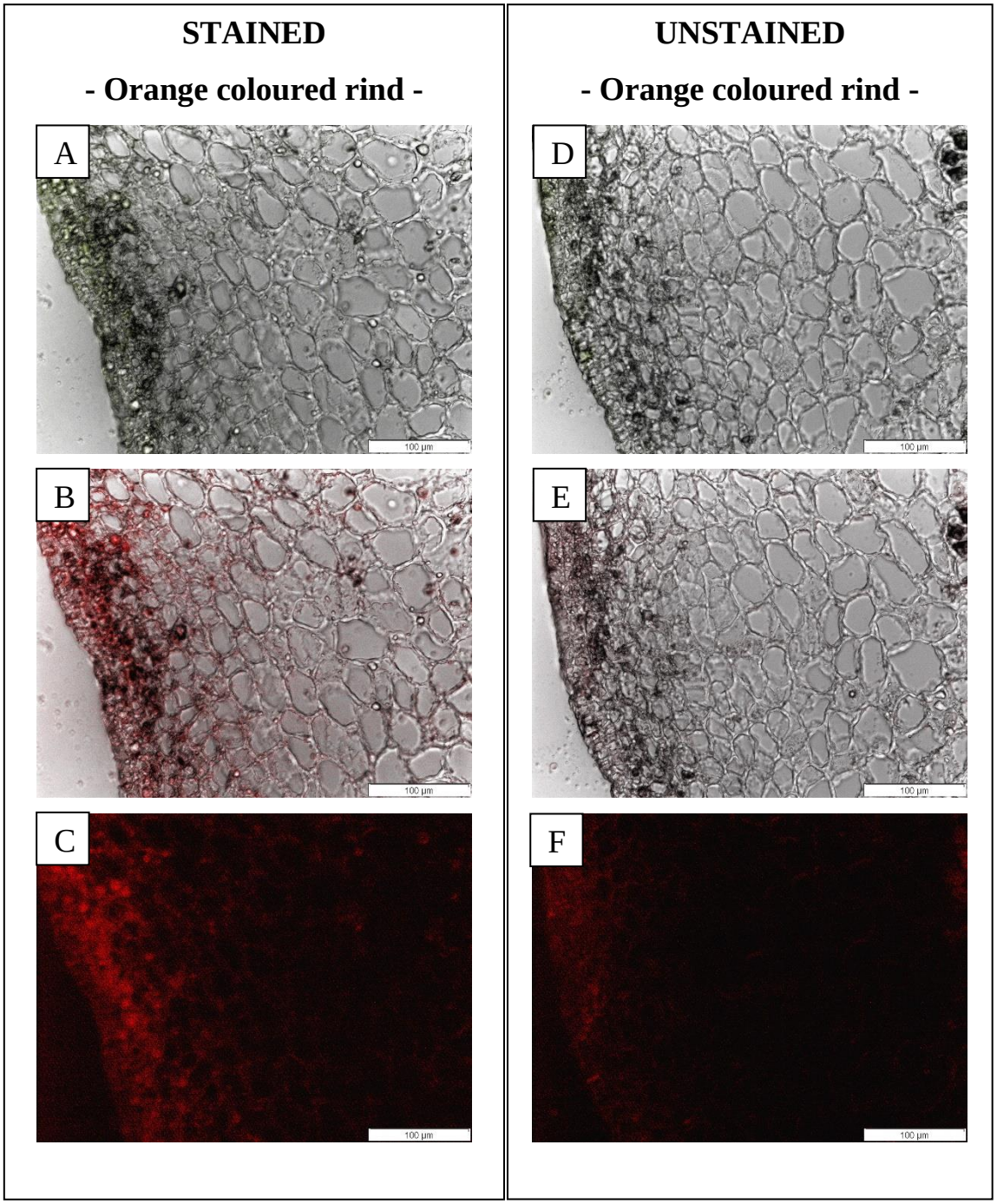

Figure 3. The Ca fluorescent dye stained (fura-2, pentapotassium salt) 'Nadorcott' mandarin rind sections: Fura340 with UBG filter (A) and Fura380 with CFP filter (B and C) using the Olympus IX81 widefield microscope. The unstained control 'Nadorcott' mandarin rind sections Fura340 with UBG filter (D) and Fura380 with CFP filter (E and F) using the Olympus IX81 widefield microscope. In these images, slight differences were found between the stained and unstained rind sections, but not to the same extent as was observed in Fig. 2.

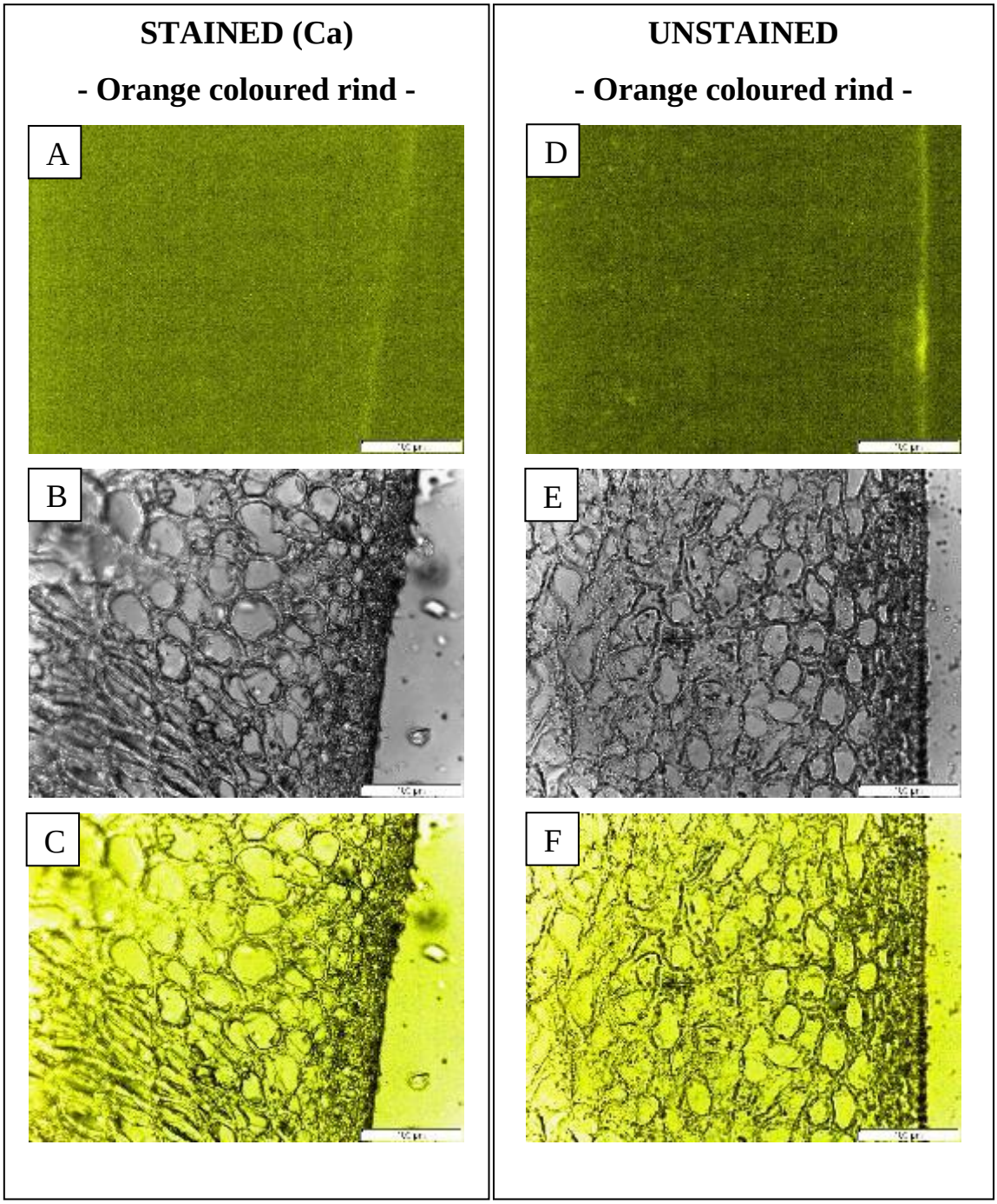

Figure 4. The Ca fluorescent dye stained (fura-2, pentapotassium salt) 'Nadorcott' mandarin rind sections using the Olympus IX81 widefield microscope (A, B and C). Control 'Nadorcott' mandarin rind sections using the Olympus IX81 widefield microscope (D, E and F). These are subsequent images taken using the same protocol but no differences between stained and unstained sections as was observed in Fig. 2 can be detected here.

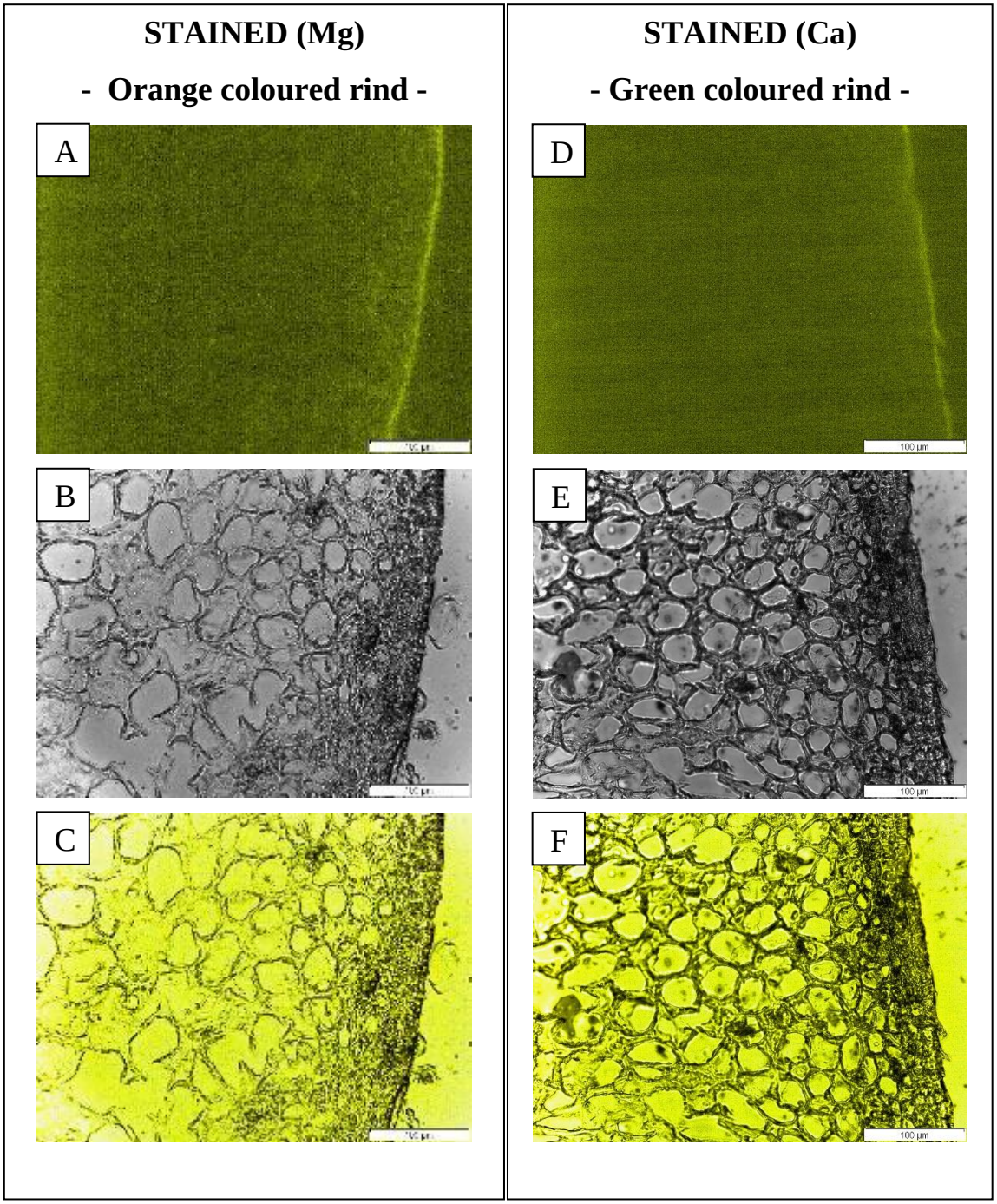

Figure 5. The Mg fluorescent dye stained (mag-fura-2, tetrapotassium salt) 'Nadorcott' mandarin rind sections using the Olympus IX81 widefield microscope (A, B and C). The Ca fluorescent dye stained (fura-2, pentapotassium salt) 'Nadorcott' mandarin rind sections using the Olympus IX81 widefield microscope (D, E and F). These are subsequent images taken using the same protocol but no differences between stained and unstained sections can be seen.

Chapter 7: General discussion and conclusion

Most of the prominent *Citrus* fertilisation studies on fruit quality was conducted in Florida, USA during the 1950s and 1960s. However, these studies could not consistently illustrate a clear pattern showing the influence of the various mineral nutrients on *Citrus* fruit quality (Quaggio et al., 2006). Certain effects of mineral elements on *Citrus* fruit quality is widely accepted, but in literature many discrepancies exist, possibly due to the interaction of the many factors directly impacting on *Citrus* fruit quality through fertilisation, with an additional range of factors that may indirectly affect fruit quality. Experimental results in this thesis reaffirmed the complexity of mineral nutrition as applicable to citriculture.

In the first experiment, mono-ammonium phosphate (MAP) foliar sprays that was applied at seven and eight weeks after full bloom showed potential in reducing the acid content of 'Nadorcott' mandarin fruit without affecting the external fruit quality. This experiment was another example of the importance of an appropriate application date with respect to a specific phenological stage to ensure the efficacy of treatments (Lovatt, 2009). If a cost-benefit analysis were to be done the late application of MAP might only be an advantage in a suspected 'on' year in alternate bearing prone cultivars. During an 'on' year the fruit are smaller and more acidic, both conditions would lead to a delayed harvest date. Such a delay in harvest could exacerbate the problem of alternate bearing. In production areas with colder winters, the lower temperatures lower the fruit respiration rate and therefore cause a decline in the breakdown of citric acid, which leads to a delay in fruit maturity and harvest. Therefore, the main benefit of MAP sprays would be when applied in production areas with colder winters during an 'on' season, which concur with a study by Quaggio et al. (2006) which reported that phosphorous decreases acidity more in 'on' years than 'off' years. This intervention, however, should be seen more as a curative measure than a preventative one. Specific preventative methods to avoid the occurrence of alternate bearing should be investigated for implementation by producers. One such option was documented by Stander and Cronjé (2016) where the use of hand thinning showed promising results to reduce the impact of alternate bearing, along with the subsequent reduction of the internal fruit quality.

The second and third experiments were corresponding studies as the one study aimed to investigate the interaction between irrigation and later applications of nitrogen (N) on mandarin fruit quality, whilst the other study only quantified the effect of the later applied N on fruit quality. The second experiment, conducted in Citrusdal, reported that the combination of the two factors, namely irrigation and additional late N application, had an impact on internal mandarin fruit quality, where

over-irrigation as main effect was mostly responsible for internal and rind quality being negatively affected. Late N application in combination with high and low irrigation regimes influenced fruit quality to a much larger extent compared to N fertilisation alone. Therefore, irrigation could be the main determinant when managing N fertilisation. The third experiment was conducted over one season in two production areas with different climatic conditions for example Citrusdal (a mediterranean area with predominantly winter rainfall) and Nelspruit (a subtropical area with summer rainfall). The data indicated that the application of additional N as soil applied fertiliser or a foliar urea spray at a later stage during fruit development did not affect mandarin internal or external fruit quality negatively. This contrasts with expectations as N is generally accepted in the citriculture community to reduce colour development. Commercial orchard practises however differed in all three experimental orchards, even though two of these sites were conducted in a similar same climatic area. For example, management practices in the first orchard where the effects of the combination of N and irrigation was studied was not ideal for experimental purposes as no measures were taken to control alternate bearing which unavoidably led to the significant impact of factors on mandarin fruit quality, other than that of the experimental treatments. This interference could thus partly explain differences in experimental results between sites.

The three experiments conducted in this study show the complexity of the interaction of horticultural practises with tree physiology on a commercial orchard level. In the first experiment, the phenomenon of alternate bearing influenced the efficacy of treatments between seasons. In the second experiment, it was clear that irrigation, or more specifically over-irrigation, could have a far greater negative impact on mandarin fruit quality (internal and external) compared to additional and late N. The interaction of these two factors did however seemed to determine whether N would be influential as seen by its impact of mandarin fruit quality. From the third experiment, it could be concluded that the impact of N over the long term could be of greater importance than a short-term fruit quality focus. Furthermore, it can be deduced that mineral nutrition may affect cultivars differently. The majority of earlier research on mineral nutrition on *Citrus* was conducted on orange cultivars, with no such studies reported on any of the new late mandarin cultivars such as ‘Nadorcott’. Genetic differences could influence the response to certain treatments either directly or indirectly. The main concern regarding late N fertilisation currently held in citriculture is on the possible detrimental impact on rind colour development. There are direct genetic differences between these late mandarin and orange cultivars. This may affect pigment composition in the rind or have an indirect influence on harvest date that could explain the lack of any negative effect on the rind colour of ‘Nadorcott’ mandarin by additional and late foliar or soil applied N.

To conclude this study confirms that the influence of mineral nutrition on *Citrus* fruit quality as a perennial crop is complex due to the large number of factors influencing fruit quality. It is near impossible to investigate all possible influential factors or control them. The impact of the tree physiology as seen in alternate bearing however can be quantified, whereas a factor such as soil characteristics is more complex and difficult to measure, however it can still have a significant impact on the final effect of soil-applied nutrients on fruit quality. This concept precisely illustrates the difference when interpreting results from the first experiment compared to that of the second and third experiments.

Recommendation resulting from this study may include the use of MAP sprays at seven and/or eight weeks after full bloom in suspected 'on' years in production areas with cold winters to achieve an earlier harvest date. The trials regarding the late application of N should be expanded to ascertain whether the application thereof has truly beneficial effects when compared to the cost of application. In a situation where the orchard in question has an N deficiency, the application of additional late N could be considered, as it apparently holds no negatively impact on fruit rind colouration or other quality aspects, unless irrigation is not managed well. The combination of irrigation and N seems to have a much larger effect on *Citrus* fruit quality than N alone and should be considered holistically to adapt irrigation and/or N management practices. Furthermore, the differences in winter and summer rainfall areas should be further investigated to include soil water content and rainfall.

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Appendix A

Chapter 3

Calculations

- *Trees per hectare*
    - Planting distance: 5 m x 2 m
      $\frac{10\ 000}{5\ \times 2} = 1000\ trees\ per\ ha$

- *Approximately 2.25 L sprayed per tree or 2250 L per ha*

- *Calcium arsenate (Ca-As) ($Ca_3(AsO_4)_2$)*
    - 855 $g.kg^{-1}$ Ca-As

- *Mono-ammonium phosphate (MAP) ($NH_4H_2PO_4$)*
    - 120 $g.kg^{-1}$ N
    - 260 $g.kg^{-1}$ P

- *Potassium nitrate (KNO_3)*
    - 135 $g.kg^{-1}$ N
    - 384 $g.kg^{-1}$ K

- *Calcium arsenate (Ca-As) per tree:*

  2015

  - 0.05% Ca-As [5.0 g.10L^{-1} = 1.125 g per tree]:

    $$1.125 \times \frac{855}{1000} = 0.961875\ g \approx 1.0\ g\ Ca - As\ per\ tree$$

  2016

  - 1% Ca-As [10.0 g.10L^{-1} = 2.25 g per tree]

    $$2.25 \times \frac{855}{1000} = 1.92375\ g \approx 1.9\ g\ Ca - As\ per\ tree$$

- *Mono-ammonium phosphate (MAP) per tree:*

  2015

  - 0.8% MAP [80 g.10L^{-1} = 18 g per tree]

    $$18 \times \frac{260}{1000} = 4.68\ g \approx 4.7\ g\ P\ per\ tree$$

    $$18 \times \frac{120}{1000} = 2.16\ g \approx 2.2\ g\ N\ per\ tree$$

  - 0.8% x 2 MAP [80 g.10L^{-1} = 18 g per tree]

    $$4.68\ x\ 2 = 9.36\ g \approx 9.4\ g\ P\ per\ tree$$

    $$2.16\ x\ 2 = 4.32\ g \approx 4.3\ g\ N\ per\ tree$$

  2016

  - 2% MAP [200 g.10L^{-1} = 45.0 g per tree]

    $$45 \times \frac{260}{1000} = 11.7\ g\ P\ per\ tree$$

    $$45 \times \frac{120}{1000} = 5.4\ g\ N\ per\ tree$$

- 1.5% x 2 MAP [150 g.10L^{-1} = 33.75 g per tree] – see 2015/2016

  $8.775\ x\ 2 = 17.55\ g\ \approx 17.6\ g\ P\ per\ tree$

  $4.05\ x\ 2 = 8.1\ g\ N\ per\ tree$

- 2% x 2 MAP [200 g.10L^{-1} = 45.0 g per tree]

  $11.7\ x\ 2 = 23.4\ g\ \approx 179\ g\ P\ per\ tree$

  $5.4\ x\ 2 = 10.8\ g\ \approx 179\ g\ N\ per\ tree$

2015/2016

- 1.5% MAP [150 g.10L^{-1} = 33.75 g per tree]

  $33.75\ \times \frac{260}{1000} = 8.775\ g\ \approx 8.8\ g\ P\ per\ tree$

  $33.75\ \times \frac{120}{1000} = 4.05\ g\ \approx 4.1\ g\ N\ per\ tree$

- 1.0% MAP [100 g.10L^{-1} = 22.5 g per tree]

  $22.5\ \times \frac{260}{1000} = 5.85\ g\ \approx 5.9\ g\ P\ per\ tree$

  $22.5\ \times \frac{120}{1000} = 2.7\ g\ N\ per\ tree$

- *Potassium nitrate per tree (KNO_3):*

  2015

  - 2.0% KNO_3 [200 g.10L^{-1} = 45.0 g per tree]

    $$45.0 \times \frac{384}{1000} = 17.28\ g \approx 17.2\ g\ K\ per\ tree$$

    $$45.0 \times \frac{135}{1000} = 6.075\ g \approx 6.1\ g\ N\ per\ tree$$

  2016

  - 4.0% KNO_3 [400 g.10L^{-1} = 90.0 g per tree]

    $$90.0 \times \frac{384}{1000} = 34.56\ g \approx 34.6\ g\ K\ per\ tree$$

    $$90.0 \times \frac{135}{1000} = 12.15\ g \approx 12.2\ g\ N\ per\ tree$$

Chapter 4

Calculations

- *Trees per hectare*
    - Planting distance: 5 m x 2 m

$$\frac{10\,000}{5 \times 2} = 1000\ trees\ per\ ha$$

- *Nitrogen per tree: LAN treatments [LAN (28): 280 g.kg^{-1} N]*
    - 2015: Nitrogen per hectare: 50 kg

$$\frac{50\ kg}{1000} = 50\ g\ N\ per\ tree$$

$$50 \times \frac{100}{28} = 178.57\ g \approx 179\ g\ LAN\ per\ tree$$

    - 2016: Nitrogen per hectare: 100 kg

$$\frac{100\ kg}{1000} = 100\ g\ N\ per\ tree$$

$$100 \times \frac{100}{28} = 357.14\ g \approx 357\ g\ LAN\ per\ tree$$

Irrigation

Table 1. Drippers per treatment, decreased irrigation (0.5X), standard irrigation (X) and increased irrigation (2X) in 2015 and 2016, and the litres (L) delivered per hour.

Treatment	2015		2016	
	Drippers	$L.h^{-1}$ delivery	Drippers	$L.h^{-1}$ delivery
0.5X	1 x 2.3 $L.h^{-1}$	2.3	2 X 1.6 $L.h^{-1}$	3.2
X	2 x 2.3 $L.h^{-1}$	4.6	4 X 1.6 $L.h^{-1}$	6.4
2X	2 x 2.3 $L.h^{-1}$ 1 x 1.6 $L.h^{-1}$	6.2	6 X 1.6 $L.h^{-1}$	9.6

Table 2. Total delivery received for each treatment, decreased irrigation (0.5X), standard irrigation (X) and increased irrigation (2X), per tree per year in litres (L) and cubic meter (m^3) and the equivalent per hectare (ha), based on the planting distance.

	$L.tree^{-1}.year^{-1}$	$m^3.tree^{-1}.year^{-1}$	$L.ha^{-1}.year^{-1}$	$m^3.ha^{-1}.year^{-1}$
2015				
0.5X	2271.74	2.27	2 271 743	2271.74
X	4543.49	4.54	4 543 486	4543.49
2X	6123.83	6.12	6 123 829	6123.83
2016				
0.5X	3173.49	3.17	3 173 486	3173.49
X	6346.97	6.35	6 346 971	6346.97
2X	9520.46	9.52	9 520 457	9520.46

Table 3. Irrigation water received in litre per day (L.day^{-1}) and litre per month (L.mo^{-1})_ for each month and treatment, decreased irrigation (0.5X), standard irrigation (X) and increased irrigation (2X), in the 2015 and 2016 season, determined using the irrigation scheduling hours per day and times per week.

			2015						2016					
			0.5X		X		2X		0.5X		X		2X	
Month of the year	h.day^{-1} X week	h.day^{-1}	L.day^{-1}	L.mo^{-1}	L.day^{-1}	L.mo^{-1}	L.day^{-1}	L.mo^{-1}	L.day^{-1}	L.mo^{-1}	L.day^{-1}	L.mo^{-1}	L.day^{-1}	L.mo^{-1}
Jan.	4 X 7	4	9.2	285.2	18.4	570.4	24.8	768.8	12.8	396.8	25.6	793.6	38.4	1190.4
Feb.	4 X 7	4	9.2	257.6	18.4	515.2	24.8	694.4	12.8	371.2	25.6	742.4	38.4	1113.6
Mar.	4 X 7	4	9.2	285.2	18.4	570.4	24.8	768.8	12.8	396.8	25.6	793.6	38.4	1190.4
Apr.	4 X 7	4	9.2	276	18.4	552	24.8	744	12.8	384	25.6	768	38.4	1152
May/Jun.	None	0	0	0	0	0	0	0	0	0	0	0	0	0
Jul.	2 X 2	0.6	1.3	40.7	2.6	81.5	3.5	109.8	1.8	56.7	3.7	113.4	5.5	170.0
Aug.	2 X 2	2	4.6	142.6	9.2	285.2	12.4	384.4	6.4	198.4	12.8	396.8	19.2	595.2
Sept.	2 X 2	2	4.6	138	9.2	276	12.4	372	6.4	192	12.8	384	19.2	576
Oct.	4 X 2	4	9.2	285.2	18.4	570.4	24.8	768.8	12.8	396.8	25.6	793.6	38.4	1190.4
Nov.	4 X 2	4	9.2	276	18.4	552	24.8	744	12.8	384	25.6	768	38.4	1152
Dec.	4 X 2	4	9.2	285.2	18.4	570.4	24.8	768.8	12.8	396.8	25.6	793.6	38.4	1190.4

Chapter 5

Calculations

Citrusdal

- *Trees per hectare*
    - Planting distance: 5 m x 2 m

$$\frac{10\,000}{5 \times 2} = 1000\ trees\ per\ ha$$

- *Nitrogen per tree: LAN treatments [LAN (28): 280 g.kg^{-1} N]*
    - Treatment 1: Nitrogen per hectare (LAN 1): 50 kg

$$\frac{50\ kg}{1000} = 50\ g\ N\ per\ tree$$

$$50 \times \frac{100}{28} = 178.57\ g \approx 179\ g\ LAN\ per\ tree$$

    - Treatment 2: Nitrogen per hectare (LAN 2): 100 kg

$$\frac{100\ kg}{1000} = 100\ g\ N\ per\ tree$$

$$100 \times \frac{100}{28} = 357.14\ g \approx 357\ g\ LAN\ per\ tree$$

- *Nitrogen per tree: Urea treatment [Urea (46): 460 g.kg^{-1} N]*
    - Treatment 3: 1% Urea treatment (100 g per 10 L)

Litres sprayed per tree: 4.8 L

$$\frac{4.8}{10} = 48\ g\ urea\ per\ tree$$

$$48 \times \frac{46}{100} = 22.08\ g \approx 22\ g\ N\ per\ tree$$

Nelspruit

- *Trees per hectare*
    - Planting distance: 5 m x 2 m

$$\frac{10\ 000}{5\ \times 2} = 1000\ trees\ per\ ha$$

- *Nitrogen per tree: LAN treatments [LAN (28): 280 g.kg^{-1} N]*
    - Treatment 1: Nitrogen per hectare (LAN 1): 50 kg

$$\frac{50\ kg}{1000} = 50\ g\ N\ per\ tree$$

$$50\ \times \frac{100}{28} = 178.57\ g\ \approx 179\ g\ LAN\ per\ tree$$

    - Treatment 2: Nitrogen per hectare (LAN 2): 100 kg

$$\frac{100\ kg}{1000} = 100\ g\ N\ per\ tree$$

$$100\ \times \frac{100}{28} = 357.14\ g\ \approx 357\ g\ LAN\ per\ tree$$

- *Nitrogen per tree: Urea treatment [Urea (46): 460 g.kg^{-1} N]*
    - Treatment 3: 1% Urea treatment (100 g per 10 L)

Litres sprayed per tree: 3.6 L

$$\frac{3.6}{10} = 36\ g\ urea\ per\ tree$$

$$36\ \times \frac{46}{100} = 16.56\ g\ \approx 17\ g\ N\ per\ tree$$