

**A PRELIMINARY STUDY ON THE EFFECT OF CLIMATIC CONDITIONS AND
FRUIT MINERAL CONCENTRATION ON THE DEVELOPMENT OF LENTICEL
DAMAGE IN 'TOMMY ATKINS' AND 'KEITT' MANGOS (*MANGIFERA INDICA*
L.) AND RIND PITTING IN 'BENNY VALENCIA' ORANGES (*CITRUS*
SINENSIS)**

By

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DECLARATION

I hereby declare that the research work reported in this thesis is as a result of my own investigation, except where acknowledged.

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ABSTRACT

The South African fresh fruit industry is a significant exporter, accounting for approximately 45% of the country's agricultural exports. Of the total exported fruit in the 2005/06 season, 60% was subtropical fruit. However, certain physiological rind disorders such as mango lenticel damage and citrus rind pitting are frequently observed, reducing the commercial value of the fruit. This thesis deals with the epidemiology of these rind physiological disorders, in an attempt to obtain basic information that could serve as a guideline to predict and manage the fruit susceptibility to these disorders. The study further investigated the relative effects that certain pre-harvest factors have on the postharvest development of these disorders. Factors of particular interest were harvest maturity, climate and the mineral content of the fruit.

Rind pitting is a physiological disorder of citrus that develops during storage. A study was conducted to investigate the relative effects that certain pre-harvest factors have on the post-harvest development of superficial rind pitting in 'Benny Valencia' oranges. Factors of particular interest were harvest maturity, climate and the mineral content of the fruit. In addition, trees were treated with two different formulations of nitrogen viz. limestone ammonium nitrate (LAN) and a slow release nitrogen fertilizer (Hortecote[®]), during March 2006. The fruit were then sampled on a two-weekly basis over a period of three months. On each sampling date a set of quality related readings, such as juice sugar and titratable acid concentration were taken, after which the mineral concentration of the exocarp and mesocarp was measured. Fruit were also stored under export simulation conditions.

The results indicate that fruit from trees that received additional N were more susceptible to rind pitting than those from control trees. Of the two N applications, fruit from trees that received slow release N were more susceptible to rind pitting than fruit from trees that received the LAN treatment. Another important observation made was that the nitrogen concentration of oranges from trees that received extra nitrogen fertilizer was lower than that from the controls. In addition, experimental fruit

were smaller than control fruit. Fruit from trees that received the slow release nitrogen treatment were smallest. A sink/source hypothesis aimed at explaining this phenomenon has been formulated and is currently being investigated. A number of control mechanisms are also being explored.

Mango lenticel damage is a serious defect that occurs on the rind of the fruit. This condition causes fruit to attain a speckled appearance and become unattractive to the buyer. Each season, the disorder reduces the packout of fresh fruit by about 16%. Several preharvest factors play a critical role in the postharvest development of lenticel damage. Preliminary studies have shown that the incidence of lenticel damage has some relationship to fruit moisture and fruit nutrient concentration, depending on which of these factors first achieve a critical threshold.

A study was conducted to develop appropriate skin moisture content parameters to predict lenticel damage potential before harvest. The study further aimed to provide certain biological markers regarding appropriate nitrogen fertilization practices to reduce lenticel damage. To do this, two trials were conducted approximately a month before harvest. Firstly, a plastic ground cover to restrict water supply was laid under 'Tommy Atkins' trees at Numbi Estates in the Hazyview area of the Mpumalanga province, South Africa. Secondly, additional nitrogen was applied as three different formulations, viz. limestone ammonium nitrate (LAN), potassium nitrate (KNO_3), and a slow release nitrogen fertilizer under trade name Horticote®, to two 'Tommy Atkins' and two 'Keitt' orchards at Bavaria Estates in the Hoedspruit area of the Limpopo province, South Africa. Fruit samples were harvested, packed and stored at different intervals after rain during January 2006.

With regard to lenticel damage on 'Tommy Atkins' fruit from Numbi, there was no significant difference between the control and plastic cover treatments. However, when compared to fruit from other localities, the Numbi fruit had the highest incidence of lenticel damage, followed by the conventional orchard at Bavaria. The Numbi fruit, which had the highest incidence of lenticel damage, also had the highest pulp and skin nitrogen concentration. Of the four treatments at Bavaria, the LAN

treatment had the highest incidence of lenticel damage, but the pulp and skin nitrogen concentrations of this treatment were comparable with the other treatments. The organic fruit had significantly lower lenticel damage incidence and also had the lowest pulp and rind nitrogen concentrations.

Similarly, 'Keitt' results showed that the intensity of lenticel damage was significantly higher in the orchard that received additional nitrogen in the form of LAN. From the results it was, however, not possible to formulate nitrogen-lenticel damage correlations. The study failed to prove the case for a direct relationship between the disorder and with nitrogen, as there were no significant or consistent correlations with nitrogen content. An interesting relationship was nevertheless observed between lenticel damage and the time of harvest before and after rainfall. The results indicated that both 'Tommy Atkins' and 'Keitt' fruit become more susceptible to lenticel damage when harvested a day after rainfall and this gradually reduces afterwards. The effect of rainfall in this regard and a sink-source hypothesis arising from these observations are also discussed.

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GENERAL INTRODUCTION

The South African fresh fruit industry is a significant contributor to the agricultural export industry, accounting for approximately 45% of the country's agricultural exports (Department of Science and Technology, 2006). Of the total exported fruit in the 2005/06 season, 60% was subtropical (57% citrus, 1.9% avocados, 0.1% litchis and 0.1% mangos) (PPECB/FPEF, 2006). From these statistics, it is clear that citrus is the biggest component of South African exported subtropical fruit. Globally, the South African citrus industry is ranked the second largest exporter of citrus after Spain (South African Citrus Growers Association, 2008). The country's citrus export industry is worth R3.2 billion a year (PPECB/FPEF, 2006). The position of the industry in the international markets is strengthened due to the production of a wide range of cultivars over an extended period of time - from March through to November (Department of Science and Technology, 2006). However, physiological rind disorders such as rind breakdown are frequently observed, reducing the commercial value of the fruit. This thesis deals with two of the important rind physiological disorders of citrus (rind pitting) mango (lenticel damage).

Compared with the South African citrus industry the mango industry is a small industry, constituting only 0.1% of the total exported subtropical fruit. In recent years mango exports have declined, in part due to poor quality especially pathological problems. There are also some physiological disorders such as internal breakdown, jelly seed and lenticel damage. Mango lenticel damage is a serious defect that occurs on the rind of mango fruit. The condition causes fruit to attain a speckled appearance and become unattractive to the buyer. Each season, the disorder reduces the packout of fresh fruit by about 16% (Kaiser *et al.*, 2004). Similarly to mango lenticel damage, citrus postharvest rind disorders such as rind pitting and peteca are responsible for heavy crop losses. In some seasons, as much as 60% of export 'Benny Valencia' fruit is rejected for rind pitting (Piner, 2006, pers. comm.).

Citrus fruit are relatively non-perishable, and can therefore be stored for fairly long periods of up to 8 weeks (Kader, 2002; Porat *et al.*, 2004). However, the development of various types of rind disorders limits the postharvest storage capability and causes commercial losses (Kruger *et al.*, 2003a; 2005). Based on the general causative factors citrus rind disorders can be classified into two main groups, namely chilling injury (or chilling related injuries) and non-chilling disorders. Chilling injury is defined as “the permanent or irreversible physiological damage to plant tissues, cells, or organs, which results from exposure of plants to temperatures below some critical threshold temperature that causes injury” (Lyons and Breidenbach, 1987). Symptoms in response to chilling damage are most apparent on the peel of citrus fruit, in various forms such as browning of the flavedo and albedo or appearance of dark sunken areas of collapsed tissues (Porat, 2003; Porat *et al.*, 2004).

Rind disorders not related to chilling develop during storage at non-chilling temperatures (Kader and Arpaia, 2002). Rind pitting falls within this category of citrus rind disorders. Over the years, it has been suggested by different researchers that the development of rind pitting and other rind disorders not related to chilling may be enhanced by increased water loss from the peel tissue (Albrigo, 1972; Vercher *et al.*, 1994). Water loss in fruit is believed to occur as the result of a water pressure gradient that prevails between the fruit, which is normally close to saturation within intercellular spaces, and the less saturated surrounding ambient environment (Kader *et al.*, 1989; Kader, 1992). From these observations, a number of investigators have studied different postharvest treatments that could possibly reduce development of rind pitting by reducing water loss. The use of different waxes and, to a greater extent, the use of modified atmosphere packaging (MAP) proved to be successful. MAP maintains a high humidity environment and may thus eliminate the development of rind disorders caused by water loss (El-Otmani and Ait-Oubahou, 1996).

Despite the evidence that postharvest treatments are capable of reducing the rind pitting disorder, it should be noted that the measures introduced to decrease moisture loss may cause anaerobic conditions that promote the development of

off-flavours. Inappropriate MAP may further promote the development of rind disorders upon removal of the fruit from the MAP system. This serves to demonstrate that the problem is still present but kept under control by the MAP system. It is therefore important to study the epidemiology of the disorder, so as to determine and control its underlying causes. Over the past six years, the Postharvest Section of the ARC-ITSC has conducted a project aimed at studying the epidemiology of rind disorders of subtropical fruit. There are various factors playing roles in physiological disorder amongst which are nutrition, harvest maturity, climate and horticultural practices such as irrigation. Previous experience in preharvest factors affecting physiological disorders has taught us that mineral elements are important in the development of physiological disorders. Therefore, one of the most important investigative techniques involved monitoring the N, P, K, Ca, Mg, Zn, Cu, Mn, Fe and B content of the skin and pulp of different crops during the maturation process. Other potential causative factors investigated were harvest maturity, climate and horticultural practices such as irrigation. The first crop to be investigated in this regard was avocado, after the industry launched an intensive investigation into the problem of poor storage potential (Kruger *et al.* 2000; 2002; 2004). As a consequence of positive results reported in avocados (Kruger *et al.*, 2004), the approach was extended to mango and citrus fruit. Preliminary results on citrus showed some interesting trends, in which rind mineral concentration (nitrogen and iron in particular) was observed to be correlated to the incidence of rind pitting. The results obtained for 'Valencia' oranges have shown that fertilizing with excess nitrogen was related to rind pitting. This was also confirmed on 'Midnight' oranges. In spite of the above, considerable superficial rind pitting still occurs each year in the correctly fertilized 'Valencia' oranges, especially in the 'Benny Valencia' cultivar (Piner, 2006, pers. comm.). In so far as mango lenticel damage is concerned, increased levels of immobile elements, including Ca, Mg, Mn, and B, were associated with increased lenticel damage. This increased level of mineral uptake was hypothesised to take place during good rains just before or during harvest. The occurrence of physiological disorders in correctly fertilized orchards implies that a proportion of disorder development can be ascribed to climatic conditions - particularly those which influence the translocation of the elements.

In this study, the epidemiology of rind pitting in 'Benny Valencia' citrus and lenticel discolouration in 'Tommy Atkins' and 'Keitt' mangos was investigated. Further, application of nitrogen towards the end of the season was investigated to provide baseline information that may serve as a guideline to predict and manage the fruit susceptibility to these disorders. In addition, the possibility of a relationship between fruit mineral content and the risk of developing lenticel damage or rind pitting disorders during subsequent storage was investigated, so that corrective measures can be implemented. Rectifying these problems would contribute towards improving the quality of export mango and citrus fruit.

CHAPTER 1

LITERATURE REVIEW

The final quality and quantity of harvested fruit is the result of fruit set, growth, development, maturation and ripening. The period from anthesis to physiological maturity is the most important physiological phase in fruit tree productivity (Childers, 1983). However, inappropriate postharvest storage and handling practices are often the final compounding factor resulting in poor quality of exported fruit. The moment of harvest is the beginning of the postharvest life of the fruit, which brings about a series of co-ordinated anabolic and catabolic events. These events result in weight (water and carbohydrate) loss, which have some negative implications for the fruit since neither water nor carbohydrates can be replaced as they would if the fruit was still attached to the tree (Turner, 1997).

FRUIT GROWTH AND DEVELOPMENT

Fruit growth consists of a sum of growth processes, which are usually used to determine the seasonal rates of growth as well as the quality of the fruit at harvest. Mango and citrus fruit growth follows a sigmoidal pattern with three distinct growth phases (Bain, 1958; Button, 1969; Davenport and Nunez-Elisea, 1997). The first phase (Phase I) is characterised by cell division and extends to the formation of the characteristic tissues of the fruit or until the end of physiological fruit drop. Phase II is the phase of cell elongation and enlargement, and is characterised by linear fruit growth and rapid changes of the morphological and anatomical characteristics of the fruit. Phase III is the maturation phase. During this phase, morphological changes decrease, although the fruit continues to grow as long as it remains on the tree (Bain, 1958; Button, 1969). Among the changes that occur during the maturation period, development of final rind colour, increase in total soluble solids (TSS) and the decrease in titratable acidity (TA) of juice are the most important (Ortuzar, 1996).

Photoassimilate influence on fruit growth

The final quality of fruit is largely influenced by seasonal fluctuations of external and internal factors, which produce various normal life cycle changes. A number of studies have indicated that yield and quality of fruit are most likely to be determined by the quantity of assimilates produced during photosynthesis (Marini and Barden, 1982; Kappel and Flore, 1984). Crop growth and productivity are directly related to the amount of carbon fixed during photosynthesis and the subsequent partitioning of carbohydrates to the various plant organs (Whiley and Schaffer, 1997). About 90-95% of the total dry weight of plants is derived from photosynthetically fixed carbon (Flore and Lakso, 1989). Depending on the stage of development, the energy required by the fruit originates from imported leaf assimilates (>90%) and less than 5% is derived from photosynthesis of the fruit (Blanke, 1996).

Carbon partitioning

Partitioning of assimilates is clearly influenced by both, the supply and demand of these assimilates by different plant organs, which is moderated by vascular connections, and the storage capacity of the leaves and pathway tissues (Wardlaw, 1992). The developing fruit acts as a strong sink for carbohydrates, and fruiting suppresses growth of other plant organs (Monselise and Goldschmidt, 1982; Goldschmidt, 1999). Since carbon assimilates are needed for nutrient acquisition and assimilation, it is apparent that any vascular constraints will affect the ability of the plant to transport and utilise distributed mineral nutrients (Bloedsoe and Orians, 2006).

Carbon transport and partitioning is largely determined by phloem source-sink relationships. Different investigators suggest that carbohydrate accumulation by the fruit is regulated at the level of the sink, and strong evidence exists that sink tissues exert an influence on the net photosynthetic rates and carbon status of source organs (Wardlaw, 1992; Etxeberria and Gonzalez, 2005). An investigation of carbon partitioning in pears suggests that an increase in the rate of

carbohydrate accumulation in fruit is responsible for an increase of fruit weight (Zhang *et al.*, 2005). This falls in line with the reasoning that photosynthate accumulation in fruit is limited by the sink strength of the fruit rather than by the transport capacity of vascular tissues.

FRUIT MATURATION

The final internal quality of fruit, which is a combination of sugars, acids, flavour, and texture, depends very much on fruit quality at harvest (Lakshminarayana, 1973; Lee and Young, 1983; Marmo *et al.*, 1985). A decision about the readiness, or stage of maturity, of the fruit for harvest is therefore a commercial one. Picking too early or too late may considerably limit fruit quality and storage potential (Brady, 1987).

Over the years there have been many studies on developing suitable and dependable maturity indices for harvesting (Kosiyachinda *et al.*, 1984; Pantastico *et al.*, 1984; Bradley and Scudamore-Smith, 1987). So far, knowledge on chemical changes in mango fruit that could be used for practical determination of maturity is insufficient. One of the major difficulties with fruit maturity studies is the strong heterogeneity in fruit physiological parameters, which culminates in uneven ripening of harvested fruit following storage (Mitra and Baldwin, 1997; Guthrie and Walsh, 1997). It is also evident that maturation and final quality of fruit varies according to variety and many other factors (Pantastico *et al.*, 1984). It is therefore important that potential maturity indices be highly correlated to fruit quality, and display minimal cultivar variability and maximum seasonal consistency. The assessment and subsequent correlation of such changes constitute the principal framework of fruit maturity standard research (Wills *et al.*, 1981).

As mango fruit mature they undergo a complex series of physiological changes, which dramatically alter their physical and biochemical properties (Wills *et al.*, 1981; White, 2002). However, it has not been possible to develop a reliable index for judging fruit maturity at harvest, as there are no visible external changes in

the fruit to indicate maturity (Oosthuysen, 1995). The accomplishment of the maturity stage in mango fruit is accompanied by changes in the accumulation of dry matter (Bradley and Scudamore-Smith, 1987; Kruger *et al.*, 2003b). Final quality of mango fruit is related to dry matter (DM) content - an indicator of starch content (Bradley and Scudamore-Smith, 1987). Kruger *et al.* (2003b) and Kruger and Magwaza (2006) showed that as mango fruit matures, the pulp moisture of mango fruit reduces from around 90% to about 80%. During the same time, the dry matter of the pulp increases from 10% to 20%. Similarly, the dry mass of the rind increases from 20% to 25%, while the moisture decreases from 80% to 75%. Based on these findings Kruger *et al.* (2003b) suggested pulp dry mass to be a more accurate maturity parameter than skin moisture.

Citrus growers use a combination of the increase in total soluble solids (TSS), decline in titratable acidity (TA) (i.e. the increase in sugar-acid ratio), and change in peel colour to specify fruit maturity (Davies and Albrigo, 1994). The stages of maturity of citrus fruit and ripening of mangos have been closely linked with the changes in peel colour (Palmer, 1971; Francis, 1980; Purvis and Barmore, 1981; Kato *et al.*, 2004). Loss of green colour in these fruit is a spectacular natural phenomenon, whereby the green fruit turns to yellow and orange. In citrus fruit, the natural colouring consists of two phases the degradation of chlorophyll and synthesis of carotenoids (Wheaton and Stewart, 1973). The yellowing of maturing and ripening citrus fruit is largely due to unmasking and partial retention, as well as synthesis of, carotenoids (Bartley and Scolnik, 1995; Rodrigo *et al.*, 2003; Kato *et al.*, 2004).

Carotenoids are a large group of lipid-soluble pigments mainly found embedded in the membranes of chloroplasts and chromoplasts. They are generally C-40 terpenoid compounds formed by the condensation of eight isoprene units (McGarvey and Croteau, 1995). In green fruit, their colour is masked by chlorophyll but in late stages of fruit development (maturation and ripening) these pigments contribute to the typical yellow colour of the fruit. In citrus, carotenoid composition and concentration varies greatly among cultivars (Gross, 1987; Goodner, *et al.*, 2001; Lee and Castle, 2001). Mandarin cultivars accumulate β -

cryptoxanthin, while mature sweet orange accumulates violaxanthin. The relative stability of the carotene compounds ensures that they remain intact in the tissue even when excessive senescence has occurred (Wills *et al.*, 1998). The activity of carotenogenic enzymes in the plastids during fruit pigmentation have neither been documented nor correlated with amounts and types of carotenoids in the fruit (Fraser *et al.*, 2004).

Although citrus maturation is associated with a decline in chlorophyll fluorescence (Matile *et al.*, 1999; Drury *et al.*, 1999), the temporal relationship between chlorophyll fluorescence and natural ethylene synthesis has not been described in detail (Goldschmidt *et al.*, 1993). Purvis and Barmore (1981) reported the rate of chlorophyll degradation of ethylene exposed 'Robinson' tangerines to be higher than that of untreated fruit. However, chlorophyll degradation in the peel of these fruit was only promoted by continuous exposure of the fruit to ethylene. Furthermore, an increase in chlorophyllase activity was also reported to be induced by continuous exposure of these fruit to ethylene. The level of chlorophyllase activity was negatively correlated with chlorophyll concentration of the tissue for all treatments and exposure times. From this study, the authors suggested the possibility that the presence of ethylene in rind tissue is an absolute requirement for chlorophyll degradation. The contribution of ethylene to the degreening of the citrus fruit flavedo was supported by Goldschmidt *et al.* (1993).

Temperature plays a critical role in the chlorophyll degradation and carotenoid biosynthesis processes. Both low and elevated temperatures have been reported to cause a marked reduction in both processes (Wheaton and Stewart, 1973; Seymour *et al.*, 1987; Hendry *et al.*, 1987; Kays, 1991). Degreening of most citrus fruit is induced by cool winter air and soil temperatures (Young and Erickson, 1961). Temperature acts indirectly in citrus fruit degreening by influencing cellular metabolism. For instance cool temperatures have been reported to reduce nitrogen uptake and translocation by citrus trees, and the pericarp of grapefruit ceases to accumulate nitrogen in winter (Wallace, 1953). In addition to influencing nitrogen metabolism, cool temperatures promote

accumulation of reducing sugars in the pericarp of grapefruit. High concentrations of reducing sugars, particularly sucrose, in the pericarp of citrus fruit usually promote degreening (Purvis and Grieson, 1982). In addition to temperature, the degreening of citrus fruit is affected by nitrogen fertilization, exogenously applied gibberellins, and certain internal factors such as cultivar, rootstocks, and number of seeds per fruit (El-zefrawi, 1977).

FRUIT RIPENING

Fruit ripening is an integrated natural and irreversible process, resulting in changes in colour, taste and texture which make the fruit acceptable for consumption (Baile, 1964; Brady, 1987; Bower and Cutting, 1988; Blanke, 1991; Marchal, 1998). It involves a series of co-ordinated anabolic and catabolic events, dominated by catabolic reactions that alter fruit anatomy, biochemistry, physiology, and gene expression (Brady, 1987; Seymour, 1993). The respiration rate is mostly used as a measure of the metabolic activity of a tissue. In fruit physiology, a high respiration rate is often associated with a short storage life. The degree to which internal reactions occur depends mostly upon external factors such as storage temperature and composition of the air surrounding the fruit (Kader, 1985; Brady, 1987). The ripening process of, particularly, climacteric fruit is highly contingent upon endogenous production and exogenous concentration of ethylene at the onset of ripening (Lelievre *et al.*, 1997).

Based on the ripening patterns and response to ethylene, fruit can be classified into two groups, climacteric and non-climacteric. In climacteric fruit, ripening is accompanied by a respiratory peak and concomitant burst of ethylene. However, the physiological role of the respiratory peak is still not fully understood (Seymour, 1993). As a result of the respiratory climacteric, most climacteric fruit tend to ripen rapidly, within 3 to 5 days after harvest depending on maturity (Dominguez and Vendrell, 1997; Dominguez *et al.*, 1998). As with other climacteric fruit, mango ripening is typified by having a climacteric rise in respiration. However, on a per weight basis the respiratory peak is relatively

small when compared with the respiration rate of the young fruit (Phan *et al.*, 1975; Blanke and Lenz, 1989).

The rise in respiration rate is linked to rapid hydrolysis of substrate reserves accumulated during fruit development (Seymour, 1993). The respiratory pathways utilised by fruit for the oxidation of sugars are those common to all plant tissues, namely glycolysis, the oxidative pentose phosphate pathway and the tricarboxylic acid cycle (Tucker, 1993). The composition of the fruit determines which substrates are utilised during respiration. In the case of mango the respiratory quotient (RQ), which is the ratio of CO₂ produced to O₂ consumed, is estimated at 1, denoting that the substrate during the respiratory climacteric is carbohydrates (Seymour, 1993; Areas and Lajolo, 1981). Citrus fruit are non-climacteric. These fruit display a gradual decline in respiration during ripening, and their ethylene production remains at very low levels (Baile and Young, 1981; McGlasson, 1985; Wills *et al.*, 1998). In climacteric fruit such as mango, the ethylene burst is required for normal fruit ripening (Oeller *et al.*, 1991; Theologis *et al.*, 1993). Although ethylene is not the dominant trigger for ripening non-climacteric fruit, it has been suggested that both ethylene-dependent and ethylene independent gene regulation pathways coexist to coordinate the ripening process of non-climacteric and climacteric fruit (Lelievre *et al.*, 1997). McMurchie *et al.* (1972) proposed the concept of two systems of ethylene regulation to operate in plants. System 1 is the ethylene production system common to both climacteric and non-climacteric fruits and is responsible for producing basal ethylene levels that are detected in all tissues, including those of non-climacteric fruit. System 2 is only functional in climacteric fruit and this system is induced by autocatalytic production of ethylene.

Though non-climacteric fruit, such as citrus, have only system 1 of ethylene production, with low levels of ethylene (Vendrell and Palomer, 1997), it does not imply that there is no ethylene interference in the ripening process of these fruit. Ethylene is indisputably involved in the induction of ripening in both, climacteric and non-climacteric fruit. Major processes associated with ethylene have spurred research methodologies for control of its synthesis and action.

RIND DISORDERS

Postharvest loss due to rind disorders is one of the most important problems affecting the South African fruit export industry. In subtropical crops, the wastage is so high that in some instances between 10 to 50% of export revenue is lost as a result of rind disorders that develop between the field and the consumer (Ben-Yehoshua, 1969; Kaiser *et al.*, 2004). Rind disorders can also lead to enhanced pathological disorders. Mango lenticel discolouration and citrus rind pitting are among the most important physiological rind disorders in subtropical horticulture. Lack of understanding of the physiology of these disorders affects both supply and profits. Intensive research aimed at eliminating these disorders has been conducted (Almela *et al.*, 1992; Kaiser *et al.*, 2004; Cronje, 2005a). However, these disorders still occur frequently and unpredictably. In order for the South African mango and citrus industries to compete with other countries producing high quality fruit, and to retain its share in the lucrative European markets, it is important to find methods of reducing the incidence of these disorders.

Mango Lenticel Damage

Lenticels are superficial macroscopic openings which occur naturally on the surface of several fruit types including pears, apples, avocados and mangoes (Dietz *et al.*, 1988; Fahn, 1974). Similar to stomatal openings, lenticels function as sites of gaseous exchange for photosynthesis, respiration and transpiration at different stages of fruit development (Maseuth, 1988). Although lenticels occur naturally as “breathing pores”, their excessive discoloration is an indicator of physiological stress (Batzli and Dawson, 1999).

Mango lenticels are hypothesized to develop from either preformed stomata or from epidermal shearing that takes place during rapid fruit growth (Dietz *et al.*, 1988; Curry, 2003). The first mechanism is supported by the discovery that a lenticel has two guard cells filled with corky tissue (Kupferman, 2005). The second mechanism is supported by the fact that the fruit cuticle is a dynamic, growing tissue system and as long as the fruit is enlarging, the cuticle, which is a

combination of wax and epidermal cells, is in a constant state of growth. As cells within the fruit enlarge, the cuticle must also expand to accommodate the enlargement, which forces the lenticel to expand (Wilson *et al.*, 1972; Curry, 2003). Under non-extreme environmental conditions this process occurs gradually. Before complete shearing and subsequent uncovering of the underlying cells takes place, a repair process begins to fill in openings that have formed due to stretching of the cuticle. Conversely, if the expansion occurs too rapidly, the cuticle and lenticel are forced to expand rapidly, rupturing vacuoles and exposing unprotected cells beneath the cuticle (Dietz *et al.*, 1988; Curry, 2003). As a result, the corky tissue in lenticels swells in an attempt to close “cracks” and lenticels therefore become pronounced. In the presence of polyphenol oxidases (PPO), the exposed cells darken due to the enzymatic oxidation of phenolic compounds (Loveys *et al.*, 1992; Robinson *et al.*, 1993). Joel *et al.* (1978) reported the presence of two types of PPO in mango fruit, one with activity of catechol oxidase-type (predominant in the skin) and the other a laccase-type enzyme (predominant in the sap). Robinson *et al.* (1993) reported that the browning of mango skin cells is predominantly catalysed by PPO present in the skin. In essence, lenticel discolouration appears to be related to microclimatic factors which directly influence fruit enlargement, particularly during the harvesting period. For example, if fruit are harvested during rapid fruit enlargement in a more turgid state, it is likely that the fruit might have a number of lenticels which would not have healed as yet. In the absence of conditions promoting rapid fruit growth, the number of darkened lenticels will be reduced since the cuticle would have been able to keep pace with cell expansion.

Citrus Rind Pitting

In citrus, physiological rind disorders, such as pitting, peteca, puffing and breakdown significantly reduce fruit quality. Peteca is a disorder of lemons that appears as deep depressions with round edges on the peel surface (Knorr, 1963; Cronje, 2005a) approximately 3 to 4 days after packing (Oberbacher and Knorr, 1965; Khalidy *et al.*, 1969; Offers, 1987). Rind breakdown occurs in “soft citrus”,

particularly clementine types, and is characterized by small, slightly sunken and discoloured patches scattered about the surface of the fruit (Gilfillan, 1990).

Postharvest rind pitting is a disorder of oranges, mandarins and grapefruit which consists of sunken areas of about 3 to 6mm in diameter on the flavedo that turn brown and dry in the severe stages of the disorder (Alferez and Burns, 2004). Sunken areas of flavedo that occur directly above and among the oil glands eventually spread and turn dark-brown and become necrotic (Gilfillan *et al.*, 1981; Almela *et al.*, 1992; Cronje, 2005a; Kruger *et al.*, 2005). Externally, this disorder may resemble the symptoms caused by postharvest chilling injury (Freeman, 1976; Arpaia *et al.*, 1991). However, rind pitting is distinguished from chilling injury in that the symptoms develop within the first 10 days of storage at non-chilling (ambient) temperatures (Petracek *et al.*, 1995). Since several inductive factors may lead to similar looking symptoms, the mechanism governing the occurrence of rind pitting and the manner it is related to other physiological rind disorders is not well understood (Agusti *et al.*, 2004).

In order to attempt to control a physiological disorder, it is essential to understand its aetiology. A healthy citrus flavedo section is characterised by an epidermis with an apolyhedral arrangement of cells, covered by a cuticle (Albrigo, 1972; Medeira *et al.*, 1999). Vercher *et al.* (1994) studied the cell orientation of pitted 'Fortune' mandarin. Internally, the damage caused by peel pitting is manifested by flattened and collapsed parenchyma cells immediately above the oil glands. Obeland *et al.* (1997) verified the collapse of parenchyma cells above oil glands. These authors are further of the opinion that this causes the oil bodies to rupture, which in turn could release oil into the surrounding cells, causing injury to citrus fruit.

Medeira *et al.* (1999) investigated the cell orientation of pitted 'Encore' mandarin fruit and found that the sub-epidermal cell alteration in affected areas resembles the damage described by Vercher *et al.* (1994) in 'Fortune' mandarin as well as the postharvest chilling injury symptoms of lemons described by Obeland *et al.* (1997). Using light microscopy Vercher *et al.* (1994) and Medeira *et al.*, (1999)

observed a similar cytoplasmic disorganization to correspond with the damage occurring in pitted areas of the peel. Damaged cell layers were found to be orientated parallel to the rind surface, extending all along the flavedo. The deterioration processes were found to begin in epidermal cells, later extending to hypodermal cells (Vercher *et al.*, 1994). According to Meideira *et al.* (1999), the first signs of cellular damage are associated with internal membrane disorganisation of the plastids, followed by vesiculation of the cytoplasm and degradation of the cytoplasm. Vitor *et al.* (1999) investigated the interactions between hydroxyl radical production and the composition of the epicarp cells associated with pitted tissues, and observed that the phosphatidylinositol content of dark stained cells was lower in unpitted than in pitted tissues. These authors also observed that the significant change of the membrane composition in pitted tissues is closely associated with acyl lipid peroxidation, mediated by hydroxyl radical production. In the light of these results, the authors concluded that degradation of fatty acids in pitted tissues was incomplete. These observations suggest that although citrus rind pitting is superficial, it begins in the cell membrane structure and progresses to epidermal cells causing these cells and oil glands to collapse.

Potential Causative Factors of Postharvest Rind Disorders

Physiological rind disorders are believed to be affected by various preharvest, harvest and postharvest conditions to which the fruit is exposed. Rind disorders are theoretically associated with preharvest ecophysiological and postharvest factors. Several preharvest factors play a critical role in the postharvest rind quality of fruit (Arpaia, 1994). Fruit mineral concentrations, position within the tree canopy, crop yield and water stress during critical stages of development are among the important preharvest factors playing a role in rind disorders (Khalidy *et al.*, 1969; Koen *et al.*, 1990; Witney *et al.*, 1990; Medeira *et al.*, 1999; Kruger *et al.*, 2000; 2002; 2003a; 2003b; 2003c; 2004; 2005; Kruger and Fraser, 2004; Kruger and Lemmer, 2006). One of the most important factors among these is the fruit mineral content, particularly the levels of N, Ca, Mg, K and some of the micro-elements (Poovaiah *et al.*, 1988). In many fruit, it is not the concentration

of individual minerals but rather the interaction between the minerals that influence quality (Penter and Stassen, 2000; van Rooyen and Bower, 2003; 2005). Good examples of where mineral balances influence fruit quality are apples (*Malus domestica* Borkh.), where mineral balance determines fruit susceptibility to bitter pit (Ferguson and Watkins, 1989), and avocado mesocarp discolouration (van Rooyen and Bower, 2003; 2005).

As far as lenticel damage is concerned, some of the causes are inherent to the fruit and cannot be changed, while others are external and can be altered (Bally *et al.*, 1996; Curry, 2003). Physiological stress, caused to a large extent by growing conditions and postharvest handling procedures may cause lenticels to darken and become more prominent. Several studies have been undertaken to investigate these aspects (Dietz *et al.*, 1988; Underhill *et al.*, 1995; Curry, 2003; Cronje, 2005b; Kupferman, 2005). Since lenticel damage may not be evident immediately after harvest, but becomes more pronounced during later handling and storing, most of these studies focused on postharvest treatments.

In terms of rind pitting, no single definite cause has been determined, but a number of factors have been associated with different citrus rind disorders. In many cases the causes of pitting are lumped with those of other disorders such as creasing and rind breakdown. An example of possible causative factors investigated are heavy cropping, age of trees, low nitrogen and potassium with high phosphorus, rootstocks and water stress (Sneath, 1987). Different researchers report tree aspects, fruit position (Wild, 1991; Almela *et al.*, 1992; Medeira *et al.*, 1999), mineral nutrition (Khalidy *et al.*, 1969; Tamin *et al.*, 2001; Kruger *et al.*, 2003a; 2005), fruit maturity (Wild, 1991; Leguizamon *et al.*, 2001), and postharvest treatments (Alferez and Zacarias, 2001; Petracek *et al.*, 1995; 1998) to be associated with the disorder.

Preharvest Factors

Much of the earlier experiments on physiological disorders of subtropical fruit such as grey pulp and black cold injury on avocado, lenticel damage on mango,

rind pitting and stem end rind breakdown on citrus, and peteca disorder on lemon fruit focused solely on the effect of postharvest factors (Alferez and Zacarias, 2001; Cronje, 2005b). However, the following preharvest determining factors have recently received attention.

Cultivar

As mentioned earlier, lenticels occur on mango fruit as sites for gaseous exchange. However, cultivars differ as to the structure of their lenticels. This makes them more or less susceptible to damage. For instance, in South Africa 'Keitt' and 'Tommy Atkins' cultivars are generally more susceptible to lenticel discolouration, while damage is seldom found on 'Kent' fruit (Bezuidenhout, 2005).

Similarly, in citrus, sensitivity to physiological rind disorders varies greatly among different species and cultivars. Although rind pitting occurs on most grapefruit and orange cultivars, 'Benny Valencia' is especially susceptible to this disorder (Kruger *et al.*, 2005).

Fruit mineral nutrition

One factor that has been shown to influence the quality, and hence the fresh fruit packout, is fertilization practice (Calvert and Reitz, 1964; Calvert, 1970). Mineral elements fulfil a vital role in plant growth and development (Tisdale *et al.*, 1985). They are involved in synthesis, heredity and energy processes and are also essential components of plant constituents, taking part in enzyme activation, osmotic regulation and membrane permeability (Mengel and Kirby, 1982; Clarkson and Hanson, 1980; Smith, 1982; Devlin and Witham, 1983; Tisdale *et al.*, 1985). Similarly to other plants, subtropical fruit trees require at least thirteen mineral elements, the so-called 'essential elements', for normal growth and reproduction. The list includes macroelements such as nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), and sulphur (S), together with microelements such as boron (B), iron (Fe), manganese (Mn), zinc (Zn), chlorine (Cl) copper (Cu) and molybdenum (Mo) (Joiner *et al.*, 1983; Marschner, 1995).

Resnizky and Sive (1993) determined that various physiological disorders arising from the extension of the storage life of a fruit are related to the mineral content present before harvest.

Although all of the above as well as certain other elements are required by plants, their importance in fruit quality is not equal. For the purposes of this review, only those known to play a key role in fruit quality such as nitrogen, calcium, potassium, phosphorus, magnesium and boron and some micro-elements will be discussed in detail. For many years, it has been (consistently) reported that nitrogen and calcium are implicated as influencing the incidence of numerous physiological disorders. For instance, bitter pit in apples has been directly linked to low fruit calcium content (Faust and Shear, 1968; Wills, 1972; Oberly, 1973; Snay and Bramlage, 1973). It would appear that most calcium-related fruit disorders are the result of a disruption of the calcium movement pattern in the plant during a critical fruit growth stage.

Nutritional levels which produce an optimum balance between vegetative and reproductive growth are important for sustained annual high quality fruit production (Childers, 1983; Weir and Cresswell, 1995). Nutritional aspects, sink-source relationships, and vegetative-reproductive balance in fruit trees form the basis of mineral involvement in fruit quality. Movement of elements within the plant system provides valuable information pertaining to conditions which contribute to their levels in plant tissues. Fruit are weaker sinks for minerals than leaves and shoots (Witney *et al.*, 1986). Immobile elements such as calcium, magnesium and iron are of particular concern, since they are lost to the plant during leaf abscission and fruit harvest. In contrast, nitrogen, phosphorus and potassium are highly mobile and are transported from the leaf or fruit prior to leaf abscission and fruit harvest (Stassen *et al.*, 1997).

The best documented relationship between the fruit mineral content and fruit disorders concerns calcium. Inter-elemental relationships also exist between minerals such as Mg, K, B, and N on the one hand and Ca on the other. As a result of this, the relative concentration of these elements also corresponds with

the incidence of certain disorders (Koen, *et al.*, 1990; Cutting and Bower, 1992; Hofman *et al.*, 2002). Plant nutritionists usually look to leaf analysis as the mainstay of their diagnostic methods (Oosthyse, 1997a). However, the most relevant relationships with respect to fruit quality appear to be those derived from fruit analyses (Kruger *et al.*, 2003a; b; c; 2004; 2005). Fruit peel has been used frequently and appears to have value in fruit quality prediction schemes (Drake *et al.*, 1966). Since rind disorders occur predominantly in the outer cortex of the fruit, analytical samples which include these tissues, either solely or predominantly, usually provide the most reliable relationship between mineral content and disorder incidence. Because mineral elements are unevenly distributed within the fruit, it is important to use enough tissue to get a representative sample of the fruit. In fruit like apples and avocados, this variation is both radial and longitudinal (Chaplin and Scott, 1980). In a longitudinal section, Ca concentration declines from the proximal to the distal calyx end of the fruit (Chaplin and Scott, 1980). The typical radial gradient is characterised by highest mineral content in the skin and lowest concentration in the pulp (Ferguson and Watkins, 1989).

Recent studies by Kruger and co-workers (Kruger *et al.*, 2000; 2002; 2003a; 2003c; Kruger and Fraser, 2004; Kruger and Grove, 2005) aimed at establishing the benefit of using fruit mineral concentration to predict and manage the storage potential of subtropical fruit crops, maintained that the mineral element concentrations of fruit play a significant role in the physiological response of these fruit to postharvest rind disorders. Mineral analysis done on fruit showed that certain key elements played a significant role in the severity of the disorders, with nitrogen and calcium levels appearing to be the most influential.

Nitrogen

Nitrogen is one of the essential elements for higher plants, and plant productivity is to a large extent determined by nitrogen nutrition. In tree crop production, large amounts of nitrogen are removed annually during harvest, resulting in this mineral being the most limiting element and consequently the one mostly applied

as fertilizer (Wolstenholme, 1989; 1990; 2004). Nitrogen is without doubt the most important nutritional element associated with the balance between reproductive and vegetative development (Weir and Cresswell, 1995). Since these two growth components are largely antagonists, particularly at the critical growth phase of flowering and fruit set, the timing and rate of nitrogen application may influence phenological and physiological plant processes and have a marked effect on postharvest fruit quality (Shear and Faust, 1980; Stassen *et al.*, 1981a; 1981b; 1997). Too much nitrogen promotes excessive vegetative growth, and sometimes results in fruit with poor postharvest quality (Calvert, 1970; Weir and Cresswell, 1995). For example, the fruit from mango trees given too much nitrogen are prone to physiological disorders such as soft nose, internal breakdown and other disorders associated with low calcium uptake by the fruit (Oosthyse, 1997a; 1997b; Oosthyse, 1998a; 1998b). Breakdown of the flesh of mango fruit has been shown to be related to low calcium and high nitrogen. The role of fruit nitrogen content on fruit softness and mesocarp discolouration of 'Pinkerton' avocado was studied by Kruger *et al.* (2000) and van Rooyen and Bower (2005). These authors reported that fruit with excessive nitrogen content are more susceptible to mesocarp discolouration.

In a study conducted on grapes, elevated nitrogen applications increased available nitrogen, potassium, calcium and magnesium, and reduced phosphorus availability in the soil. A similar trend was reported on apples by Fallahi *et al.* (2001), who studied the influence of different rates of nitrogen on leaf mineral content and fruit quality. These investigators observed a general increase in leaf magnesium and manganese and a decrease in leaf potassium when increasing nitrogen application rates. This confirms that nitrogen has an important influence on the uptake of other nutrients and their distribution within the plant.

In citrus fruit, Calvert (1970) reported nitrogen fertilization to increase yields but decrease fruit size, soluble solids and the acid content of the juice. The peel of fruits from trees with high nitrogen content were distinctly greener and coarser than peel of fruit from lower nitrogen trees. Similar results were obtained on

lemons by Koo *et al.* (1974), where increased rates of nitrogen increased production of green fruit and decreased juice acid content.

Kruger *et al.* (2003a; 2003c; and 2005) and Kruger and Fraser (2004) highlighted the benefits of using fruit nitrogen content as a supplementary tool to predict and manage occurrence of rind disorders, and increase storage potential of subtropical fruit crops. Preliminary results (Kruger *et al.*, 2003a; 2003c) showed that the incidence of mango lenticel damage has some bearing on fruit moisture and nutrients, particularly nitrogen content. Subsequent results confirmed that a relationship exists between the mineral composition of the fruit and the incidence of lenticel damage (Kruger and Fraser, 2004). Both the rind and pulp of less susceptible fruit was found to contain significantly lower levels of immobile elements such as calcium, manganese, iron and copper.

A similar study was conducted on citrus fruit (Kruger *et al.*, 2005). From this study, it was reported that over-fertilizing with nitrogen is the most important contributing factor to rind pitting in grapefruit and oranges. Rind pitting was not only experimentally induced by overfertilizing trial trees, but it was also eliminated when the leaf nitrogen content of problematic commercial grapefruit orchards was reduced. A similar survey in 'Midknight' oranges further maintained that fertilizing with excess nitrogen is a major cause of rind pitting (Kruger *et al.*, 2004).

Calcium

Calcium is known to have a considerable influence on fruit physiology, and the effect that this element has on important aspects of fruit quality has been of particular interest for many years. Due to its role in cell wall structure and membrane function, calcium has been implicated in physiological disorders of many fruits (Poovaiah *et al.*, 1988). Calcium metabolism is described as the most complex of all mineral elements, the reason being that calcium interaction with other mineral elements such as N, Mg, K, B, Zn and P would seem to be

more important than the absolute tissue concentrations of these elements (Ferguson and Watkins, 1989; Thorp *et al.*, 1997; Penter and Stassen, 2000).

Role of Ca in fruit physiology

The roles of calcium in terms of enzymes, membranes, cell walls and its interactions with plant growth regulators, all of which can be related to fruit quality and ripening, has been reviewed in a number of publications (Ferguson, 1984; Poovaiah *et al.*, 1988; Bower and Cutting, 1988). Accumulated literature consistently shows a direct association between calcium content and the incidence of physiological disorders. Early studies on the role of calcium in fruit quality focused on the relationship between tissue calcium content and the occurrence of physiological disorders (DeLong, 1936 cited by Poovaiah *et al.*, 1988). Since this initial study, more than thirty calcium-related disorders have been identified in various crops (Shear, 1975; Bower and Cutting, 1988; Poovaiah *et al.*, 1988). Low calcium levels have been directly coupled with physiological disorders such as bitter pit, internal breakdown and lenticel discolouration in apples (Faust and Shear, 1968; Wills, 1972; Bramlage *et al.*, 1973; Oberly, 1973; Snay and Bramlage, 1973), blossom-end rot in pepper (Ehert and Ho, 1986; Morley *et al.*, 1993; Li *et al.*, 2004) and tomatoes (Bangerth, 1979), cuticle cracking in cherries (Seske, 1995) and sweet pepper (Shear, 1975; Shear and Faust, 1975; Poovaiah *et al.*, 1988; Opara *et al.*, 1997; Aloni *et al.*, 1998), as well as vascular and pulp browning in avocado (Chaplin and Scott, 1980; Bower and Cutting, 1988; Thorp *et al.*, 1997).

In terms of tropical and subtropical fruit crops, considerable literature dealing with the relationship between calcium levels and internal disorders is available. Bower (1985) reported that avocado fruit with a low calcium concentration had a greater potential for physiological disorders and poor postharvest quality. In later studies, Witney *et al.* (1990) and Cutting and Bower (1992) showed that avocado fruit on trees with reduced vegetative vigour accumulated more calcium than fruit from vegetatively vigorous trees, and that the former fruit had improved postharvest quality. Postharvest calcium infiltration has also been shown to have a positive

effect on the shelf life of avocados (Eaks, 1985; Witney *et al.*, 1990). In mangos, physiological disorders characterized by internal breakdown, such as jelly seed and soft nose, have been associated with a lack of calcium. A distinct correlation between low calcium levels and internal breakdown has also been recorded in mangos (Singh *et al.*, 1993; Hermoso *et al.*, 1996).

Calcium is known to reduce physiological disorders through stabilising the cell membranes of the fruit. In addition, calcium has been reported by Ohta and Deguchi (1969) and Joham and Johanson (1973) to play a role in the translocation of photosynthates. However, although the role of calcium in subtropical fruit such as mango and avocado is well established, how to correct the disorders that occur as a result of calcium deficiency is still not well understood (Penter and Stassen, 2000). Nevertheless, several workers have reported the benefit of direct calcium applications in reducing the incidence of physiological disorders (Mason *et al.*, 1974; Poovaiah and Moulton, 1982; Penter and Stassen, 2000).

Calcium uptake and translocation

Calcium uptake and distribution has been extensively studied in a number of plants. Different investigators consistently maintain that calcium is relatively immobile in the soil and not translocatable within the plant system and, therefore, the problem is one of distribution rather than uptake (Greene and Smith, 1979; Marschner, 1983; Saure, 2005). In the soil, calcium moves mainly by mass-flow and uptake by plants is passive and restricted to the tip of young roots. Calcium transport within the plant is dependant upon a positive pressure in the xylem, which is sometimes facilitated by exchange sites where Ca^{2+} is momentarily adsorbed (Bell and Biddulph, 1963; Guttridge *et al.*, 1981). It also takes place after chelation with organic acids in the xylem sap (Bell and Biddulph, 1963; Ohta and Deguchi, 1969; Joham and Johanson, 1973; Guttridge *et al.*, 1981).

Saure (2005) is of the opinion that plants have developed physiological mechanisms to restrict calcium transport to fruits in order to maintain rapid fruit

growth. The mechanisms involved are not well understood, but Saure (2005) postulates that some hormones, possibly gibberellins, control calcium translocation. Gibberellic acid application has been reported to stimulate shoot growth and this has been shown to limit calcium translocation to the fruit while promoting translocation to vigorous and actively transpiring vegetative shoots. This is supported by the fact that calcium generally moves unidirectionally in the xylem to areas of young, actively growing tissues (Bangerth, 1979; Pauls *et al.*, 1982). Excessive gibberellin-induced shoot growth inhibits calcium translocation to the fruit, with preferential movement to the shoots. An interaction between calcium and other growth regulators, such as auxin, ethylene and cytokinins seems to exist. The role played by auxin and the effect that auxin transport has on calcium transport has been reported by Bangerth (1976). Cytokinins have also been reported to play some role in regulating calcium concentration and movement (Ferguson, 1984). An interaction between ethylene and calcium also seems to exist based on the effect that calcium has on membrane structure (Poovaiah and Leopold, 1976). Extracellular signals are mediated in plants through transient increase in cytosolic calcium, which is normally maintained at submicromolar levels by membrane-borne calcium transport system (Bush, 1995).

Relationship between organic acids and Ca

Citrus fruit contain considerable quantities of organic acids. The predominant organic acid in orange juice is citric acid (98%), although other organic acids such as malic (1%), tartaric (0.05%) benzoic and succinic acids are present (Sinclair, 1961; Clements, 1964a; 1964b; Davies and Albrigo, 1994). Oxalic acid predominates in the peel and malic acid exists in measurable concentrations in the peel. As the season advances and the citrus fruit develops and matures, the titratable acidity shows a marked decrease while the sugar content increases. The decrease in concentration of acids in citrus fruit juice is believed to be caused by the growth of the fruit and the consequent dilution of the acids present for, as the fruit increases in size, the acids must be distributed through an increasing volume of juice (Clements, 1964b).

Clements (1964a) found that organic acids account for about 50% of the total anions in the peel. Calcium and magnesium have been reported to be the only inorganic cations which exist in the peel in concentrations sufficiently high to form salts with the organic acids. The calcium in the peel normally combines with pectin to form calcium pectate. If calcium is present in the peel in quantities above that required to form calcium pectate, the excess will immediately be converted to insoluble calcium salts.

The peel contains comparatively small amounts of organic acids when compared with the pulp. These low concentrations in the peel strongly suggest that acid synthesis occurs in the pulp. According to Sinclair (1961) citrus fruit have no fleshy centres that are permeated by the vascular system. Instead the vascular system to transport water and solutes from the tree to pulp vesicles is limited to the mesocarp, the spongy parenchyma of the peel. If the organic acids are synthesized in the leaves and translocated to the pulp through the mesocarp, higher concentrations of these acids would be present in the mesocarp.

Potassium

Potassium is the third most important macro-nutrient required for plant growth. This element has an osmo-regulatory function in plants and therefore has a role in plant water utilization and control. It is the most common cation of protoplasm and, as such, balances the charges of cytoplasmic anions. In doing so, potassium is used to maintain turgor and water movement, by regulating opening and closing of stomatal guard cells (Salisbury and Ross, 1992; Marschner, 1995). Street and Opik (1984) noted that a known metabolic role of potassium is in enzyme activation for the synthesis of proteins and sugars. Fairly high potassium concentrations may be needed to make potassium-enzyme complexes. Therefore, potassium deficiencies in fruit crops will harm important enzyme-driven physiological processes and will inhibit fruit development.

Mode of action

Potassium has been reported to play a significant role in quality parameters of many crops. For instance, increasing potassium in litchi fruit increased the total soluble solids and vitamin C content and decreased fruit acidity (Lal *et al.*, 1996). Similar results were obtained on citrus fruit, where increasing potassium improved the peel colour and thickness, juice content, total soluble solid content, as well as the vitamin C content, while fruit creasing and acid content were reduced (Cohen, 1976; Koo, 1985; Dutta *et al.*, 2003). However, at very high levels, the benefits of potassium are compromised due to rind coarseness, decreased juice content, increased titratable acidity and reduced brix/acid ratio. Low potassium reduces citrus fruit production, by producing small fruit and increasing premature fruit drop (Bar-Akiva, 1975; Koo and Reese, 1977; Morgan *et al.*, 2005). A number of physiological disorders on citrus fruit such as creasing, plugging and fruit splitting have consistently been shown to be associated with smaller fruit with thin, weak rinds (Calvert, 1969; Bar-Akiva, 1975).

Potassium interaction with other elements

The balance between calcium, magnesium, and potassium is known to influence fruit quality (Usherwood, 1985). Witney *et al.* (1990) observed a relationship between the fruit potassium level and the occurrence of avocado fruit disorders. This was possibly due to interactions with calcium and magnesium within the fruit (Ferguson, 1980; Hofman *et al.*, 2002). Cutting and Bower (1992) linked high magnesium concentrations and a high (Ca+Mg)/K ratio in 'Hass' fruit with an increased potential for physiological disorders developing, due to increased polyphenol oxidase activity. Similar observations were made by Koen *et al.* (1990) who reported that a high potassium status in the soil and leaf was related to low levels of vascular browning and pulp spot in avocado fruit. However, Thorp *et al.* (1997) reported that fruit calcium concentration alone was a better indicator of fruit susceptibility to vascular browning than the (Ca+Mg)/K ratio. Similar results were obtained by Ferguson and Watkins (1989) for bitter pit in apples.

In citrus, high potassium applications usually lower calcium uptake, producing calcium deficiency symptoms expressed as different physiological disorders (Calvert, 1970; Boman, 2005). This is due to cation antagonism. Tamin *et al.* (2001) reported that an increased leaf potassium concentration significantly reduces the incidence of rind pitting in 'Shamouti' oranges. In a study aimed at establishing the effect of rind potassium concentration on rind physiological disorders, Tamin *et al.* (2001) reported that fruit suffering from rind pitting had a lower rind potassium content than unaffected fruit. These authors also found that additional potassium sprays raised potassium levels and significantly reduced the incidence of rind pitting. From this study these authors hypothesised that "potassium deficiency leads to malfunction of biomembranes, causing water loss, followed by enhanced senescence, cell collapse and local necrosis in the external layer of the rind, visibly revealed as rind pitting". In an epidemiological study of lemon peteca, a disorder similar to rind pitting, Storey and Treeby (2002) reported a similar trend, whereby potassium concentration of affected fruit was lower than that of unaffected regions of the rind. The lower potassium in cells might be indicative of loss of cell integrity and ion leakage, as K is, generally, the most abundant ion of plant cells.

Phosphorus

After nitrogen, phosphorus is usually the most limiting nutrient in crop production, although crop uptake and harvest of phosphorus is only a fraction of that of nitrogen or potassium. Phosphorus is required for all forms of life, being a structural constituent of nucleic acids, phospholipids as well as being involved in metabolic energy transfers through adenosine triphosphate (Ozanne, 1980). As part of the adenosine phosphates (adenosine triphosphate, ATP, Adonesine diphosphate, ADP and adenosine monophosphate, AMP) the phosphate group takes part in energy transduction. Intermediary metabolism includes phosphorylation, the addition of phosphate groups from ATP to carbohydrates and their intermediary products. ATP produced in the light dependent photosynthesis phase is used in the Calvin cycle to produce hexose sugars. As ATP is continuously made and used within cells, there is a constant conversion of inorganic phosphate to organic forms and *vice versa* (Street & Opik, 1984). A

shortage of phosphorus means a breakdown of the transmission of energy in plants and, as such, prevents growth. Much of the reason for continued need for P additions is due to the slow conversion of P to plant-unavailable forms, or P fixation.

Studies on phosphorus translocation in mangos have documented that there is a preferential movement of phosphorus through the stem to differentiating buds. During fruit set there is a rapid translocation of phosphorus from the leaves to the fruit, which decreases as the fruit develops (Narwadkar and Pandey, 1992). Weir *et al.* (1981) studied the effect of phosphorus, nitrogen and potassium on fruit quality of 'Valencia' oranges and reported that high phosphorus and potassium had a positive effect while high nitrogen negatively affected fruit quality.

Boron

Boron is an essential nutrient in fruit tree physiology. Its primary role is linked to its presence and function in the primary cell wall structure. A close relationship between the primary cell wall and boron nutrition has been consistently observed by different researchers. Up to 90% of the cellular boron has been found in the cell wall (Blevins and Lukaszewiski, 1998), and the first symptoms of boron deficiency disorders include disorganisation of the cell wall and middle lamella (Hu and Brown, 1994). The mechanism in which boron bonds with cell wall polymers has been proposed by Loomis and Durst (1992) to involve formation of borate esters with hydroxyl groups of cell wall carbohydrates and/or glycoproteins. In addition to its existence and function in the primary cell wall structure, boron is also closely linked to membrane functioning. The most significant documented function of boron in the cell membrane is its role during uptake of ions such as rubidium (Rb) and phosphorus (Tanada, 1983). Boron has been reported to increase translocation of sugars and plant growth regulators in plants (Mitchel *et al.*, 1953; Dugger *et al.*, 1957).

Boron uptake and translocation

Boron is absorbed from the soil solution by roots, mainly as undissociated boric acid, and behaves as a mobile element in the soil (Blevins and Lukaszewski, 1998). However, Sanchez and Righetti (2005) reported that early in the season, soil-applied boron is less available to aerial parts of the plant. These investigators noted that only about 2% of the boron in the leaves was derived directly from soil-applied fertilizer. Roots retained most of the boron absorbed, with about 60% of the root boron being fertilizer derived, and this becomes available to aerial parts, particularly flowers, later in the season. Once in the leaves, boron is generally considered immobile since its translocation is restricted and it becomes fixed in the apoplast (Blevins and Lukaszewski, 1998). However, some investigators have reported foliar-applied boron to be highly translocatable within the plant (Hanson *et al.*, 1985; Shu *et al.*, 1993; Shu *et al.*, 1994). Sanchez and Righetti (2005) suggested that flower buds are a preferential sink for boron mobilization after foliar applications. It is therefore clear that boron is more mobile than previously thought. In the long run, soil applications provide adequate boron to trees, but routine additions of boron in fertigation systems and foliar fertilization are becoming increasingly popular and are generally successful.

Boron interaction with calcium

Clarkson and Hanson (1980) studied how boron interacts with mineral calcium in the cell wall, and proposed that boron protects calcium in the cell wall through forming crosslinks in pectin. Results to support this hypothesis were obtained by Yamauchi *et al.* (1986) who observed less calcium in boron-deficient cell walls of tomatoes. From the analysis of these documented trials, Blevins and Lukaszewski (1998) hypothesized that “the hydroxyl H-bonding and borate ester formation may pull carboxylate groups of polymers into close proximity and allow calcium or magnesium binding by polymers”.

Liang *et al.* (2000) studied the effect of boron sprays on citrus fruit quality. From this investigation, it was reported that boron and calcium in the leaves, peel and pulp were positively related to boron application rate at all stages of fruit

development. It was also reported that boron treatments decreased the incidence of physiological disorders. These observations suggest that increasing the boron content in plant tissues reduces physiological disorders, directly or indirectly, by increasing the fruit calcium content of the fruit.

Seasonal mineral element distribution

Seasonal changes in mineral concentration have been witnessed in different fruit species. The flesh calcium, magnesium, and potassium concentrations of apple fruit decline during development, at a rate which lessens as the season progresses (Wilkinson and Perring, 1964; Quinlan, 1969). The reason for this decline is that the rate of fruit expansion is higher than the rate of mineral uptake, resulting in a dilution of the mineral concentration. In essence, as fruit becomes larger, the mineral concentration becomes diluted (Kruger *et al.*, 2000). However, when these minerals are expressed in terms of total fruit concentration, they usually increase over the season. In the case of apple, Ca content increases most rapidly during the early stages of development while it ceases to increase during later stages of growth (Jones *et al.*, 1983).

In citrus fruit, Kruger *et al.* (2005) noticed that mineral element distribution exhibits three patterns during the growing season. The first pattern is characterized by having a gradual decrease in fruit mineral content over the season and this levels out towards the end of the season. Examples of elements that showed this first type of pattern are N, P, K, Cu, Zn and B. In the second pattern, fruit mineral content shows an initial increase followed by a peak. This peak levelled towards the end of the season. Calcium exhibited this type of pattern. In the third pattern it was noticed that the fruit mineral content remained relatively stable during the beginning of season and peaked for a short period towards the end of the season, before returning to the initial lower level. Iron and manganese typified the third seasonal distribution of mineral elements (Kruger *et al.*, 2005; Kruger and Lemmer, 2006).

From the same investigation a correlation between fruit nitrogen and iron content was observed. These authors reported that trees which received additional nitrogen had significantly higher fruit nitrogen and iron content than that of control fruit. When late season rains fall, uptake of nitrogen and associated iron occurs and immature fruit respond by entering a late growth phase. Studies of rind physiology have shown that cell division of albedo tissue ceases after 8 to 9 weeks and that subsequent growth is by cell expansion (Grieson, 1986). Late growth may result in the occurrence of newly developed cells during the late season that causes unhardened rind areas that die-off during subsequent storage. In this study nitrogen and iron contents of 'Valencia' fruit affected by rind pitting were revealed to be lower than that of unaffected fruit. This was explained by dilution of the two elements which resulted from late growth of susceptible fruit.

A similar seasonal distribution of elements has been observed in other crops such as mangos and avocados, where a three-fold increase in fruit iron content approximately three weeks before harvest was recorded (Kruger *et al.*, 2003a; 2003c; 2004). In avocado fruit, the concentration of calcium decreased by a factor of two while boron concentration increased by approximately 40% during the season. In contrast to citrus fruit, after the late season elemental peak, iron was the only element that returned to initial concentration before harvesting. This was only realized in avocado orchards with high risk of rind chilling disorder. In a similar study conducted on mangos, Kruger *et al.* (2003c) and Kruger and Fraser (2004) reported manganese as a good indicator of how an immobile element changes in concentration during the growing season. The seasonal distribution of manganese in the pulp of mango fruit was found to gradually decrease as the fruit volume increases, while the rind manganese content increased gradually as the fruit matured. It was further noticed that the proportional increase in manganese content of the rind after good rains was considerably higher than in the pulp.

Fruit position and maturity

Almela *et al.* (1992) studied the occurrence of rind pitting in 'Fortune' mandarins and noted that fruit were highly sensitive to rind pitting during ripening, and that different microclimates influenced this sensitivity. They verified that fruit susceptibility to rind pitting varies from year to year and according to orchard location. These fluctuations could also be observed among fruits from the same tree, the incidence being higher in fruit exposed to the sun than non-exposed fruits, and higher on exposed than the non-exposed side of individual fruits. Fruit oriented to the North-West in canopy were most affected by the disorder. However, a different situation was found with peteca disorder on lemon fruit, where fruit hanging on the east side of trees are found to be more susceptible than fruit on the west side (Wild, 1991).

Kruger *et al.* (2005) studied the possible relationship between the incidence of citrus rind disorders and the mineral composition of citrus fruit. This study indicated fruit mineral composition to be an important factor causing postharvest rind pitting, and both mineral composition and rind pitting were found to vary in relation to fruit position in the tree canopy. Inside fruit were found to have a significantly higher concentration of mobile elements than outside fruit. Outside fruit were found to contain higher levels of calcium and boron. In lemons, similar mineral elements, calcium and boron, have been identified by Khalidy *et al.* (1969) to play a role in the development of the peteca disorder.

The mineral composition of citrus (Kruger *et al.*, 2003a; 2005; Kruger and Lemmer, 2006) and mango (Kruger *et al.*, 2003c; Kruger and Fraser, 2004) fruit was found to vary in relation to position in the tree canopy. In citrus, inside fruit were found to have a significantly higher concentration of mobile elements (e.g. potassium and phosphorus) than outside fruit. It is interesting to note that visual symptoms of rind pitting appear on the regions of the rind most exposed to external factors such as radiation (Medeira *et al.*, 1999). Medeira *et al.* (1999) maintained that South (Northern hemisphere) and South-western areas of the canopy were most severely affected. The same applied for immobile elements where outside fruit were found to contain significantly higher levels of calcium

and boron. Elemental differences between outside and inside mango fruit were readily explainable although this was not as obvious in mangoes as it was in citrus fruit. In mangoes, the rind of light-deprived inside fruit contained higher concentrations of magnesium and lower concentrations of calcium than outside fruit.

Solar radiation may be involved in the structural alteration of the cuticle, and high rind temperature over a long period may induce localised dehydration in the epidermal and subepidermal cells, leading to plasmolysis and membrane collapse. The fruit hanging on the eastern side of the tree (Northern hemisphere) had a mineral concentration higher than fruit on the western side of the tree. Tissue nitrogen concentration per dry weight decreases with increasing irradiance (Niinemets, 1996). In a study conducted on grapes (*Vitis vinifera*), light limitation reduced tissue concentrations of phosphorus, potassium, and magnesium in the xylem sap by about 50% (Rodriguez-Lovelle and Gaudillere, 2002). Since a considerable fraction of leaf and fruit magnesium is contained in chlorophyll, a decrease in leaf magnesium concentration with increasing irradiance, and a linear relationship between concentrations of leaf nitrogen and magnesium, provides evidence that partitioning of foliar nitrogen and magnesium occurs towards enhanced light capture with decreasing irradiance.

In citrus and mango fruit, Kruger *et al.* (2003a; 2003b; 2005) showed the inside fruit to mature at a considerably slower rate than the outside fruit. From the same set of results, these authors also reported the western fruit to mature at a faster rate than eastern fruit. Subsequent results (Kruger and Fraser, 2004) confirmed that the moisture content of faster maturing outside fruit was found to decrease at a faster rate than inside fruit, while the pulp moisture content of western fruit was reduced at a faster rate than eastern fruit. Cohen (1988) and Genizi & Cohen (1988) showed that maturity characteristics of large orange fruit harvested from the upper external side of the tree were better than those of small fruit harvested from the lower, internal and northern side of the tree. This is in line the suggestion that sun-exposed fruit mature faster than shaded fruit (Barry *et al.*, 2003).

Fruit maturity is believed to play a critical role in the development of postharvest rind disorders (Wild, 1991; Kruger *et al.*, 2005). Duarte and Guardiola (1995) and Undurraga *et al.* (2006) reported a positive correlation between grapefruit fruit maturity and rind pitting, with fruit harvested with yellow rinds developing more rind pitting than green fruit. These results consistently support the hypothesis that pitting susceptibility develops with maturity and peel pigmentation.

Rainfall and fruit water potential

In addition to the above-mentioned potential causative factors, the amount and distribution of rain over a season is also important in the development of rind disorders. Kruger and Classens (1997) showed that high rainfall during fruit maturation increases internal disorders in avocado fruit. In this regard, grey pulp incidence in 'Pinkerton' avocados was influenced by the rainfall pattern in the quarter before harvest. In as far as mango lenticel damage is concerned, a strong correlation was found between wet orchard conditions and the incidence of lenticel damage (Oosthyse, 1998a). Kruger *et al.* (2003c) and Cronje (2005b) confirmed this trend by showing that lenticel damage incidence was reduced in 'Tommy Atkins' mangoes harvested during dry periods.

It is well known that the risk of lenticel damage in mangoes is considerably higher after rain. It is thought that this is due to increased turgor in the rind. Kruger *et al.* (2005) showed that skin manganese concentrations were very stable during the season and started to increase only after heavy rain. They also observed that the manganese content of mango fruit correlated with the lenticel damage incidence pattern during the two weeks after rain. Both of these parameters increased after rain and then decreased. From these results the authors suggested that a rise in the concentration of immobile elements, particularly manganese, is indicative of turgid fruit with high risk of developing lenticel damage. In citrus fruit, Undurraga *et al.* (2006) reported that extending the days of harvest after rainfall reduces rind pitting intensity. The effect of water potential on rind disorders has been

confirmed in citrus fruit by showing that alteration of peel water potential of citrus may be a triggering factor related to rind pitting.

Postharvest Factors

For many years lenticel damage disorder has been associated with postharvest treatments. Postharvest treatments which have been shown to increase the incidence of lenticel damage include, among others, dipping fruit in hot, dirty, latex-contaminated water and holding fruit too long in detergent water (Johnson *et al.*, 1997). Oosthyse (1997a; 1998a) reported the incidence of lenticel damage in mango fruit to generally increase with delays prior to storage. In a subsequent study Oosthyse (1998b) demonstrated that cool humid conditions on the date of harvest strongly favour the postharvest occurrence of lenticel damage while dry, hot conditions discourage the postharvest occurrence of lenticel damage. In addition, lenticel damage becomes more pronounced on surfaces with sap flow marks.

In an attempt to control fungal diseases McLauchlan *et al.* (1990) showed that fruit exhibit increased lenticel damage following irradiation at 300 or 600 Gy but not at 75Gy. This was supported by Johnson *et al.* (1990), who confirmed that irradiation at dosages in excess of 600 Gy cause unacceptable lenticel damage, which becomes severe after storage. In subsequent studies Lonsdale (1992) and Lonsdale *et al.* (1991) used mild irradiation (0.75Gy) with hot water treatment and reported this combination to increase the risk of lenticel damage. In addition to above mentioned lenticel damage possible causative factors, hot water treatment also plays a significant role. Dipping fruit for 3 minutes in hot water treatment at 45°C accentuates lenticel discolouration (Jacobi *et al.*, 1996).

Recently, Cronje (2005b) showed that the disorder becomes aggravated by brushing on the packing line. To determine which points in harvesting and postharvest handling operations contribute most to these blemishes, Cronje (2005b) and Bally *et al.* (1996) investigated mango handling systems of different packing houses. The operations that contributed most to lenticel damage were

those associated with detergent, de-sapping operations, excessive brushing and hot dips. Duvenhage (2000) maintained that warm water baths with detergent increase levels of lenticel damage. In a study to determine at which points within the packing line most lenticel damage occurs, Oosthyse (2000) observed that brushing was the most influential stage, followed by soap washing and hydroheating. In general the net effect of all packline operations is an increase in lenticel damage incidence (Oosthyse, 2000).

Several theories have been developed suggesting the potential causes of the rind pitting disorder (Arpaia, 1994; Petracek *et al.*, 1995; 1998). Initial results reported that waxing may aggravate the disorder by modification of internal gas concentration (Petracek *et al.*, 1995; 1998). Petracek *et al.* (1998) reported rind pitting to be stimulated by waxing, as a result of decreasing O₂ and increasing CO₂ internal levels. However, subsequent results obtained by Alferez and Zacarias (2001) showed that the rind pitting index was lower in waxed fruit than non-waxed fruit, but, as expected, internal CO₂ and ethylene concentrations were higher than in non-coated fruit. The fact that waxing the fruit on its own is not detrimental resulting in the development of rind pitting, but alteration in relative humidity of waxed fruit may be deleterious support this idea (Alferez and Zacarias, 2001; Alferez and Burns, 2004).

Water Loss

Citrus fruit is characterized by high postharvest weight loss; this is essentially due to water loss by transpiration, as this accounts for 90% of total weight loss (Ben-Yehoshua, 1969). Water loss from the fruit occurs through a passive process, resulting from a water pressure gradient prevailing between the fruit peel, which is close to saturation with water, and the less saturated outer atmosphere (Kader *et al.*, 1989; Kader, 1992; Ben-Yehoshua *et al.*, 1994; Macnish *et al.*, 1997). Postharvest water loss has some negative implications to the fruit, since lost water is never replaced as it would be when the fruit was still attached to the tree (Turner, 1997). Initial signs of excessive water loss are shrivelling, which is immediately visible on the peel, and affected fruit loses its

shine, softens and senesces. For instance, in mangoes and citrus fruit, a 5 to 10% weight loss results in reduced crispness and early ripening (Macnish *et al.*, 1997). Moisture loss is also important in the postharvest softening of fruit such as bell pepper, lemon (Ben-Yehoshua *et al.*, 1983) and oranges (Ben-Yehoshua, 1969). Albrigo (1972) reported the total concentration of wax on the surface to be inversely correlated to postharvest weight loss.

Accumulated research suggests that the peel water status is a factor prevailing in the susceptibility of citrus fruit to rind pitting disorder. Cohen *et al.* (1994) suggested that low humidity during storage may be responsible for citrus rind pitting. However, coating fruit with commercial waxes, coupled with warm temperature storage has been reported to promote rind pitting (Wild, 1991). Alferez and Burns (2004) showed that the effect was greater on fruit exposed to prolonged periods of dehydration at lower relative humidity. These authors concluded that waxing fruit enhanced the severity of the damage only if there was a previous dehydration period. Therefore, it was suggested that keeping constant water relations in the peel of Marsh grapefruit is a key factor in the prevention of postharvest peel pitting.

In this study, the epidemiology of rind pitting in 'Benny Valencia' citrus and lenticel discolouration in 'Tommy Atkins' and 'Keitt' mangos was investigated. Further, application of nitrogen towards the end of the season was investigated to provide baseline information that may serve as guideline to predict and manage the fruit susceptibility to these disorders. In addition, the relationship between fruit mineral content and the risk of developing lenticel damage or rind pitting disorders was investigated, so that corrective measures can be implemented. Rectifying these problems would contribute towards improving the quality of export mango and citrus fruit.

CHAPTER 2

A Preliminary Study on the Effect of Climatic Conditions and Fruit Mineral Concentration on the Development of Lenticel Damage in 'Tommy Atkins' and 'Keitt' Mangos (*Mangifera indica* L.)

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ABSTRACT

Mango lenticel damage is a serious defect that occurs on the rind of the fruit. This condition causes fruit to attain a speckled appearance and become unattractive to the buyer. Each season, the disorder reduces the packout of fresh fruit by about 16%. Several preharvest factors play a critical role in the postharvest development of lenticel damage. Preliminary studies have shown that the incidence of lenticel damage has some relationship to preharvest climatic conditions, fruit moisture and fruit nutrient content, depending on which of these factors first achieves a critical threshold.

A study was conducted to determine certain climatic factors to predict lenticel damage potential before harvest. The study further aimed to provide fruit mineral content “pointers” for the development of lenticel damage. Therefore, two trials were conducted approximately a month before harvest. In the first, a plastic ground cover to restrict water supply was laid under 'Tommy Atkins' trees at Numbi Estates in the Hazyview area of the Mpumalanga province, South Africa.

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In the second, additional nitrogen was applied as three different formulations, viz. limestone ammonium nitrate (LAN), potassium nitrate (KNO_3), and a slow release nitrogen fertilizer under the trade name of Horticote®, to 'Tommy Atkins' and 'Keitt' orchards at Bavaria Estates in the Hoedspruit area of the Limpopo province, South Africa. Fruit samples were harvested, packed and stored at different intervals after rain during January 2006.

In both cultivars, the rind mineral concentration of relatively mobile elements such as N, P, Mg, Cu, Mn and B was higher than that of the pulp, while the opposite was observed regarding immobile elements such as Ca, Zn, and Fe. With regard to lenticel damage on 'Tommy Atkins' fruit from Numbi, there was no significant difference between the control and plastic cover treatments. However, when compared to fruit from Bavaria, the Numbi fruit had the highest incidence of lenticel damage. It was interesting that the Numbi fruit, which had the highest incidence of lenticel damage, also had the highest pulp and skin nitrogen concentration. Of the four treatments at Bavaria the LAN treatment had the highest incidence of lenticel damage, but the pulp and skin nitrogen concentrations of this treatment were comparable with the other treatments.

Similarly, 'Keitt' results showed that the intensity of lenticel damage was significantly higher in the orchard that received additional nitrogen in the form of LAN. From the results it was however not possible to formulate, nitrogen-lenticel damage correlations. An interesting relationship was nevertheless observed between lenticel damage and the time of harvest before and after rainfall. The results indicated that both 'Tommy Atkins' and 'Keitt' fruit become more susceptible to lenticel damage when harvested a day after rainfall and this gradually reduces afterwards. The effect of rainfall in this regard and a sink-source hypothesis arising from these observations are also discussed.

INTRODUCTION

In order to be profitable, the South African mango export industry needs to present high quality fruit to the international market. The major constraints facing the industry include unreliable fruit quality (Kruger, 2002). The most important problems are of a pathological nature, but certain physiological ailments also exist. Lenticel damage is among the more important physiological disorders.

Lenticels are superficial macroscopic openings which occur naturally on the surface of several fruit types including pears, apples, avocados and mangos (Dietz *et al.*, 1988; Fahn, 1974). Similar to stomatal openings, lenticels function as sites of gaseous exchange for photosynthesis, respiration and transpiration, the importance of each differing at different stages of fruit development (Maseuth, 1988). Although lenticels occur naturally as gas exchange sites, excessive discoloration of lenticels is an indicator of physiological stress (Batzli and Dawson, 1999). Several causative factors play a critical role in the development of postharvest lenticel damage. Physiological stress, related to growing conditions and postharvest handling procedures, may cause lenticels to darken and become more prominent. While some factors such as cultural practices and postharvest handling can be altered, some of the causative factors are genetic and cannot be changed (Bally *et al.*, 1996; Curry, 2003). Cultivars differ in lenticel structure, which makes them more or less susceptible to damage. 'Keitt' and 'Tommy Atkins' are the most susceptible cultivars. Lenticel discolouration is seldom found on 'Kent' fruit (Bezuidenhout, 2005).

Mango lenticels are hypothesized to develop from epidermal shearing due to rapid fruit growth (Dietz *et al.*, 1988; Curry, 2003). This mechanism is supported by the discovery that the fruit cuticle is a dynamic growing tissue system, and as long as the fruit is enlarging the cuticle is in a constant state of growth (Curry, 2003). As cells within the fruit enlarge, the cuticle, which is a combination of wax and epidermal cells, must also expand to accommodate the enlargement, which forces the lenticel to expand (Wilson *et al.*, 1972; Curry, 2003; Kupferman, 2005). Under non-extreme environmental conditions this process occurs gradually.

Before complete shearing and subsequent uncovering of the underlying cells takes place, a repair process begins to fill in openings that have formed due to stretching of the cuticle. Conversely, if the expansion occurs too rapidly, the cuticle and lenticel are forced to expand rapidly, rupturing vacuoles and exposing unprotected cells beneath the cuticle (Dietz *et al.*, 1988; Curry, 2003). As a result, the corky tissue in lenticels swells in an attempt to close “cracks” and lenticels therefore become pronounced. In the presence of polyphenol oxidase (PPO), the exposed and damaged cells darken due to the enzymatic oxidation of phenolic compounds (Loveys *et al.*, 1992; Robinson *et al.*, 1993).

In essence, lenticel discolouration appears to be correlated with preharvest ecophysiological factors which directly influence fruit growth rate, as well as postharvest factors. Several investigators maintain that climate (particularly rainfall), fruit turgidity (Oosthyse, 1998a; Cronje, 2005; 2006), fruit mineral content (Kruger and Fraser, 2004), and postharvest handling procedures (Cronje, 2005) play a significant role in lenticel discolouration. Fruit mineral status, particularly the levels of nitrogen (N), calcium (Ca), magnesium (Mg), potassium (K) and some of the micro-elements (B, Mn and Fe), is among the most important factors playing a role in postharvest quality of a number of fruit types (Witney *et al.*, 1990; van Rooyen and Bower, 2005; Wolstenholme, 2004; Poovaiah *et al.*, 1988). In fruit physiology, it is not the concentration of individual minerals but rather the interaction between the mineral elements within the fruit that determine fruit susceptibility to physiological disorders (Penter and Stassen, 2000). Examples in which the balance between mineral elements determines quality are apples and avocados, where this balance determines fruit susceptibility to bitter pit (Ferguson and Watkins, 1989) and mesocarp discolouration (van Rooyen and Bower, 2003; 2005) respectively.

Most studies examining the possible causes of the lenticel disorder focused solely on the effect of postharvest factors, since lenticel damage may not be evident immediately after harvest but becomes more pronounced during later handling and storing (Dietz *et al.*, 1988; Underhill *et al.*, 1995; Curry, 2003; Cronje, 2005; Kupferman, 2005). However, preharvest determining factors such

as cultivar, fruit mineral nutrition, fruit maturity and fruit water potential have recently received attention (Oosthyse, 1998a; 1998b; Kruger *et al.*, 2003b; Kruger & Fraser, 2004; Cronje, 2005; 2006). Kruger *et al.* (2003a; b; c) highlighted the benefits of using fruit mineral concentration as a supplementary tool to predict and manage the occurrence of lenticel damage. These authors showed that fruit moisture and nutrient content, particularly nitrogen, might have some bearing on the incidence of mango lenticel damage. Subsequent results confirmed that a relationship exists between mineral composition of the fruit and the incidence of lenticel disorder (Kruger and Fraser, 2004). From the latter study, it appears that the rinds and pulp of less susceptible fruit contain significantly lower levels of less mobile elements such as calcium and manganese. Distribution of elements within the fruit system (rind and pulp) during a growing provided valuable information pertaining to the effect of climatic conditions on their levels in fruit tissues. Importantly the pulp and rind manganese concentration, as well as rind moisture content, was found to significantly increase after rain. It was noteworthy that lenticel damage increased during the same period. In the same token, Cronje (2005) conducted a mulching study and maintained that soil and fruit water potential plays a significant role in lenticel damage potential. The later study supports the role played by water potential in the development of the disorder. From these findings the authors hypothesised that immobile mineral element composition, soil water potential and moisture levels of the pulp and rind of mangos can be used to predict transient sensitivity of an orchard to lenticel damage. It is unclear from these findings, whether all or some of these factors play a role in lenticel damage, and before predictions of the disorder can be made, which will be very useful, some clarity on the roles of the various factors is necessary.

The first aim of the present study was to determine the effect of mulching and rainfall before harvest on the postharvest development of lenticel damage. The second aim was to determine whether the mineral concentration of the pulp and rind can serve as a guideline when assessing the lenticel damage risk of a specific orchard. In such a study, it is customary to first address elements known to have a marked effect on fruit quality (*viz* N). The study further aimed at

assessing two mango growing regions with different climatic conditions for the risk of developing lenticel damage.

MATERIALS AND METHODS

Locations

Field trials were laid out at Bavaria Estates, located in the Hoedspruit area of the Limpopo province (24°21'S, 30°58'E), as well as at Numbi Estates in the Hazyview area (25°02'S, 31°07'E) of the Mpumalanga province, South Africa. The two locations are characterised by hot and humid climatic conditions. The yearly average maximum temperature for Hoedspruit is 29°C; the annual average minimum is 16°C with average annual rainfall of 450mm. Hazyview on the other hand is characterised by an average rainfall of 590mm per annum with an average daily minimum and maximum temperatures of 13 and 28°C respectively (ARC – Soil Water and Climate Institute).

Cultivars and orchards

Two cultivars, namely 'Tommy Atkins' and 'Keitt', were studied. In the case of 'Tommy Atkins', the conventional orchards 3B at Numbi Estates and AL5 at Bavaria Estates were studied. Two 'Tommy Atkins' orchards were used for location comparison. In the case of 'Keitt', only one conventional orchard, AE7 located at Bavaria Estates was used.

Treatments, replications and application dates

Two experiments were conducted. Approximately one month before harvest a plastic ground cover was laid under the trees for one experiment, while, in the second experiment, additional nitrogen was applied in various formulations.

a) Plastic ground cover trial

The trial was conducted in orchard 3B at Numbi Estates ('Tommy Atkins' conventional orchard). This trial consisted of two treatments namely, plastic ground cover and the uncovered control. During the second week of December

2005, 4 m wide strips of 90 micron black polyethylene plastic sheeting were placed under the trees. In the experiment each treatment was replicated 5 times with each replicate consisting of 5 trees, of which the 3 middle trees served as data trees.

b) Nitrogen application experiment

Three different formulations of nitrogen (LAN @ 200 g/tree, Horticote® 100 @ 300 g/tree and KNO₃ @ 150 g/tree) were applied in the conventional 'Tommy Atkins' and 'Keitt' orchards at Bavaria Estates during the second week of December 2005. The trial also included an untreated control for comparison to nitrogen treatments. Each treatment provided a different rate of nitrogen per tree. Horticote®100 (40) provided 120g N while LAN (28) and KNO₃ (13) respectively provided 56g and 19.5g of nitrogen per tree. A decision on these rates was based on the form of application and the time of nitrogen availability to the tree. KNO₃ was applied as foliar spray, while Horticote®100 and LAN were top dressed to the soil. It was suggested by a soil scientist (Abercrombie, pers. comm.) that by the time of harvest, each treatment would have received relatively similar amounts of nitrogen. The statistical layout of the trial was a completely randomized design. Each treatment consisted of 5 replicates of 5 trees each, of which the three middle trees served as data trees.

Sampling

Ten fruit samples per tree were harvested from all representative sides of the tree on 13, 16, 17 and 18 January 2006. The fruit were then subjected to normal packinghouse procedures at Bavaria Packhouse and packed in 4 kg cartons. The boxes were transported to the ARC-ITSC's Burgershall Research Station where they were stored in a cold room at 8°C for 26 days.

Sample analysis

From each treatment sample, five additional fruit were retained for maturity determination and mineral content analysis. Moisture content assessments, storage trials as well as the preparation of samples were executed at the ARC-

ITSC's laboratory at Burgershall Research Station, while mineral analysis was executed by the ARC-ITSC's soil science laboratory in Nelspruit. The maturity of the mango fruit was determined by the dry weight method described by Kruger and Magwaza (2006). The fruit were peeled and the peel cut into 1 cm² squares. The peeled fruit were dissected into two seed-to-skin pulp sections from the two longitudinal sides of the fruit. These were then grated with a domestic grater. The prepared pulp and peel samples with initial fresh weight of 10 grams was placed into a plastic petri dish and dried in an oven set at 35°C until constant mass was reached.

After drying, the samples were sent to the ARC-ITSC Soil Science Laboratory in Nelspruit where the N, P, K, Ca, Mg, Zn, Cu, Mn, Fe, and B contents were determined. Nitrogen concentration was determined by colorimetric auto analyzing after wet digestion with 98% sulphuric acid and 30% hydrogen peroxide (Adrian, 1973; Giron, 1973; Novozamsky *et al.*, 1983). The concentrations of the other minerals were determined after digestion and extraction with 2:1 55% nitric acid and 70% perchloric acid (Adrian, 1973; Giron, 1973; Novozamsky *et al.*, 1983). The concentrations of other minerals were determined after digestion and extraction with 2:1 55% nitric acid and 70% perchloric acid (Adrian, 1973; Giron, 1973; Novozamsky *et al.*, 1983). The Concentrations of Ca, Mg, Zn, Cu, Mn and Fe were determined by atomic absorption flame spectroscopy using air-acetylene (Price, 1972). Potassium was determined by flame atomic emission spectroscopy using air-acetylene (Poluektov, 1973). Phosphorus concentrations were measured colorimetrically using an auto analyzer. The Boron concentration was determined colorimetrically by auto analyzer.

Quality evaluations

The number of days each fruit took to reach the ready-to-eat stage was recorded. Lenticel damage and disease development at ripeness were scored on a scale between 0 and 5, where 0 was a clean fruit and 5 a severely damaged fruit (Plate 1). The sugar content was also measured using an Atago® Palette-Style digital refractometer.

Statistical analysis

Statistical analysis included the Student's T-test ($P = 0.05$) as well as analysis of variance (ANOVA) using GenStat® version 6 (VSN International Hemel Hempstead, UK).

RESULTS AND DISCUSSION

The data presented in this study had certain limitations in that there was no information as to the mineral content of small fruit at an earlier stage of development. Soil and leaf mineral tests to give an indication of the mineral status of each block were not executed. Nevertheless, a number of trends of lenticel damage in different treatments, as well as pulp and rind mineral concentration of different growing localities emerged.

'Tommy Atkins'

The rind and pulp nitrogen concentration of 'Tommy Atkins' fruit from both experimental sites was not significantly different between any of the treatments (**Table 1 & 2**). However, in the case of other elements, the differences were in some cases significant although inconsistent. For instance in a mulching trial at Numbi Estates rind Mg, Cu, Mn and pulp P, K and Mn were significantly different between treatments. Other elements were not consistent between rind and pulp except in the case of Mn. The difference in the Mn concentration between ground cover treatment and the control at Numbi Estates could be the result of differences in fungicide application. The mulching treatment probably interfered with the spraying procedure. In a nitrogen trial at Bavaria Estates, the differences in mineral concentration between treatments were not significant except in the case of Mn which was significantly higher in the KNO_3 treatment.

Certain trends with regard to evaluated parameters at different locations were observed. In the plastic cover trial at Numbi estates, the difference between the lenticel damage of fruit from the plastic cover treatment and the control was not significant (**Table 3**). It was however interesting to note that the control fruit had

the highest rind and pulp manganese concentrations. The lower manganese concentration on water-restricted fruit suggests that water content played a critical role in the manganese translocation to the fruit. However, there was no connection between increasing Mn concentration and development of lenticel damage. The lack of direct correlation between these items rather implies that Mn is not important for the development of the disorder. The strongest evidence of a factor playing a role in lenticel damage was that of rind moisture content, as related to the rainfall data (**Figure 1**). Unfortunately, soil and tree water status of the treatments was not measured. However, the month during which treatments were applied was characterised by very good rainfall (a total of 124.8mm, ARC-Soil Climate and Water Institute). This may suggest that there was no difference in tree water status, as the trial may have started too late. Since mangoes have a deep root system (Whiley and Schaffer, 1997) and the soil moisture status was good before application, there might have not been enough drying out by the time fruit were picked. The pulp moisture did not show a difference, so perhaps this explains the lack of clear results in this experiment. It also supports the implication that elements such as Mn play no clear role in the development of the disorder.

The results of the nitrogen trial at Bavaria are presented in **Table 4**. Of the four treatments at Bavaria, the LAN treatment had the highest incidence of lenticel damage, but the pulp and skin nitrogen concentrations of this treatment were comparable with the other treatments. The results suggest that nitrogen may have some influence on lenticel damage, in that the disorder seems to be higher in the nitrogen application treatments than the control. However, the effect of nitrogen was not direct. Nitrogen is known to be the most important nutritional element associated with the balance between reproductive and vegetative development. Since these two growth components are largely antagonists, results obtained in this study suggest that nitrogen applied in the soil late in the season would either result in high tree nitrogen content, which would in turn enhance vegetative growth at the expense of fruit (Calvert, 1970; Weir and Cresswell, 1995) or nitrogen was not available to the fruit and the tree. Nitrogen-stimulated vegetative growth would create a cascade of other events, such as

partitioning of carbohydrates and calcium to actively transpiring shoots (Shear and Faust, 1980; Stassen *et al.*, 1981a; 1981b; 1997). However, as nitrogen may have been applied too late in the growth cycle of the fruit supports the possibility that nitrogen was not available to the fruit or tree.

When comparing the lenticel damage of fruit from two locations with different orchard management practices and climatic conditions, it was found that control fruit from the Numbi orchard had a higher incidence of lenticel damage than the orchard at Bavaria (**Table 5**). It is noteworthy that fruit from the Numbi orchard, which had the highest incidence of lenticel damage, also had the highest rind and pulp nitrogen and manganese concentrations. However, in terms of mineral association with the disorder, there is not enough evidence to support the contention that fruit N and Mn concentrations are important in the development of the disorder. The lack of this evidence highlighted the importance of fruit origin rather than fruit mineral concentration on the development of lenticel damage. It therefore seems that the role played by nitrogen on lenticel damage is not as significant as that played by orchard location. These findings are in agreement with those reported by Kaiser *et al.* (2004). These authors reported that fruit rind nitrogen concentration does not play a direct role in the development lenticel discolouration. It is therefore suggested that, in addition to nitrogen, other factors such as climatic conditions and perhaps orchard management practices play a significant role in development of the disorder.

The lenticel damage scores of fruit from different nitrogen treatments at Bavaria Estates harvested one day before rainfall, on the day of rainfall, one and two days after rainfall are visually portrayed in **Figure 2**. In all four treatments, the incidence of lenticel damage was relatively low at the start of the evaluation period before the rainfall. It then increased threefold after the first rain for all nitrogen treatments. It then either remained high (LAN and Horticote®100 treatments) or reduced slightly (control and KNO₃) towards the end of the observation period. This is in agreement with the results observed by Cronje (2005), who reported that fruit harvested during a period of soil moisture saturation (immediately after rain or irrigation) were more susceptible to lenticel

damage. This author also reported that the incidence of lenticel damage was reduced as the soil dried off.

In a later study, Cronje (2006) reported that fruit with high turgor are more susceptible to the disorder. The risk of lenticel damage in mangoes is considerably higher after rain (Oosthyse, 1998a; Kruger and Fraser, 2004; Cronje, 2005). It is thought that this is due to increased turgor in the rind. Cronje (2006) supported this by showing that fruit turgor at harvest plays an important role in the development of lenticel damage, as it renders lenticels susceptible to damage in the packhouse.

'Keitt'

The rind and flesh mineral concentration of 'Keitt' fruit is portrayed in **Table 6**. Similarly to what was observed in 'Tommy Atkins' fruit, except rind Mn which was statistically different in three treatments, none of the analysed mineral elements was significantly different. However, this was not consistent between rind and pulp.

The mean lenticel damage scores and the pulp and skin nitrogen concentration readings are shown in **Table 7**. In the 'Keitt' orchard at Bavaria, the results were fairly similar to that of 'Tommy Atkins', in that the intensity of lenticel damage was significantly higher in the orchard that received additional nitrogen in the form of LAN. However, the nitrogen concentration of the LAN treatment with the highest lenticel damage was not statistically significant from other treatments. This observation suggests that the direct effect of nitrogen application of a particular form was not important to the development of lenticel damage and fruit nitrogen concentration. Wolstenholme (2004) reviewed nitrogen budgets, particularly in soils high in soil organic matter, and reported that soil organic matter contains about 95% of potentially available nitrogen, which can either be immobilised or protected in clays as amorphous compounds of aluminium and iron. Nitrogen in the amorphous form is temporarily unavailable, and as long as it occurs in this form both nitrogen and iron are unavailable to the plant. Nitrogen uptake by a mango tree as a whole occurs during two specific periods. One is in spring as a

result of inflorescence and fruit development and the other one occurs after harvest as a result of increased number of leaves (Stassen *et al.*, 1997). It is therefore apparent that in order to have acceptable fruit nitrogen levels, nitrogen availability should synchronise with these periods. Therefore the nitrogen that was applied one month before harvest was not indicative of normal nitrogen application and uptake by the tree. Since nitrogen partitioning in fruit trees is largely affected by application time and, preferentially, to the vegetative parts (Stassen *et al.*, 1981a; 1981b; Khemira *et al.*, 1998; Sanchez *et al.*, 1992), it is suggested that additional nitrogen was either not taken up by the time of harvest or was partitioned to vegetative parts at the expense of the fruit. Evidence seems to support the earlier statement.

The daily lenticel damage incidence scores of 'Keitt' and rainfall data for the sampling period are shown in **Figure 3** and the corresponding skin moisture content scores are shown in **Figure 4**. As was the case with 'Tommy Atkins', the severity of lenticel damage was relatively low at the beginning of the sampling period. It increased three fold shortly after rain and then decreased again. LAN treated fruit was an exception to this trend. Instead of dropping back to the initial score, it remained at double this value. This means that the residual effect of nitrogen induced by LAN treatment was more persistent than that of other nitrogen treatments. These observations suggest nitrogen applications shortly before harvest may influence some unknown factors that perhaps change the fruit reaction to available soil water content. However, the physiology of this reaction is difficult to hypothesize at this stage.

The moisture content of the skin was found to increase and then decrease in a pattern that closely resembled the lenticel damage pattern. Important observations were made with regard to the skin moisture content, in that before the rain the fruit skin moisture content of all treatments was below 75%. However, shortly after the rain, the skin moisture content of all treatments increased to around 77% to 78%. With the exception of the LAN treatment, all fruit returned below 75% towards the end of the observation period. This strengthens the hypothesis made by Kruger and Fraser (2004) that fluctuations in

fruit moisture content are significantly influenced by the rainfall pattern. This also supports the idea that the risk of lenticel damage in mangoes is considerably higher after rain, due to increased turgor in the rind (Oosthyse, 1998a; 1998b; Cronje, 2005; 2006).

In general, the data does not consistently support fruit mineral content having a direct effect on the development of the disorder. There is also no strong evidence that nitrogen plays a clear role, and the only element that seems to change was Mn. However, whether this is of consequence or not, is difficult to say. It may just be as a result of other changes, and not a driver. The Numbi ground cover where Mn increased but not lenticel damage rather implies it is not of importance. The strongest evidence is that of rind moisture content, as related to the rainfall data. However, the mulching trial failed to show this, but perhaps there was no difference in tree water status, as the trial may have started too late. The month, during which treatments were applied, the orchard received a total of 124.8mm of rainfall. This may suggest that there was no difference in tree water status, as the trial may have started too late. Since mangoes have a deep root system and the soil moisture status was good before application, there might not have been enough drying out by the time fruit were picked.

Nitrogen seems to have some influence in lenticel damage disorder, in that where applications were made the damage seems to be higher than in the control. However, when considering the lack of evidence that nitrogen plays a direct role, due to the lack of significant differences in the fruit, it would seem that nitrogen applications shortly before harvest may influence some unknown factors that perhaps change the fruit reaction to available soil water content.

Therefore, the strongest evidence explaining lenticel damage relates to water relations, where high water contents, as may occur after rain or irrigation enhance damage. There seems to be some evidence that nitrogen applications within a month of harvest may modify this relationship, but by what means is unknown, as there is no evidence of fruit nitrogen being changed, and only one other element was modified, which may not be meaningful. Overall, the cause of

lenticel susceptibility to damage seems more complex than expected, but the best evidence thus far available points to tree and fruit water relations at the time of harvest. Production area or climatic differences may be related in the same manner.

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TABLE 1

Rind and pulp mineral concentrations of fruit from the plastic cover trial in the 'Tommy Atkins' orchard on Numbi Estates (& ** P Sign. at 1 and 5% levels)*

<i>Treatment</i>	<i>N</i>	<i>P</i>	<i>K</i>	<i>Ca</i>	<i>Mg</i>	<i>Zn</i>	<i>Cu</i>	<i>Mn</i>	<i>Fe</i>	<i>B</i>
	%	%	%	%	%	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
<u><i>Rind</i></u>										
Numbi Plastic cover	0.50	0.07	0.69	0.32	0.11	23.33	16.00	49.33	56.00	19.13
Numbi Control	0.55	0.07	0.66	0.42	0.15	18.33	25.00	90.33	42.00	22.67
LSD (0.05) n = 10	ns	ns	ns	ns	0.48	ns	8.47	19.28	ns	ns
<u><i>Pulp</i></u>										
Numbi Plastic cover	0.48	0.07	0.78	0.22	0.09	25.00	14.67	60.67	121.67	21.33
Numbi Control	0.50	0.09	1.03	0.09	0.06	34.67	9.00	22.67	103.33	17.00
LSD (0.05) n = 10	ns	0.01	0.19	ns	ns	ns	ns	33.02	ns	ns
Rind pulp significance	ns	ns	*	**	**	**	*	**	**	ns

TABLE 2

Rind and pulp mineral concentrations of fruit from four nitrogen treatments in the 'Tommy Atkins' orchards on Bavaria Estates (& ** P Sign. at 1 and 5% levels)*

<i>Treatment</i>	<i>N</i>	<i>P</i>	<i>K</i>	<i>Ca</i>	<i>Mg</i>	<i>Zn</i>	<i>Cu</i>	<i>Mn</i>	<i>Fe</i>	<i>B</i>
	%	%	%	%	%	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
<u><i>Rind</i></u>										
Control	0.46	0.05	0.66	0.52	0.17	9.75	20.75	38.50	33.75	23.75
Horticote (100)	0.46	0.04	0.76	0.51	0.17	15.75	16.75	44.25	31.25	22.55
KNO ₃	0.44	0.05	0.78	0.57	0.16	11.75	22.75	52.00	35.00	24.15
LAN	0.46	0.04	0.70	0.50	0.18	17.00	22.00	49.00	38.75	24.50
LSD (0.05) n = 20	ns	ns	0.11	ns	0.02	ns	6.31	11.66	ns	ns
<u><i>Pulp</i></u>										
Control	0.36	0.05	0.83	0.10	0.06	31.50	5.25	10.75	46.50	10.35
Horticote (100)	0.31	0.04	0.85	0.10	0.06	25.25	4.75	11.25	78.25	10.33
KNO ₃	0.39	0.06	0.89	0.10	0.06	24.00	5.00	12.25	115.25	11.53
LAN	0.37	0.05	0.86	0.10	0.06	35.25	5.25	12.50	72.50	11.45
LSD (0.05) n = 20	ns	0.010	ns	ns	ns	ns	ns	ns	ns	ns
Rind pulp significance	**	ns	*	**	**	**	**	**	**	**

TABLE 3

Mean lenticel damage scores and pulp and skin nitrogen percentage of fruit from two treatments on Numbi Estates (* & ** *P* Sign. at 1 and 5% levels)

Treatment No.	Orchard	Treatment	Lenticel damage (0-5)	Pulp N (%)	Skin N (%)	Flesh Mn (mg/kg)	Skin Mn (mg/kg)	Pulp Moisture content (%)	Skin Moisture content (%)
1	Numbi 3B	Plastic cover	3.9 a	0.48 a	0.50 a	22.7 b	49.3 b	85.4 a	74.09 a
2	Numbi 3B	Control	4.0 a	0.50 a	0.55 a	60.7 a	90.3 a	86.1 a	74.7 a
LSD (0.05) n=1			ns	ns	ns	ns	19.28	33.02	ns

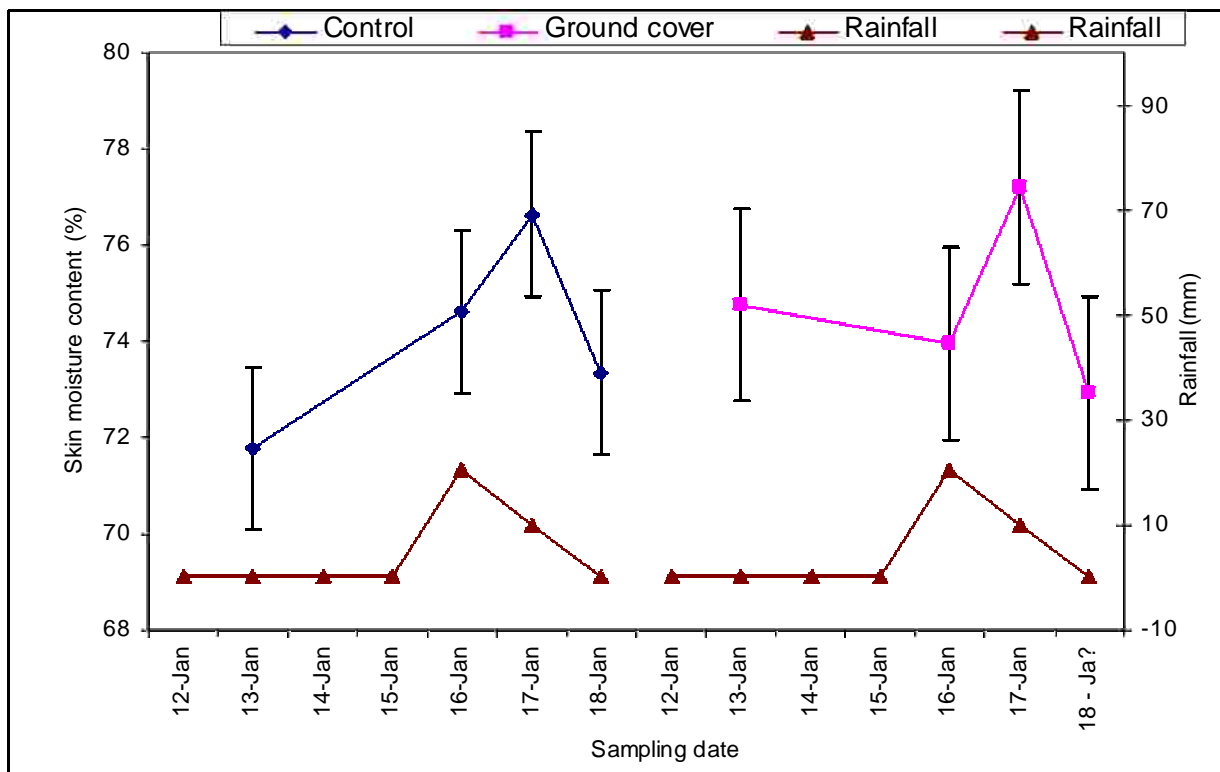


FIGURE 1

Daily skin moisture percentage for fruit from two treatments in the 'Tommy Atkins' orchard on Numbi Estates.

TABLE 4

Mean lenticel damage scores and pulp and skin nitrogen percentage of 'Tommy Atkins' fruit from four nitrogen treatments on Bavaria Estates

Treatment No.	Treatment	Lenticel damage (0-5)	Pulp N (%)	Skin N (%)	Flesh Mn (mg/kg)	Skin Mn (mg/kg)	Pulp Moisture content (%)	Skin Moisture content (%)
1	200g LAN	3.3 a	0.37 a	0.46 a	12.5 a	49.0 a	86.8 a	76.4 a
2	300g Horticote 100	2.9 b	0.31 a	0.46 a	11.3 a	44.3 a	86.4 a	76.1 a
3	150 g KNO ₃	2.8 b	0.39 a	0.44 a	12.3 a	52.0 a	85.6 ab	74.7 a
4	Control	2.6 c	0.36 b	0.46 a	10.8 a	38.5 a	86.1 a	75.4 a
LSD (0.05) n=20		0.377	ns	ns	ns	ns	ns	ns

TABLE 5

Mean lenticel damage scores, pulp and skin nitrogen and manganese concentration readings of fruit from two mango growing localities on Numbi Estates and Bavaria Estates

Location	Orchard	Lenticel damage (0-5)	Pulp N (%)	Skin N (%)	Flesh Mn (mg/kg)	Skin Mn (mg/kg)	Pulp Moisture content (%)	Skin Moisture content (%)
Numbi	3B	4.0 a	0.50 a	0.55 a	60.7 a	90.3 a	86.1 a	74.1 a
Bavaria	AL5	2.6 b	0.31 b	0.46 b	11.3 b	44.3 b	86.4 a	75.4 a
LSD (0.05) n=10		0.827	0.084	0.082	39.37	36.73	ns	ns

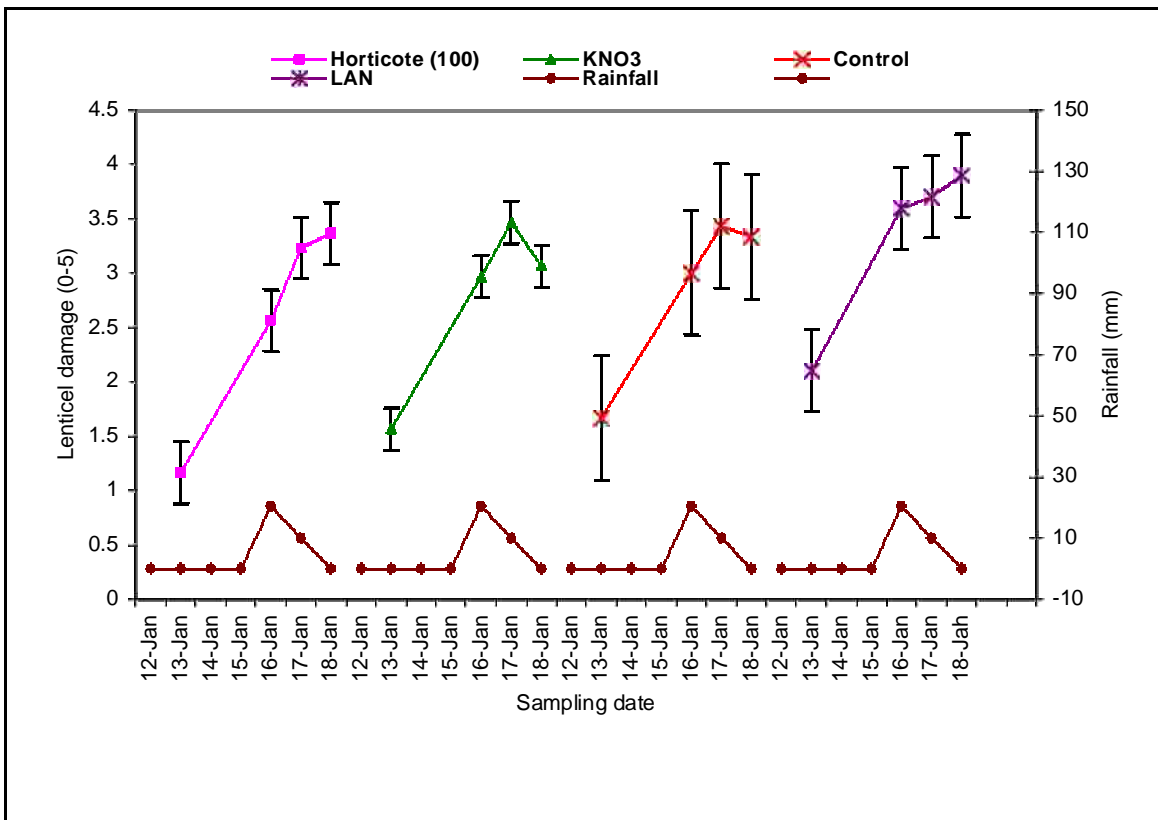


FIGURE 2

Daily lenticel damage scores for four nitrogen treatments in the 'Tommy Atkins' orchard at Bavaria Estates.

TABLE 6

Rind and pulp mineral concentrations of fruit from four nitrogen treatments in 'Keitt' orchard at Bavaria Estates (& ** P Sign. at 1 and 5% levels)*

Treatment	N	P	K	Ca	Mg	Zn	Cu	Mn	Fe	B
	%	%	%	%	%	Mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
<i>Rind</i>										
Control	0.49	0.09	0.89	0.27	0.15	13.50	21.75	30.25	185.50	19.33
Horticote (100)	0.51	0.09	0.95	0.28	0.14	17.75	28.25	37.75	96.75	20.08
KN ₃	0.51	0.09	0.87	0.29	0.14	14.25	20.50	30.75	255.75	19.08
LAN	0.51	0.09	0.84	0.31	0.14	19.75	23.25	34.00	51.25	19.40
LSD (0.05) n=20	ns	ns	0.102	ns	ns	ns	ns	6.040	ns	ns
<i>Pulp</i>										
Control	0.35	0.06	1.02	0.07	0.06	30.25	5.00	13.50	795.25	16.58
Horticote (100)	0.35	0.05	1.06	0.09	0.06	20.00	4.50	16.25	851.50	15.65
KN ₃	0.40	0.06	1.03	0.08	0.06	16.50	4.25	12.00	181.75	11.15
LAN	0.40	0.06	0.98	0.09	0.06	24.25	4.75	13.00	136.00	10.15
LSD (0.05) n=20	ns	ns	ns	0.019	ns	ns	ns	ns	ns	ns
Rind pulp significance	**	**	**	**	ns	**	**	**	ns	*

TABLE 7

Mean lenticel damage scores and pulp and skin nitrogen percentage of fruit from five treatments in the 'Keitt' orchard on Bavaria Estates

Treatment No.	Treatment	Lenticel damage (0-5)	Pulp N (%)	Skin N (%)
1	200g LAN	2.3 a	0.40 a	0.51 a
2	150 g KNO ₃	1.8 b	0.40 a	0.51 a
3	300g Horticote (100)	1.6 b	0.35 a	0.51 a
4	Control	1.4 b	0.35 a	0.49 a
LSD (0.05) n=20		0.530	ns	ns

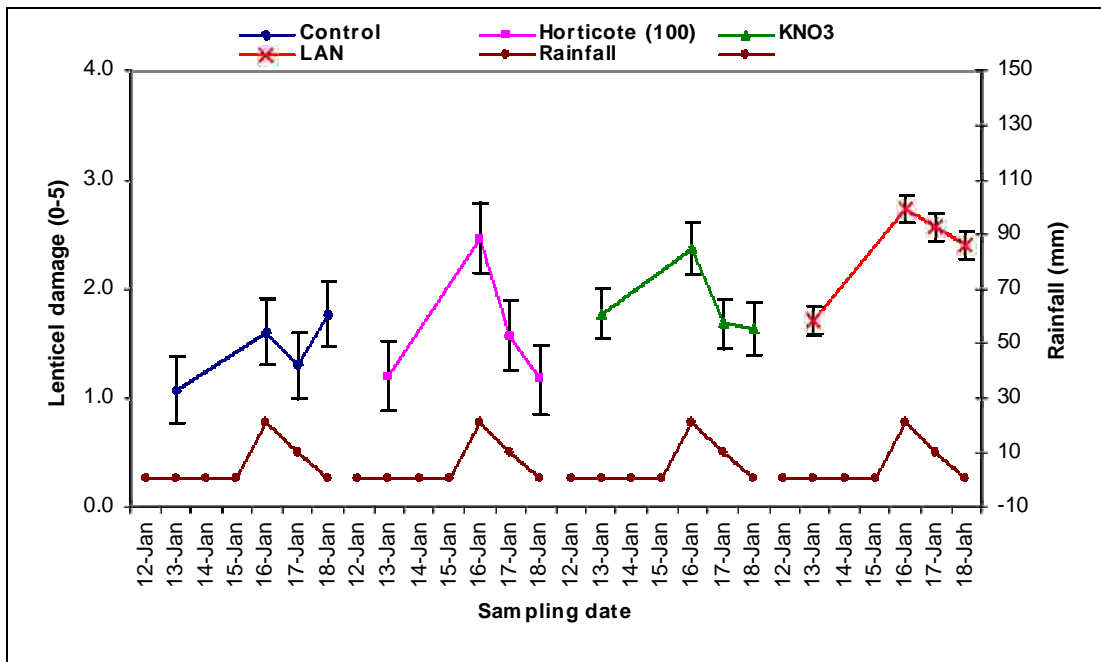


FIGURE 3

Daily lenticel damage scores for fruit from five treatments in the 'Keitt' orchard on Bavaria Estates.

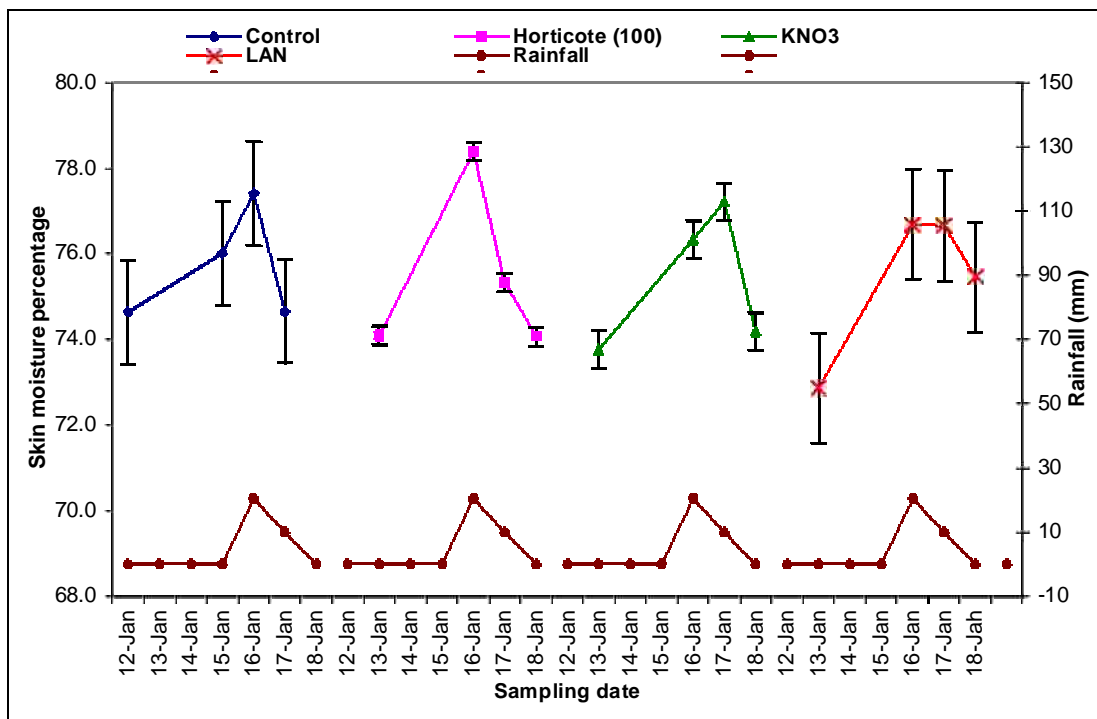


FIGURE 4

Daily skin moisture percentage for fruit from five treatments in the 'Keitt' orchard on Bavaria Estates.

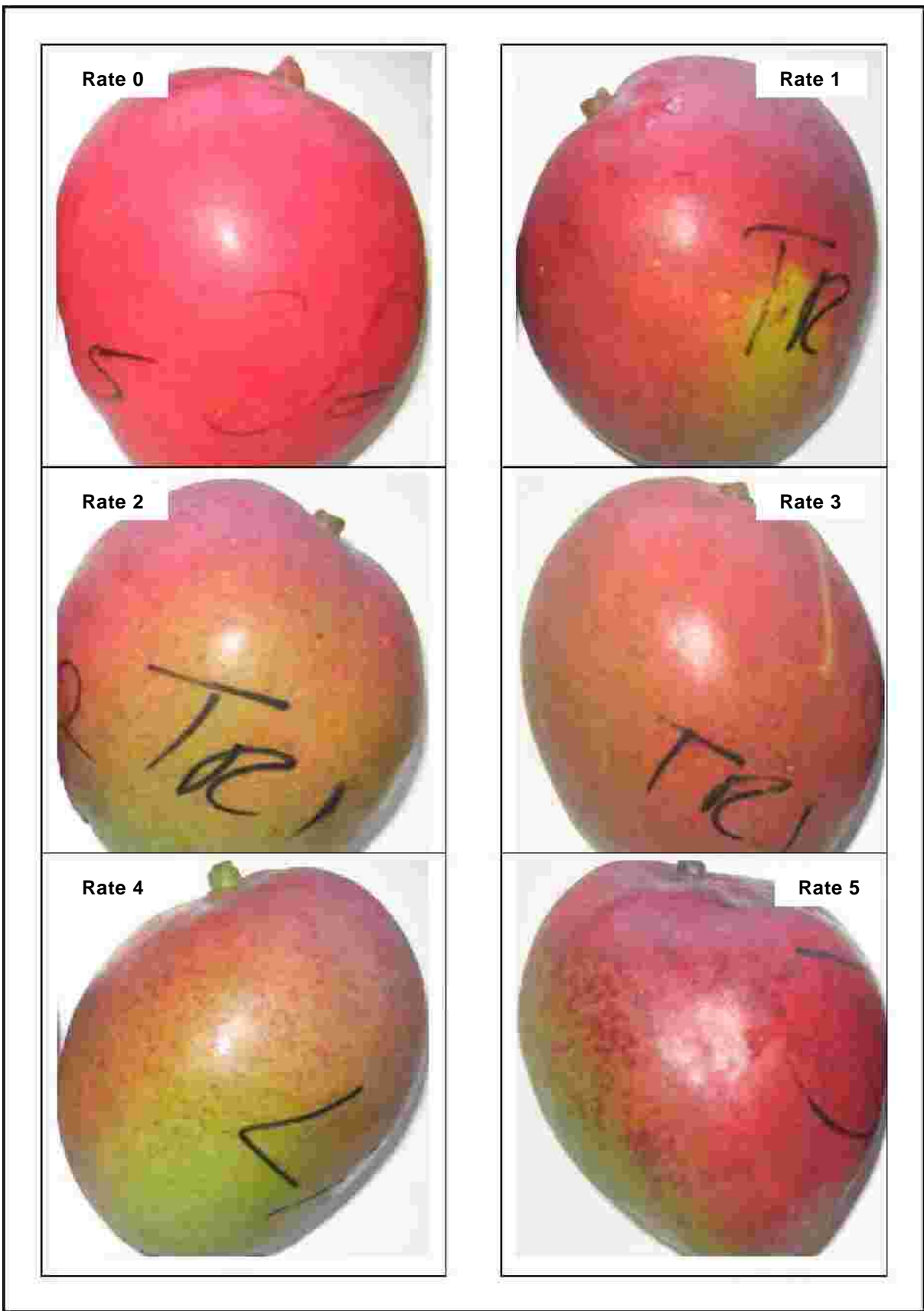


PLATE 1:

Photographs illustrating the rating scale for lenticel damage.

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CHAPTER 3

A Preliminary Study on the Effect Climatic Conditions and Fruit Mineral Status on Assimilate Distribution and Ultimate Development of Rind Pitting in 'Benny Valencia' oranges (*Citrus sinensis*)

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ABSTRACT

Rind pitting is a physiological disorder of citrus that develops during storage. A study was conducted to investigate the relative effects that certain pre-harvest factors have on the post-harvest development of superficial rind pitting in 'Benny Valencia' oranges. Factors of particular interest were harvest maturity, climate and the mineral content of the fruit. In addition, trees were treated during March 2006 with two different formulations of nitrogen viz. Limestone Ammonium Nitrate (LAN) and a slow release nitrogen fertilizer, under the trade name Horticote[®]. The fruit were then sampled on a two weekly basis over a period of three months. On each sampling date a set of quality related readings such as juice total soluble solids and titratable acid were taken, after which the mineral content of the exocarp and mesocarp was analysed. Fruit were also stored for 40 days at room temperature and rated for rind pitting.

The results indicated that fruit from trees that received additional N were more susceptible to rind pitting than those from control trees. Of the two N applications,

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fruit from trees that received slow-release N were more susceptible to rind pitting than fruit from trees that received the LAN treatment. Another important observation made was that the nitrogen content of oranges from trees that received extra fertilizer was lower than that from the control trees. In addition, the fruit from trees receiving additional nitrogen were smaller than the control fruit. Fruit from trees that received the slow release nitrogen treatment were smallest. A sink/source hypothesis aimed at explaining this phenomenon has been formulated and is currently investigated. A number of control mechanisms are also explored.

INTRODUCTION

The South African fresh fruit industry is a significant exporter, accounting for approximately 45% of the country's agricultural exports (Department of Science and Technology, 2006). Of the total exported fruit in the 2005/06 season, 60% was subtropical (57% citrus, 1.9% avocados, 0.1% litchis and 0.1% mangos) (PPECB/FPEF, 2006). From these statistics, it is clear that citrus is the biggest component of South African exported subtropical fruit. Globally, South Africa is ranked the second largest exporter of citrus, after Spain (South African Citrus Growers Association, 2008). The position of the industry in the international markets is strengthened due to the production of a wide range of cultivars over an extended period of time - from March through to November (Department of Science and Technology, 2006). However, physiological rind disorders such as stem end rind breakdown, peteca spots (lemons), chilling injury and rind pitting are frequently observed, reducing the commercial value of the fruit.

Postharvest rind pitting occurs on oranges, mandarins and grapefruit and is manifested as sunken areas of about 3 to 6mm in diameter on the flavedo (Gilfillan, 1990). These sunken areas on the flavedo occur directly above and among the oil glands (Medeira *et al.*, 1999; Alferez and Burns, 2004). In severe stages of the disorder sunken areas spread, turning dark-brown and becoming necrotic (Gilfillan *et al.*, 1981; Almela *et al.*, 1992; Cronje, 2005; Kruger *et al.*, 2005). The mechanism governing the occurrence of this disorder and how it is related to other rind physiological disorders is not well understood, since several inductive factors may lead to similar symptoms (Agusti *et al.*, 2004). Based on general causative factors, citrus rind disorders can be classified into two main groups, viz. chilling related (or chilling injury) and non-chilling disorders. Chilling injury is defined as "the permanent or irreversible physiological damage to plant tissues, cells, or organs, which results from exposure of plants to temperatures below some critical threshold temperature that causes injury" (Lyons and Breidenbach, 1987). Symptoms in response to chilling damage are most apparent on the peel of citrus fruit, in various forms such as browning of the

flavedo and albedo or appearance of dark sunken areas of collapsed tissues (Porat, 2003; Porat *et al.*, 2004).

Rind disorders not related to chilling develop during storage at non-chilling temperatures (Kader and Arpaia, 2002). Rind pitting falls within this category of citrus rind disorders. Citrus rind pitting is associated with a multitude of preharvest ecophysiological factors and postharvest treatments (Petracek *et al.*, 1995; 1998; Alferez and Zacarias, 2001). Different researchers report tree aspect, fruit position within a canopy (Wild, 1991; Almela *et al.*, 1992; Medeira *et al.*, 1999), mineral nutrition (Khalidy *et al.*, 1969; Tamin *et al.*, 2001; Kruger *et al.*, 2003a; 2005; Kruger and Lemmer, 2006), fruit maturity (Wild, 1991; Leguizamon *et al.*, 2001), crop yield (Leguizamon *et al.*, 2001), adverse climatic conditions and water stress during critical stages of fruit development (Wild, 1991; Tamin *et al.*, 2001; Kruger *et al.*, 2003a; 2005) to be some of the important preharvest causative factors of rind pitting.

It has been suggested by different researchers that the development of rind pitting and other rind disorders not related to chilling may be enhanced by excessive water loss from the peel tissues (Albrigo, 1972; Vercher *et al.*, 1994; Porat *et al.*, 2004). Water loss in fruit is believed to occur as a result of a water pressure gradient that prevails between the fruit, which is normally close to saturation within intercellular spaces, and the less saturated surrounding ambient environment (Kader *et al.*, 1989; Kader, 1992). From the above documented facts, a number of investigators have studied different postharvest treatments that could possibly reduce development of rind pitting by reducing water loss. The use of different waxes and, to a greater extent, the use of modified atmosphere packaging (MAP), has been shown to reduce the incidence and intensity of the disorder (Albrigo, 1972; Vercher *et al.*, 1994; Alferez and Zacarias, 2001; Agusti *et al.*, 2004; Porat *et al.*, 2004). MAP maintains a high humidity environment, and thus reduces development of rind disorders caused by water loss (El-Otmani and Ait-Oubahou, 1996). Despite the evidence that the above-mentioned postharvest treatments reduce the rind pitting disorder, it should be noted that measures introduced to decrease moisture loss may

increase CO₂ and reduce O₂ and ethylene concentrations which in turn may cause anaerobic conditions that promote development of off-flavours (Kader, 1992). Inappropriate MAP may further promote the development of rind pitting upon removal of fruit from the MAP system (Alferez and Burns, 2004). This serves to demonstrate that the problem is still present but kept under control by the MAP system. It is therefore important to study the epidemiology of the disorder, so as to determine and deal with its underlying causes.

Over the past decade, the Postharvest Section of the ARC-ITSC has initiated a project aimed at studying the epidemiology of rind disorders of tropical and subtropical fruit and nut crops. Various preharvest factors playing roles in physiological disorders were studied, amongst which were nutrition, harvest maturity, climate and horticultural practices such as irrigation. Previous experience has taught us that fruit mineral status during certain times of fruit development is important in the development of physiological disorders. Therefore, one of the most important investigative techniques involved monitoring the N, P, K, Ca, Mg, Zn, Cu, Mn, Fe and B content of the skin and pulp of different crops during the maturation process. Other potential causative factors investigated were harvest maturity, climate and horticultural practices such as irrigation and fertilization. The first crop to be investigated in this manner was avocado, after the industry launched an intensive investigation into the problem of poor storage potential (Kruger *et al.*, 2000; 2002; 2004). As a consequence of positive results reported in avocados, the approach was extended to citrus fruit. Preliminary results on citrus showed some interesting trends, in which rind mineral concentration (nitrogen and iron in particular), were observed to be correlated to the incidence of rind pitting (Kruger *et al.*, 2005). The results obtained on 'Valencia' oranges have shown that fertilizing with nitrogen in excess of the recommended dosage was related to rind pitting. This was also confirmed on 'Midnight' oranges. In spite of the above, considerable superficial rind pitting still occurs each year on normally fertilized 'Valencia' oranges, especially in the 'Benny Valencia' cultivar (Piner, pers. comm.). The occurrence of physiological disorders in apparently correctly fertilized orchards implies that a proportion of the disorder development can be ascribed to other

influences. Climatic conditions that influence the translocation of the elements may be involved.

In this study, the epidemiology of rind pitting in 'Benny Valencia' citrus was investigated. In order to investigate the possible role of nitrogen in the development of rind pitting, application of the element towards the end of the season was made. The study further investigated whether a relationship between the rind mineral concentration and the risk of developing rind pitting during subsequent storage exists.

MATERIALS AND METHODS

Experimental site

A field experiment was carried out in a 'Benny Valencia' orchard at Crocodile River Valley citrus farm, situated in the Nelspruit area of Mpumalanga, South Africa (25°27'S, 30°58'E). This area is characterised by a subtropical climate with average rainfall of 767mm per annum, an average daily minimum and maximum temperatures of 13 and 27°C respectively (ARC – Soil Water and Climate Institute).

Treatments and replications

The treatments included the addition of two different formulations of nitrogen (LAN at a rate of 200 grams per tree, slow release nitrogen fertilizer traded as Hortecote[®]100 at a rate of 300 grams per tree), as well as the untreated control. Each treatment provided a different amount of nitrogen per tree during the experimental period. Hortecote[®]100 (40) provide 120g N while LAN (28) provide 56g of nitrogen per tree. A decision on these rates was based on the form of application and the time of nitrogen availability to the tree. Hortecote[®]100 is a slow-release nitrogen fertilizer which takes about 100 days for 80% of nitrogen to be released at constant temperature of 20°C. LAN on the other hand is dissolved into the soil solution and immediately available to the tree. It was therefore

suggested by soil scientist (Abercrombie, pers. comm.) that by the first date of harvest, each treatment would have received relatively similar amounts of nitrogen. These calculations were based on the principle of the half life of Horticote® compound of which the amount of nitrogen released from the compound after a certain number of days is presented in **Table 1**. On the table it is shown that by the first sampling date (04/05/2006), the amount of nitrogen received from the Horticote® treatment will be equal to the N received from the LAN treatment. The treatments were applied on the 14th of March 2006. Fruit sampling was conducted on the day of treatment as well as at two-week intervals from the 4th of May 2006 until commercial maturity was reached on the 10th of July 2006. When designing the trial, the authors were acutely aware of the changing nitrogen availability to the slow release treated trees while LAN nitrogen becomes available some time before harvest. The idea was to compare the continuous with the once off supply of nitrogen.

TABLE 1
Amount of nitrogen released from LAN and Horticote® treatments during the sampling period.

Sampling No.	Sampling date	N Days	Grams N released Horticote®	Grams N released from LAN
	12/05/2006	28	43.55	56.00
1	04/05/2006	42	58.97	56.00
2	18/05/2006	56	71.29	56.00
3	01/06/2006	70	81.12	56.00
4	14/06/2006	84	88.97	56.00
5	28/06/2006	98	95.23	56.00
6	10/07/2006	112	100.23	56.00

The statistical layout of the trial was a completely randomised block design consisting of 4 replications of 5 trees each, of which the 3 middle trees served as data trees.

Sampling

As from the 4th of May 2006, a total of 42 fruit (21 from eastern and 21 from western side of each tree) were sampled from each tree on each sampling date. Twelve of these fruit per treatment, 6 from the east and 6 from the west side of each tree, were retained for maturity determination and mineral analysis. The remaining sample of 30 fruit per tree was packed in commercial boxes and stored at room temperature for 40 days. At two week intervals, fruit samples were weighed and evaluated for development of superficial rind pitting. The incidence of rind pitting was scored on a subjective scale from 0 to 3, where 0 was an unaffected fruit and 3 was a severely affected fruit. Rind pitting was recorded and correlated with fruit weight loss and preharvest conditions. For each sampling date, rind pitting data from the final evaluation was plotted against time. Sampling continued until commercial harvesting of the experimental block took place.

Sample analysis

Upon arrival at the Burgershall laboratory, the exocarp (flavedo), mesocarp (albedo) and endocarp (juice vesicles) of retained samples were separated. The exocarp and mesocarp samples were oven-dried at 70°C, after which they were sent to the ARC-ITSC Soil Science Laboratory in Nelspruit where mineral analysis was executed using atomic absorption spectrometry. The mineral content measured included N, P, K, Ca, Mg, Zn, Cu, Mn, Fe, and B. Nitrogen concentration was determined by colorimetric auto analyzing after wet digestion with 98% sulphuric acid and 30% hydrogen peroxide (Adrian, 1973; Giron, 1973; Novozamsky *et al.*, 1983). The concentrations of the other minerals were determined after digestion and extraction with 2:1 55% nitric acid and 70% perchloric acid (Adrian, 1973; Giron, 1973; Novozamsky *et al.*, 1983). The concentrations of Ca, Mg, Zn, Cu, Mn and Fe were determined by atomic absorption flame spectroscopy using air-acetylene (Price, 1972). Potassium was determined by flame atomic emission spectroscopy using air-acetylene (Poluektov, 1973). Phosphorus and boron concentrations were measured colorimetrically using an auto analyzer.

Fruit maturity at each sampling date was determined by measuring total sugars and titratable acid (TA) of the juice. Total sugars was measured using a hand-held digital refractometer (Atago® Pallete series, PR-32a) and was expressed as °Brix while TA measurements were executed by titrating 10ml of juice against 0.1N sodium hydroxide (NaOH), and results calculated as a percentage of the juice.

Statistical analysis

Statistical analysis included the analysis of variance by Fischer's method where means of treatments were compared using least significant difference (LSD, $P = 0.05$) and Student's t-test ($P = 0.05$) using GenStat® version 6 (VSN International Hemel Hempstead, UK).

RESULTS AND DISCUSSION

Rind pitting disorder

Rind pitting results indicated that throughout the sampling period fruit from trees receiving additional nitrogen tended to be more susceptible to rind pitting than control fruit (**Figures 1 and 2**). Of the two nitrogen treatments, fruit from trees that received the slow release formulation of nitrogen also appeared to be affected to a greater degree, although the differences between treatments were only statistically significant on one of the six sampling dates. For fruit from both the western and eastern sides of the tree, the statistically significant difference between treatments was observed on the sampling date 14/06/2006. The severity of pitting also varied with sampling date. The incidence of rind pitting was low at the beginning of the sampling period, followed by a peak and a decline towards the end of the sampling period. Again, the control tended towards the lowest intensity of rind pitting.

With regard to fruit position within a canopy, the severity of the disorder was not affected by fruit position (**Figure 3**). These results disagree with the findings

reported by Almela *et al.* (1992), where the incidence of rind pitting was higher in fruit exposed to the sun than non-exposed fruits, and higher on the exposed than the non-exposed side of individual fruits. These authors also reported that fruit oriented to the North-West (Northern hemisphere) of the canopy were most affected by the disorder. A similar situation was found with peteca disorder on lemon fruit, where fruit hanging on the east side of trees are found to be more susceptible than fruit on the west side (Southern hemisphere) (Wild, 1991). Continuous evaluation of harvested fruit showed that the intensity of the disorder in fruit rind increased with storage duration (data not shown). It has been suggested by different researchers that the development of rind pitting may be enhanced by excessive water loss from the peel tissues (Albrigo, 1972; Vercher *et al.*, 1994; Porat *et al.*, 2004). However, no differences in weight loss were observed between treatments. It therefore appears that certain preharvest factors augment development of the disorder.

Mineral nutrient composition of the rind

Certain trends with regard to rind mineral content were observed. The nitrogen concentration of fruit harvested from the eastern side of the trees was not significantly different (**Figure 4**). On fruit from the western side, the nitrogen concentration in the exocarp of oranges from trees that received Horticote[®] was significantly lower ($P < 0.05$) than that of the LAN and control treatments (**Figure 5**). The difference between nitrogen concentration of nitrogen treatments and the control was influenced by fruit position (**Figure 6**). Fruit located on the western side of the tree had higher nitrogen concentration than fruit on the eastern side, especially on the LAN and control treatments ($P < 0.01$). However, there was no difference in rind pitting. These observations seem to imply that nitrogen concentration in the exocarp is not significant in the development of the disorder. The average difference between nitrogen content of fruit from east and west side of the trees treated with Horticote[®] was not significant. In addition, the fruit from nitrogen treated trees were significantly smaller than the control fruit (**Figures 7 and 8**). Fruit from trees that received the LAN nitrogen treatment were the smallest.

The nitrogen results in relation to rind pitting seem to show that application of additional nitrogen seems to influence rind pitting, but the influence of nitrogen concentration in the rind is not strong. It is suggested that nitrogen applied late in the season may have been directed to vegetative growth at the expense of the fruit (Witney *et al.*, 1990; Khemira *et al.*, 1998; Toselli *et al.*, 2000). Similar results have been described by Sanchez *et al.* (1992) and Khemira *et al.* (1998), who reported that nitrogen applied to pear fruit 3-6 weeks before harvest increased nitrogen in above ground vegetative tissues but not in fruits. Previously, Kruger *et al.* (2005) hypothesised that late season rainfall enhances increased nitrogen uptake and subsequent late growth, which may result in the occurrence of newly developed cells that die-off during subsequent storage. From the results obtained in this study, it would seem that this hypothesis does not hold true. It has been reported that carbohydrate distribution within a plant is affected by nitrogen supply, which strongly influences the processes of carbon assimilation, allocation and partitioning (Kaiser, 1997). Sink/source relations may have changed in response to late nitrogen application, resulting from enhanced vegetative growth which could have established an alternative sink competing with fruit for assimilates and mineral elements. Both the nitrogen and carbohydrate status have great impact on fruit maturity and postharvest quality (Traub *et al.*, 1933; Kallsen, 1999; Garcia-Luis *et al.*, 2002). Obtained maturity data, however, do not show significant differences between different treatments. This may also be related to the fact that nitrogen treated fruit were smaller. Hence, although total carbohydrates were lower, concentration was high enough to be comparable to that of bigger control fruit.

Mineral elements that would have been directed to vegetative plant parts instead of fruit, as observed by Fallahi *et al.* (2001), include, among others, calcium, magnesium and manganese. Unexpectedly, the mean of calcium (**Figure 9**), magnesium (**Figure 10**) and manganese (**Figure 11**) concentrations in the rind of fruit receiving extra nitrogen were consistently higher than the controls. In this case, it is important to note that fruit from nitrogen treatments were smaller than the control fruit. The decrease in concentration of these minerals in bigger fruit

could have resulted from fruit expansion and consequent dilution of the present mineral concentration (Jones *et al.*, 1983; Kruger *et al.*, 2000; 2005).

The exocarp mineral concentration of three treatments is shown in **Table 2** while the mineral concentration of mesocarp is shown in **Table 3**. As is the case with a number of subtropical fruit such as mango (Kruger *et al.*, 2003c; Kruger and Fraser, 2004) and avocado (Kruger and Lemmer, 2006), the exocarp of these fruit contained significantly higher concentrations of minerals than the mesocarp of the fruit (**Table 4**). However, zinc was the exception to this observation in that the mesocarp had a higher content of zinc than the exocarp.

With regard to fruit position in a canopy, results similar to those reported by Kruger *et al.* (2005) on citrus were observed. The mineral composition of fruit differed between tree aspects in the canopy and is summarised in **Tables 5 and 6**, where the mean mineral content for the whole sampling period and eastern:western ratios of mineral elements are respectively shown. Fruit from the eastern side of the tree were found to contain significantly lower concentrations of more mobile N, P and K than western fruit. However, the mean eastern:western ratio for nitrogen of treated fruit was close to 1 while that of control fruit was significantly lower than 1. Thus, nitrogen content of treated fruit (Hortecote[®] treatment in particular) was evenly distributed within the canopy. In contrast to mobile elements, less mobile Ca, Mg, Cu, and B were found to be consistently lower in fruit positioned on the western side than fruit on the eastern side of the canopy. However, other immobile elements such zinc, manganese, copper and iron, an opposite trend was noticed in that rind of fruit from the eastern side contained lower concentrations of these elements than the western side. These results are in agreement with those reported by Kruger *et al.* (2005), where although the ratio of outside:inside fruit of other immobile elements was consistently higher than 1, the indices for iron and zinc were not. It is suggested that this may be as a result of the light and heat dependent transport of these immobile elements that fruit on the eastern side of the tree had more immobile elements (Curie *et al.*, 2000; Curie and Briat, 2003).

Kruger *et al.* (2003a) reported that exposure to radiation heat improves uptake of immobile elements and the daily distribution pattern of the heat units may possibly play a role. Fruit from the hotter western side (Barry *et al.*, 2003) of the tree would be expected to be more mature and have higher immobile elements (Kruger *et al.*, 2003a; 2005). However, high afternoon temperatures to which the western side was exposed to, nearly always occur during periods of high water stress that may lead to the cessation of transpiration by stomatal closure (Fitter and Hay, 1987). Gernama and Sardo (1996) reported a correlation between xylem water potential and net photosynthesis in orange trees. Stomatal closure would in turn decrease the transpiration stream, reducing water movement and subsequently reducing translocation of relatively immobile elements into the fruit. Kamota *et al.* (1974) found higher water use in 'Satsuma' in the early morning and afternoon as compared to midday. The effects of water stress during this period may be linked to net carbohydrate accumulation resulting from photosynthesis in adjacent leaves and fruit (Blanke and Lenz, 1989). Over a day the cooler eastern side is less subject to stress, thus transpiration and photosynthesis are higher due to stomata staying open for longer. In this case, the cooler eastern side of the tree was arguably subject to higher transpiration and photosynthesis, hence it had more mature fruit and higher immobile elements such as calcium, magnesium and boron than the western side of the tree.

Magnesium is the metal moiety of the chlorophyll molecule and is thus indispensable for photosynthesis. Usually shade leaves contain more chlorophyll per unit leaf mass than sun leaves, in accordance with their ability to make use of low light intensities (Boardman, 1977; Dorenstouter *et al.*, 1985). The magnesium concentration of plant tissues varies not only with exposure to radiation, but also at different ages of the same plant and plant parts (Marschner, 1995). The magnesium concentration of citrus fruit was reported to decline as the fruit matures (Kruger *et al.*, 2005) and it is thus assumed that fruit on the eastern side were more mature than those on the western side.

With regard to manganese, literature reports agree that manganese accumulates preferentially in leaves exposed to higher temperature or higher light intensity (Heenan and Carter, 1975; Ruffy *et al.*, 1979; McCain and Markley, 1989), and sun leaves contain more Mn per unit area than shade leaves (McCain and Markley, 1989). In this study, it was noted that control fruit which had a higher concentration of nitrogen on the western side consistently had lower manganese on this side of the tree (**Table 5**). Robertson (1951) reported an association between manganese with the uptake of nitrogen by the roots. Investigations reviewed by Wood (1953) confirm that manganese is necessary for nitrogen reduction and assimilation. North and Wallace (1959) suggested that if nitrogen is applied as ammonium nitrate, nitrification of the ammonium ions takes place so that mostly nitrate is absorbed by the plant and this may account for the increasing manganese with increasing nitrogen levels. However, if not nitrified, ammonium competes with manganese for uptake in the root zone. This could explain why, in this study, fruit with high nitrogen concentration had lower manganese concentration than low nitrogen fruit.

When comparing the rind mineral concentration of fruit with rind pitting (pitted) to those without rind pitting (non pitted), the mean concentration of immobile elements in pitted fruit was higher ($P < 0.05$) than that of non pitted fruit (**Table 7**). Irrespective of rind pitting, the control fruit had a higher concentration of immobile elements than treated fruit. The mean elemental ratio of pitted/non pitted fruit shows that all indices were around 1 except for calcium, magnesium and boron that were considerably higher than 1. Indices greater than 1 imply that the concentrations of these elements were significantly ($P < 0.005$) higher in pitting-affected fruit than in unaffected fruit. High concentration of immobile elements in pitted fruit as well as control fruit, which had lower pitting suggest that if mineral elements play a role in the disorder, the effect is not direct and the physiology may be complex and still not known. In many fruits low, rather than high, calcium concentration has been implicated in the incidence of physiological disorders (Poovaiah *et al.*, 1988). However, gold speck of tomato fruit is an example of a disorder associated with excess calcium (De Kreij *et al.*, 1992). In peteca, a lemon rind disorder, Khalidy *et al.* (1969) and Storey and Treeby (2002) reported

an increase in calcium levels in peteca-affected regions. Storey and Treeby (2002) suggested that high levels calcium on lemon rinds causes lemon rind pitting.

Conversely to immobile elements, the mobile mineral content in pitted fruit was significantly lower ($P < 0.001$) than that of non pitted fruit. What is also important to note is that nitrogen and zinc were the only two elements with indices equal to 1. In contrast to current observations, Kruger *et al.* (2005) found non pitted fruit to have higher rind nitrogen content than affected fruit. It is, however, important to note that these authors only analysed holdback samples from export consignments with fruit divided into pitted and non pitted groups. In addition, potassium and phosphorus concentration were significantly ($P < 0.005$) lower in pitting affected fruit compared with unaffected fruit. Hence phosphorus and potassium indices were below 1. Similar results were observed for lemon peteca by Storey and Treeby (2002) and rind breakdown on clementines by Kruger *et al.* (2005). Storey and Treeby (2002) explained that lower potassium in cells might be indicative of loss of cell integrity and ion leakage as potassium is, generally, the most abundant solute of plant cells.

Overall, it appears that while some trends were evident, there is not sufficient statistical evidence to demonstrate the effects of nitrogen application on rind pitting, nor the mechanism or involvement of other mineral elements, and thus results are inconclusive as to the effect of late nitrogen application on the development of rind pitting. It is concluded that while there was a tendency for late nitrogen applications to enhance the incidence and intensity of rind pitting, the mechanism may be indirect. It is therefore impossible to draw any clear conclusions regarding the relationship between nitrogen and rind pitting disorder. Since nitrogen partitioning in fruit trees is largely affected by application time and, preferentially, to the vegetative parts (Stassen *et al.*, 1981a; 1981b; Khemira *et al.*, 1998; Sanchez *et al.*, 1992), it would seem that late nitrogen application may have promoted vegetative growth, hence partitioning minerals and assimilates to actively growing and transpiring shoots at the expense of the fruit. However, whether any of these elements played a direct role in development of rind pitting,

or whether other unknown factors were influences and were the primary drivers, is not known. Of the two nitrogen treatments, fruit that received slow release fertilizer tended towards highest incidence of the disorder, implying that the residual effect of the once off LAN treatment was shorter than that of continuous supply provided by the slow release Hortivate[®] treatment.

The study further demonstrated that the incidence and severity of the disorder varied with sampling dates, with relatively low incidence at the beginning of the sampling period followed by a peak and then a drop towards the end of the period. The reason for this is unknown, and may relate to some fruit maturity factor or environmental conditions. Nevertheless, despite the uncertain effect of nitrogen application on rind pitting, the demonstrated tendency to enhance the disorder should indicate caution, and in the absence of other clear causative evidence, it is suggested that late (near harvest) nitrogen applications should be avoided.

ACKNOWLEDGEMENTS

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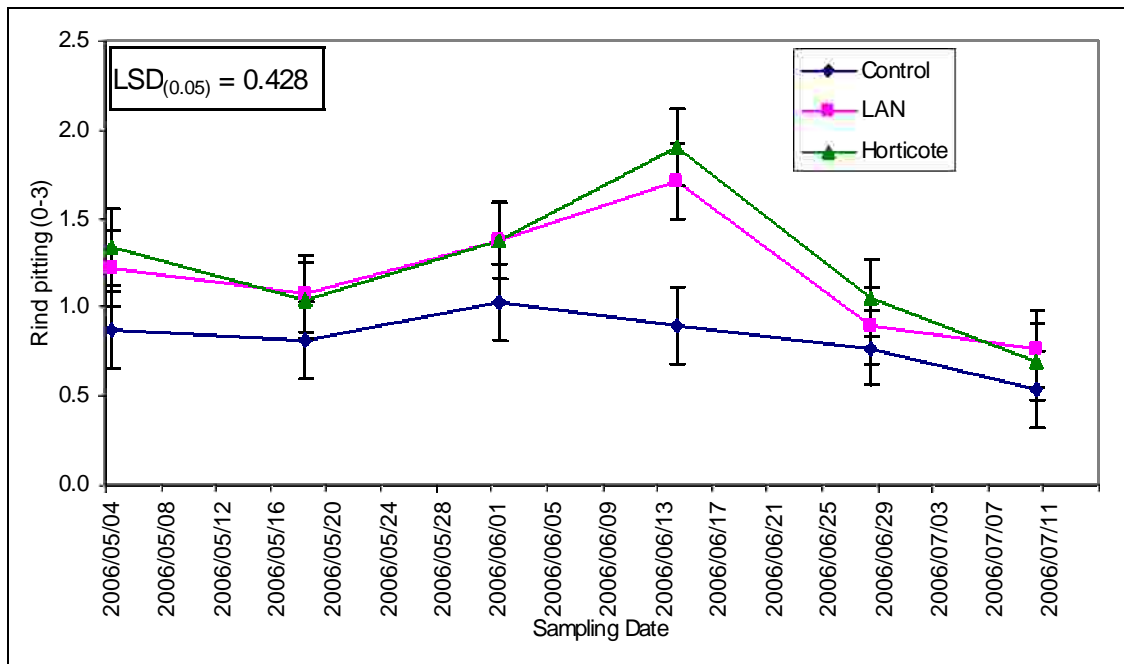


FIGURE 1

Severity of rind pitting on fruit positioned on the eastern side of the tree, as affected by nitrogen treatments and sampling dates. Error bars represent standard error of the mean.

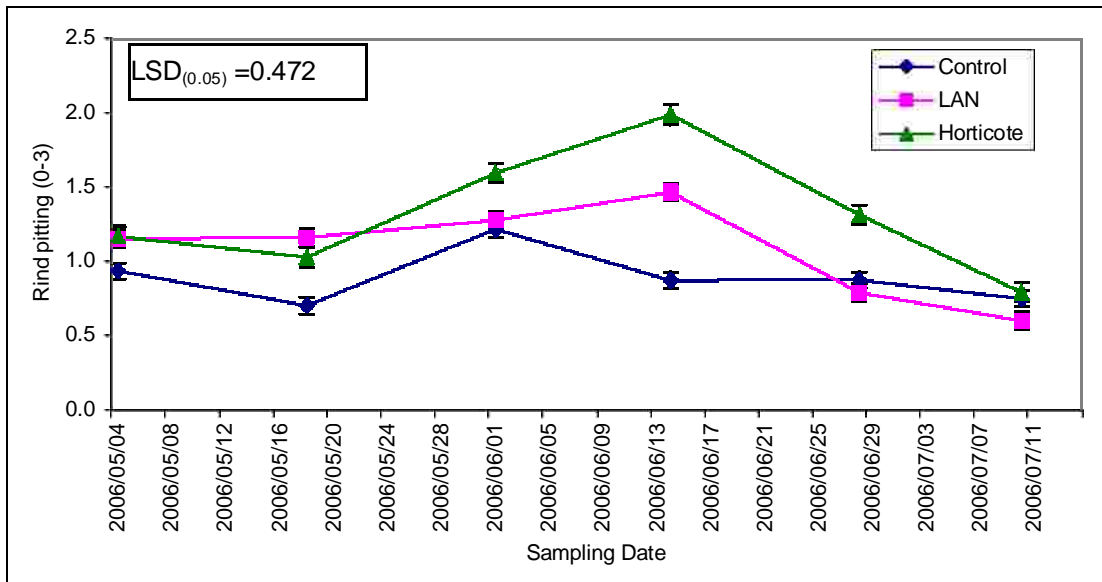


FIGURE 2

Severity of rind pitting on fruit positioned on the western side of the tree, as affected by nitrogen treatments and sampling dates. Error bars represent standard error of the mean.

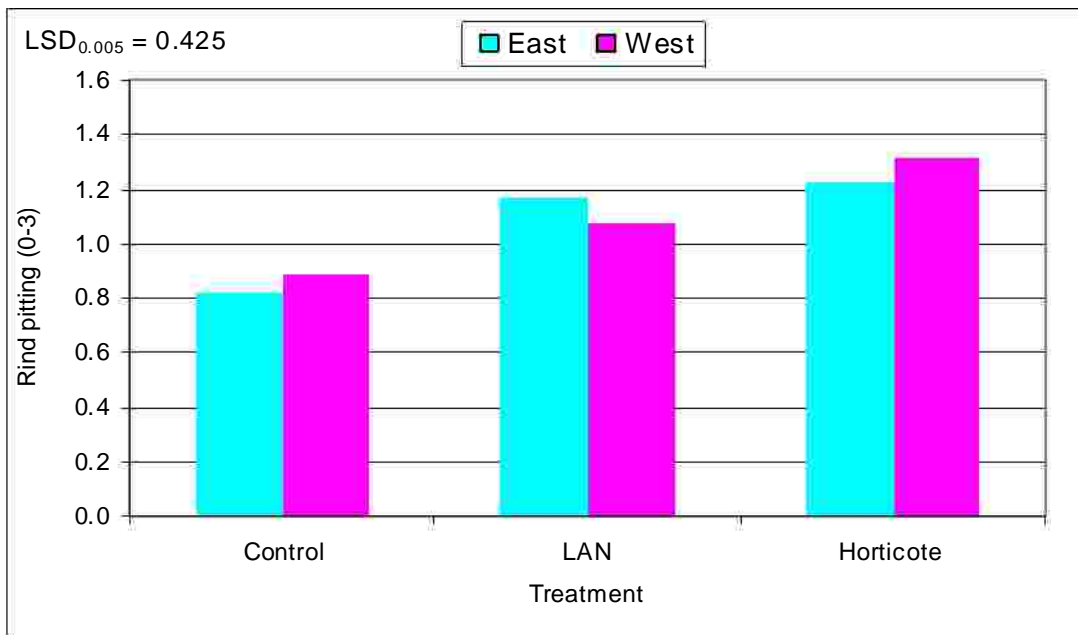


FIGURE 3

Severity of rind pitting on fruit positioned on eastern and western sides of the tree, as affected by nitrogen treatments. Error bars represent standard error of the mean.

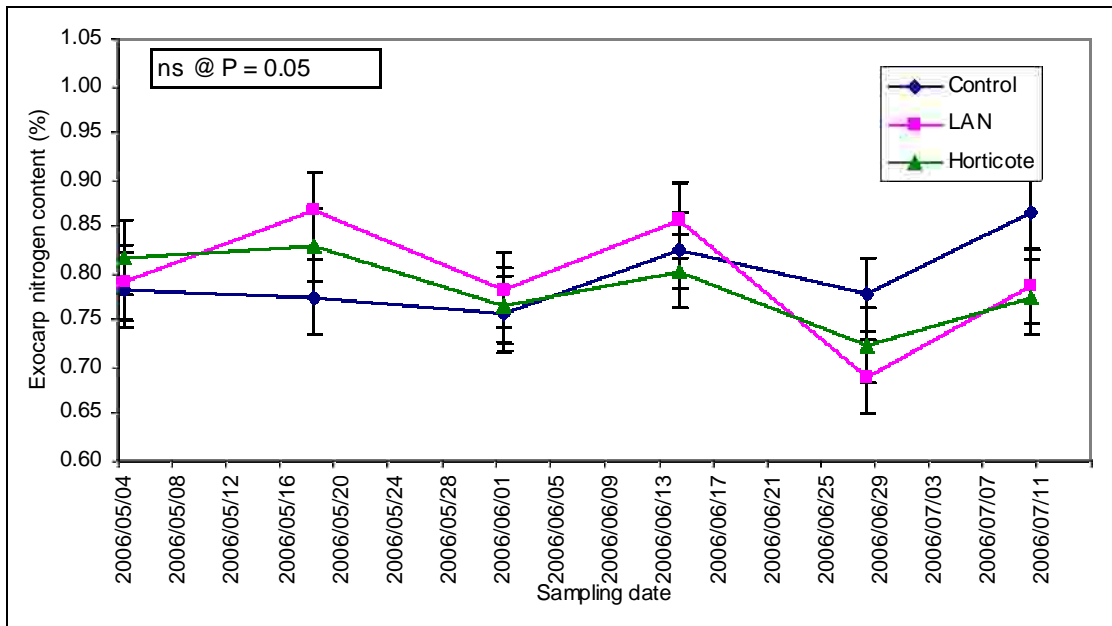


FIGURE 4

Exocarp nitrogen concentration of oranges harvested on different sampling dates from the eastern side of the tree. Error bars represent standard error of the mean.

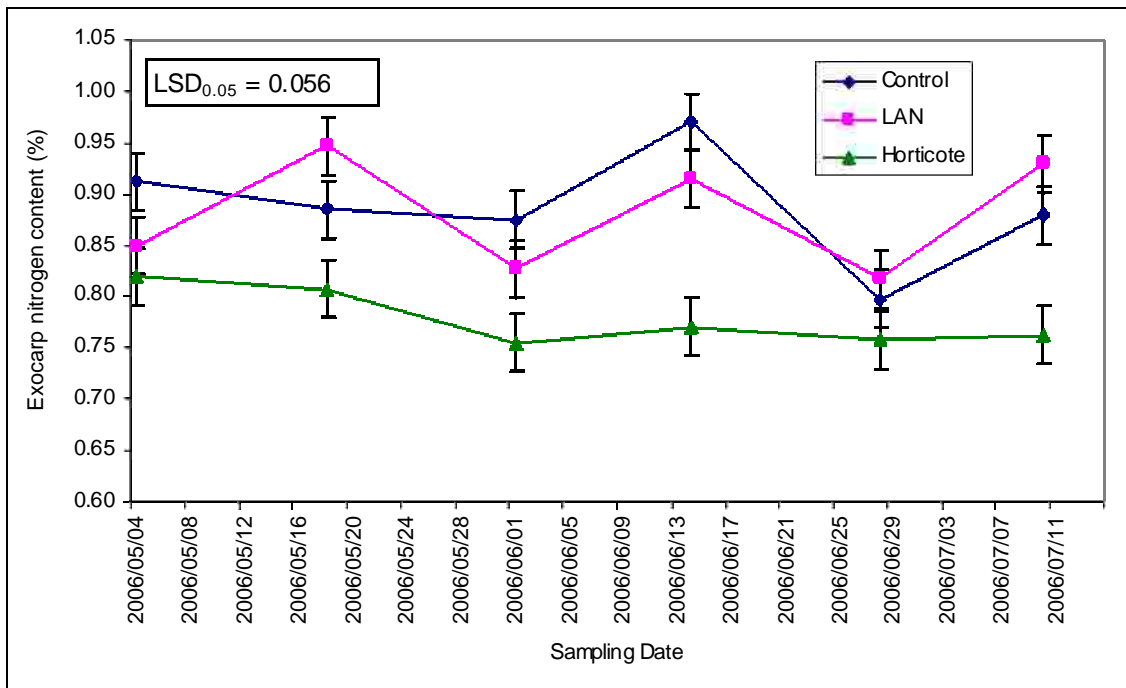


FIGURE 5

Exocarp nitrogen concentration of oranges harvested on different sampling dates from the western side of the tree. Error bars represent standard error of the mean.

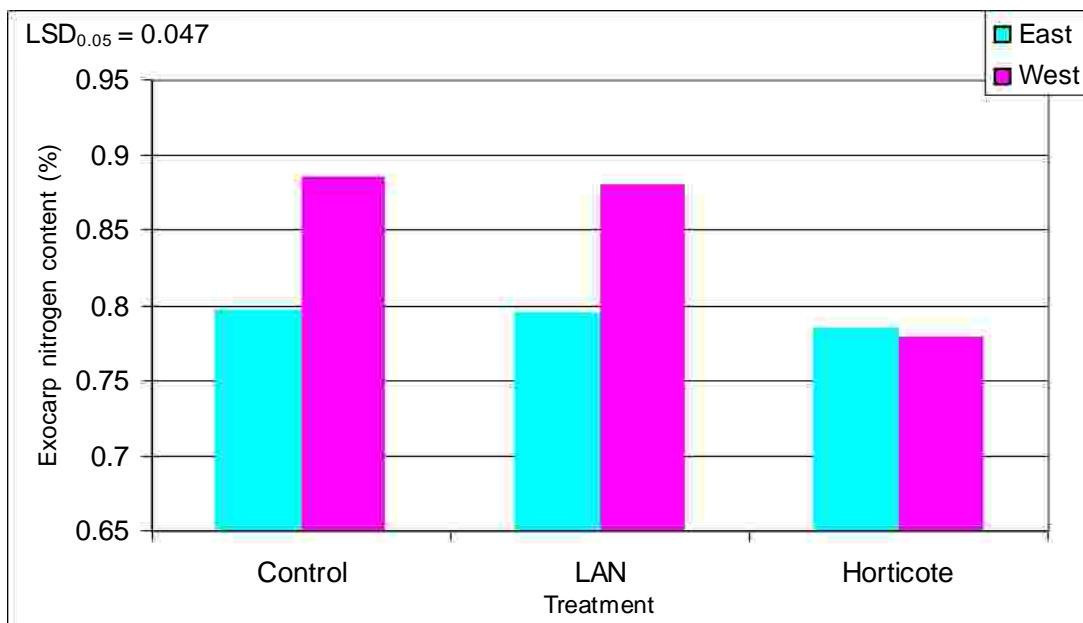


FIGURE 6

Mean nitrogen concentration on the exocarp of oranges harvested from western and eastern sides of the tree. Error bars represent standard error of the mean.

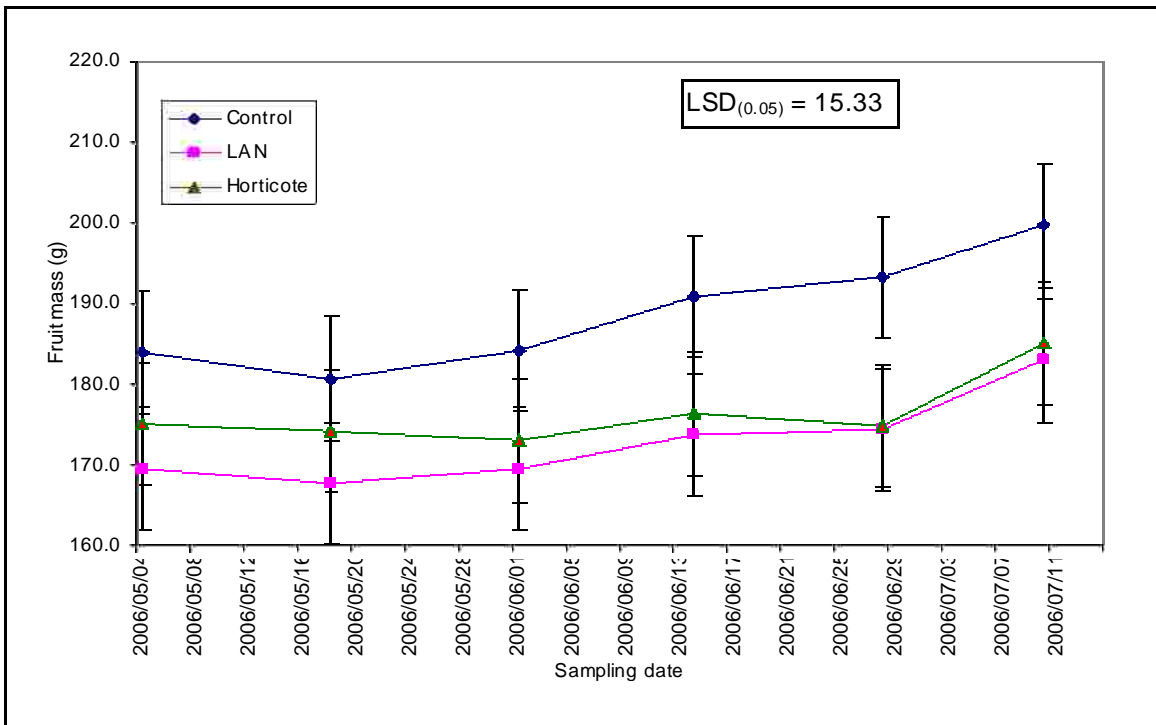


FIGURE 7

Fruit mass of oranges harvested from the eastern side of the tree at different sampling dates. Error bars represent standard error of the mean.

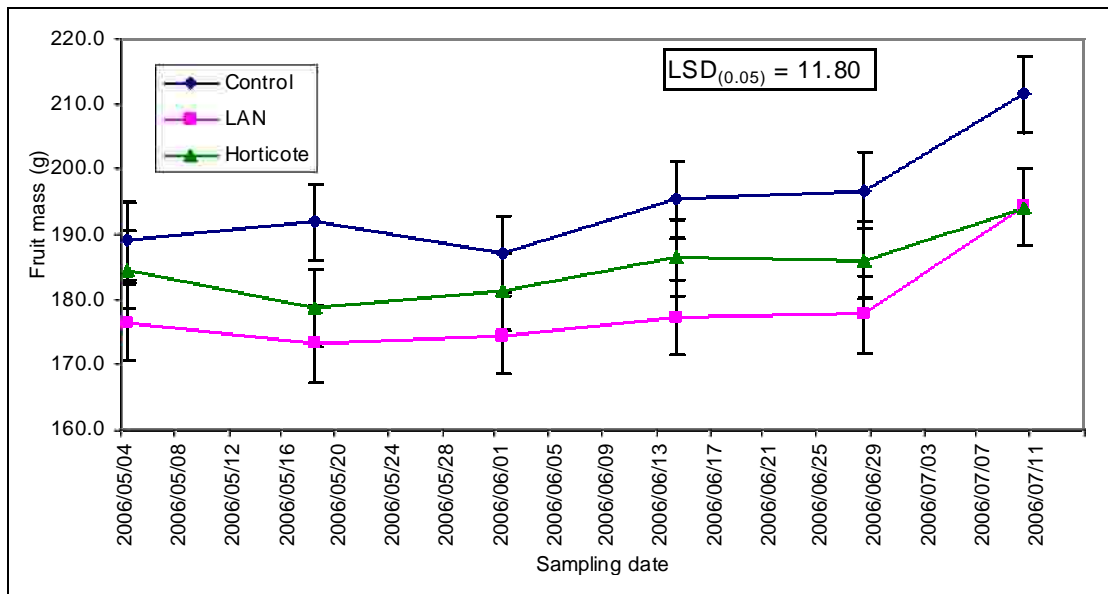


FIGURE 8

Fruit mass of oranges harvested from the western side of the tree at different sampling dates. Error bars represent standard error of the mean.

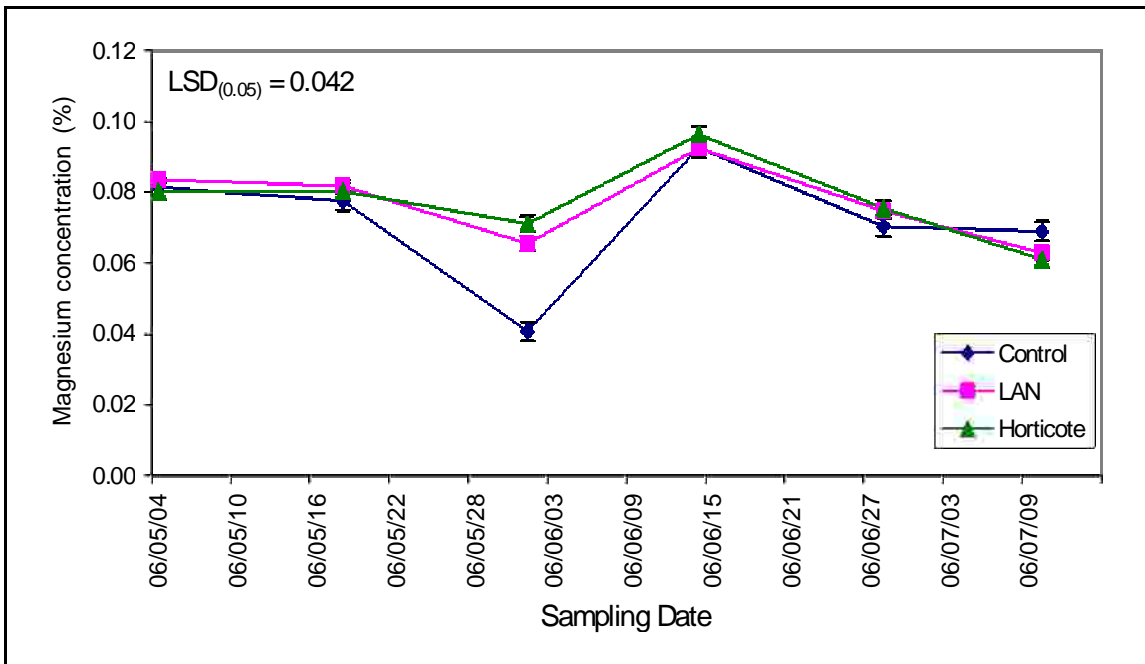


FIGURE 9

Means of mesocarp magnesium concentrations of oranges from three nitrogen treatments. Error bars represent standard error of the mean.

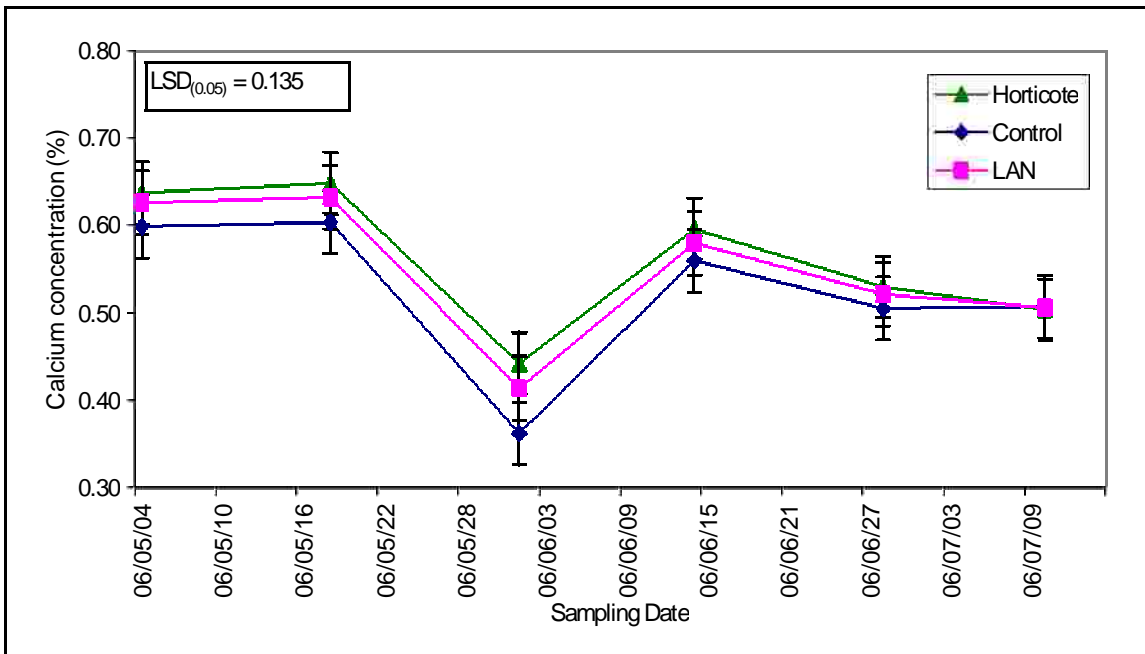


FIGURE 10

Means of mesocarp calcium concentrations of oranges from three nitrogen treatments. Error bars represent standard error of the mean.

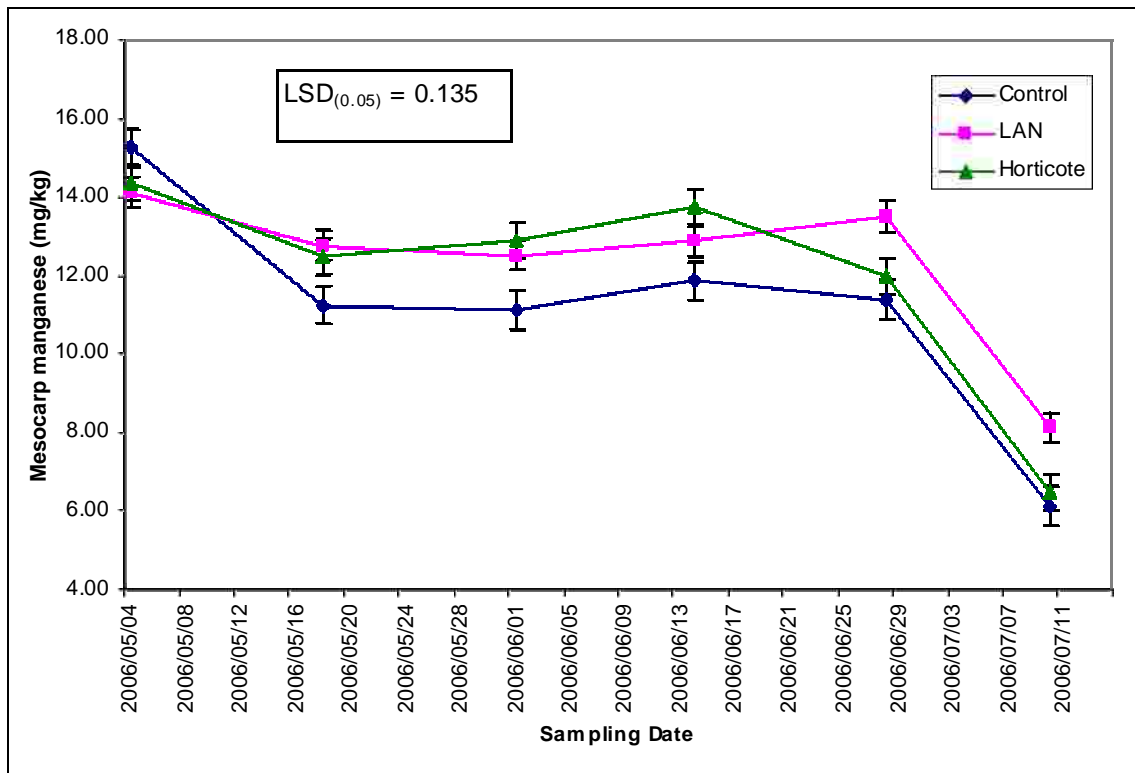


FIGURE 11

Mean mesocarp manganese concentration of oranges harvested on different sampling dates from three nitrogen treatments. Error bars represent standard error of the mean.

TABLE 2
Exocarp mineral concentrations in fruit of three treatments from different sampling dates.

Sampling date	N %	P %	K %	Ca %	Mg %	Zn Mg/kg	Cu mg/kg	Mn mg/kg	Fe mg/kg	B mg/kg
<u>Control</u>										
04/05/2006	0.848	0.053	1.480	0.648	0.132	6.750	1.500	27.375	57.750	26.200
18/05/2006	0.830	0.057	1.421	0.678	0.123	5.750	3.500	26.625	41.250	26.813
01/06/2006	0.816	0.059	1.498	0.591	0.107	9.000	4.000	31.875	45.125	23.400
14/06/2006	0.898	0.074	1.680	0.650	0.140	6.625	3.125	30.875	58.250	19.850
28/06/2006	0.788	0.070	1.454	0.623	0.125	9.625	2.000	33.500	45.500	18.988
10/07/2006	0.873	0.073	1.395	0.591	0.105	9.750	2.250	32.000	56.750	23.938
<u>LAN</u>										
04/05/2006	0.820	0.049	1.410	0.690	0.152	7.125	1.500	26.625	55.625	28.263
18/05/2006	0.908	0.057	1.499	0.803	0.157	5.875	3.250	30.750	45.250	25.175
01/06/2006	0.805	0.054	1.466	0.639	0.143	8.500	1.875	28.750	40.750	18.825
14/06/2006	0.886	0.064	1.513	0.705	0.156	8.125	4.250	39.125	67.000	19.713
28/06/2006	0.754	0.066	1.441	0.621	0.130	9.875	2.375	33.625	46.875	17.525
10/07/2006	0.859	0.066	1.477	0.633	0.130	10.000	2.000	32.875	58.125	22.138
<u>Horticote</u>										
04/05/2006	0.819	0.053	1.365	0.765	0.140	7.625	1.500	27.500	62.125	26.650
18/05/2006	0.819	0.058	1.563	0.814	0.140	6.250	3.125	33.375	51.500	21.100
01/06/2006	0.760	0.060	1.530	0.555	0.109	8.000	1.750	28.750	43.625	22.263
14/06/2006	0.786	0.061	1.499	0.695	0.133	6.625	2.875	33.875	58.125	18.775
28/06/2006	0.740	0.067	1.440	0.659	0.124	10.000	1.625	33.000	47.000	17.088
10/07/2006	0.769	0.067	1.490	0.640	0.117	8.875	2.000	25.250	53.875	18.238
<u>LSD (0.05) n=24</u>										
04/05/2006	ns	ns	ns	ns	ns	ns	ns	ns	ns	1.093
18/05/2006	ns	ns	ns	0.136	ns	ns	ns	2.902	ns	4.327
01/06/2006	0.0362	ns	ns	ns	ns	ns	ns	ns	ns	ns
14/06/2006	0.0862	ns	ns	ns	ns	ns	ns	6.04	ns	ns
28/06/2006	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
10/07/2006	0.0842	ns	ns	ns	ns	0.661	ns	5.834	ns	4.291

TABLE 3
Mesocarp mineral concentrations in fruit of three treatments from different sampling dates.

Sampling date	N %	P %	K %	Ca %	Mg %	Zn mg/kg	Cu Mg/kg	Mn mg/kg	Fe Mg/kg	B mg/kg
<u>Control</u>										
04/05/2006	0.446	0.024	0.398	0.599	0.081	15.750	2.000	15.250	30.750	22.613
18/05/2006	0.489	0.024	0.376	0.604	0.078	23.000	8.875	11.250	27.000	18.813
01/06/2006	0.398	0.024	0.306	0.363	0.041	16.375	1.250	11.125	22.375	25.325
14/06/2006	0.413	0.024	0.335	0.560	0.093	15.375	2.375	11.875	26.000	17.450
28/06/2006	0.394	0.034	0.449	0.505	0.070	19.750	1.250	11.375	22.875	16.913
10/07/2006	0.415	0.026	0.489	0.508	0.069	16.250	2.000	6.125	23.875	15.988
<u>LAN</u>										
04/05/2006	0.455	0.019	0.325	0.626	0.084	18.875	1.875	14.125	38.500	22.088
18/05/2006	0.466	0.019	0.333	0.633	0.082	21.000	6.125	12.750	26.500	26.750
01/06/2006	0.415	0.022	0.300	0.414	0.066	21.000	1.250	12.500	26.875	24.813
14/06/2006	0.416	0.022	0.338	0.580	0.093	19.000	2.500	12.875	26.375	17.688
28/06/2006	0.395	0.027	0.445	0.521	0.075	20.250	1.500	13.500	24.625	16.538
10/07/2006	0.405	0.025	0.445	0.506	0.063	21.375	2.000	8.125	33.250	15.775
<u>Horticote</u>										
04/05/2006	0.444	0.022	0.370	0.638	0.080	17.625	1.750	14.375	24.750	21.400
18/05/2006	0.450	0.021	0.371	0.649	0.080	17.375	3.750	12.500	30.125	24.650
01/06/2006	0.404	0.028	0.369	0.443	0.071	20.250	1.625	12.875	25.125	28.213
14/06/2006	0.385	0.024	0.415	0.596	0.096	17.125	2.750	13.750	29.250	19.463
28/06/2006	0.405	0.026	0.454	0.530	0.076	20.500	1.250	12.000	23.625	16.013
10/07/2006	0.378	0.025	0.455	0.504	0.061	18.875	2.125	6.500	27.500	18.388
LSD _{(0.05) n=24}										
04/05/2006	ns	0.00236	ns	ns	ns	ns	ns	ns	ns	ns
18/05/2006	ns	ns	ns	ns	ns	2.591	ns	ns	ns	ns
01/06/2006	ns	0.00562	ns	ns	ns	ns	ns	ns	ns	ns
14/06/2006	ns	ns	0.0525	ns	ns	ns	ns	ns	ns	ns
28/06/2006	ns	ns	ns	ns	ns	ns	3.266	ns	ns	ns
10/07/2006	ns	ns	ns	ns	0.02219	ns	ns	ns	ns	ns

TABLE 4

The mean difference in mineral concentration between the exocarp and mesocarp of 'Benny Valencia' oranges.

Rind portion	N %	P %	K %	Ca %	Mg %	Zn mg/kg	Cu Mg/kg	Mn mg/kg	Fe Mg/kg	B mg/kg
Exocarp	0.821	0.0616	1.4789	0.6667	0.1313	8.0208	2.4722	30.875	51.917	21.941
Mesocarp	0.421	0.024	0.387	0.543	0.076	18.875	2.569	11.826	27.188	20.493
LSD _{(0.05) n=24}	0.0542	0.0081	0.0951	0.1094	0.0189	2.5804	2.2475	4.5004	9.0501	5.5931

TABLE 5

Exocarp mineral concentrations of oranges sampled from three nitrogen treatments.

	Exocarp		
N%	East	West	LSD
Control	0.805	0.889	0.048
LAN	0.799	0.892	
Horticate	0.790	0.784	
P %			
Control	0.063	0.068	0.006
LAN	0.059	0.062	
Horticate	0.060	0.062	
K %			
Control	1.258	1.715	0.121
LAN	1.288	1.648	
Horticate	1.311	1.632	
Ca %			
Control	0.683	0.593	0.061
LAN	0.746	0.635	
Horticate	0.740	0.690	
Mg %			
Control	0.149	0.100	0.015
LAN	0.169	0.120	
Horticate	0.151	0.111	
Zn mg/kg			
Control	7.650	7.750	ns
LAN	7.950	8.450	
Horticate	7.700	8.050	
Cu mg/kg			
Control	2.400	2.550	ns
LAN	2.700	2.650	
Horticate	2.150	2.300	
Mn mg/kg			
Control	30.850	29.300	ns
LAN	31.700	33.500	
Horticate	29.350	31.850	
Fe mg/kg			
Control	50.650	53.150	7.246
LAN	54.150	55.000	
Horticate	50.200	58.850	
B mg/kg			
Control	25.365	22.305	3.119
LAN	23.015	22.110	
Horticate	21.445	19.295	

TABLE 6

Exocarp East/West ratio of oranges from different sampling dates. Ratio is the quotient of east to west of each mineral concentration from each of the three different treatments.

Sampling										
Date	N	P	K	Ca	Mg	Zn	Cu	Mn	Fe	B
Control										
04-May	0.86	0.92	0.67	1.19	1.62	0.86	0.71	1.03	0.86	1.00
18-May	0.88	0.78	0.68	1.11	1.47	1.09	1.00	0.99	0.90	0.97
01-Jun	0.87	0.90	0.72	1.16	1.74	0.85	0.52	1.11	0.88	1.20
14-Jun	0.85	0.91	0.78	1.18	1.53	0.96	0.92	1.01	1.02	1.08
28-Jun	0.97	0.89	0.80	1.11	1.36	0.97	1.00	1.02	0.93	1.72
10-Jul	0.98	1.09	0.74	1.17	1.46	1.05	1.00	1.23	1.05	1.12
Horticote										
04-May	1.00	1.01	0.78	1.05	1.29	0.85	0.71	0.88	0.72	1.06
18-May	1.03	0.99	0.80	1.10	1.34	0.92	1.08	0.92	0.91	1.11
01-Jun	1.01	1.01	0.81	0.93	1.18	0.94	1.00	0.97	0.92	1.05
14-Jun	1.04	0.97	0.79	1.09	1.34	1.04	0.92	0.96	0.88	1.04
28-Jun	0.95	0.87	0.77	1.13	1.49	0.95	0.86	0.91	0.89	1.01
10-Jul	1.02	0.99	0.87	0.99	1.35	1.03	1.00	0.92	0.92	1.40
LAN										
04-May	0.93	0.98	0.90	1.23	1.37	1.04	1.40	0.94	0.96	1.08
18-May	0.92	0.94	0.75	1.31	1.45	0.96	0.86	0.98	1.10	1.04
01-Jun	0.95	0.97	0.71	1.21	1.74	0.94	0.88	0.95	0.92	1.15
14-Jun	0.94	1.06	0.78	1.08	1.26	0.86	1.27	1.05	1.06	1.11
28-Jun	0.84	0.91	0.76	1.10	1.43	0.98	0.73	0.88	0.91	0.90
10-Jul	0.85	0.89	0.73	1.14	1.56	0.90	1.00	0.88	0.90	1.05
LSD <small>(0.05) n =24</small>										
04/05/2006	0.068	ns	0.123	ns	ns	ns	ns	ns	ns	ns
18/05/2006	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
01/06/2006	ns	ns	ns	ns	ns	ns	0.026	ns	ns	ns
14/06/2006	0.120	ns	ns	ns	ns	ns	ns	ns	ns	ns
28/06/2006	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
10/07/2006	0.065	ns	ns	ns	ns	0.086	ns	0.209	ns	ns

TABLE 7

Element concentrations and ratio of affected versus unaffected fruit from three treatments. Values are means (n=24). An asterisk indicates a significant difference between same treatments of affected and unaffected fruits. # indicates a significant difference between the means of the affected and unaffected fruit. Means with same letters are not significantly different (student t-test, $P = 0.05$).

	N	P	K	Ca	Mg	Zn	Cu	Mn	Fe	B
	%	%	%	%	%	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
<u>Non pitted fruit</u>										
Cont	1.02 a	0.078 a*	1.77 a*	0.79 a*	0.16 b*	7.75 b	3.25 b	33.13 b*	52.50 b	22.68 a*
LAN	1.04 a	0.076 b*	1.73 b*	0.79 a*	0.17 a*	8.38 a	3.13 c	35.50 a*	52.38 b	22.05 b*
Hort	1.00 a	0.077 b*	1.73 b*	0.87 a*	0.18 a*	8.63 a	3.38 a	39.63 a	58.38 a	22.10 b*
<u>Pitted fruit</u>										
Cont	1.04 x	0.069 x*	1.41 x*	0.99 z*	0.23 y*	7.88 y	3.38 y	39.63 y*	56.75 y	26.90 x*
LAN	1.01 x	0.064 y*	1.30 y*	1.09 y*	0.26 x*	8.38 x	3.50 x	40.63 x*	56.38 z	24.50 y*
Hort	1.01 x	0.065 y*	1.24 y*	1.15 x*	0.25 x*	8.63 x	3.25 y	38.00 y	59.38 x	22.74 y*
Mean pitted	1.02	0.070 #	1.32 #	1.07 #	0.24 #	8.29	3.38	39.42 #	57.50	24.71 #
Mean non pitted	1.02	0.080 #	1.74 #	0.82 #	0.17 #	8.25	3.25	36.08 #	54.42	22.28 #
Pitted/ non pitted	1.0	0.9	0.8	1.3	1.4	1.0	1.0	1.1	1.1	1.1
N	24	24	24	24	24	24	24	24	24	24

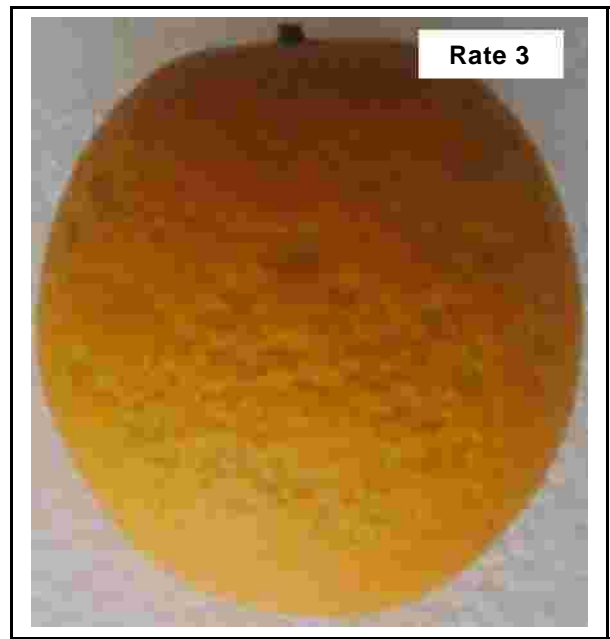
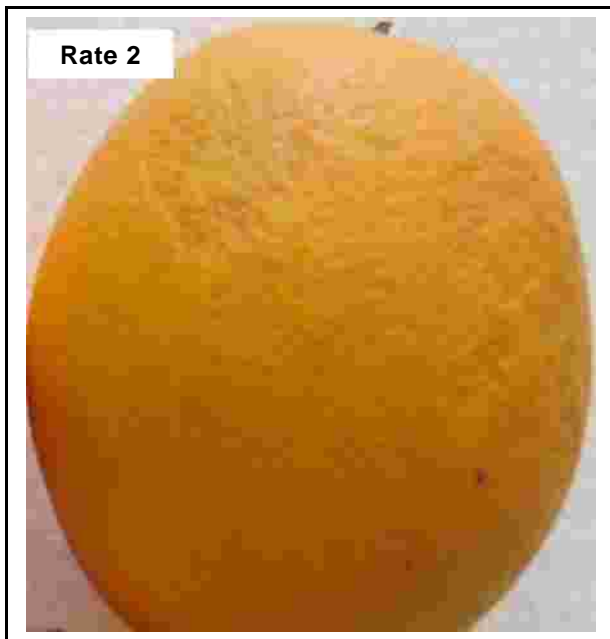
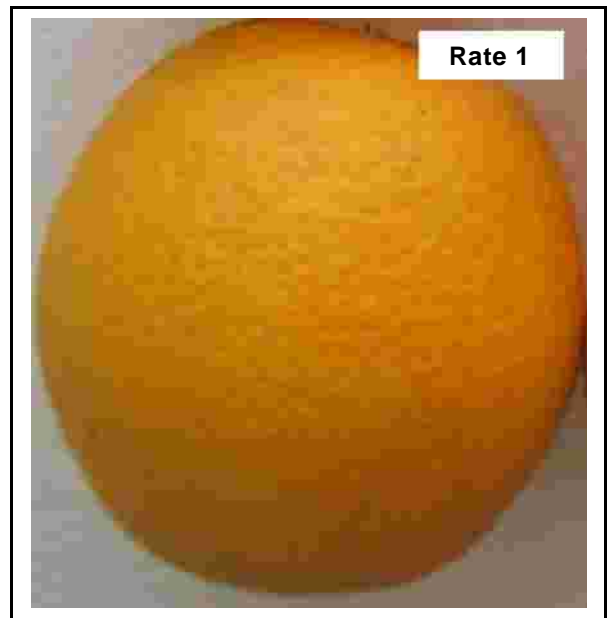
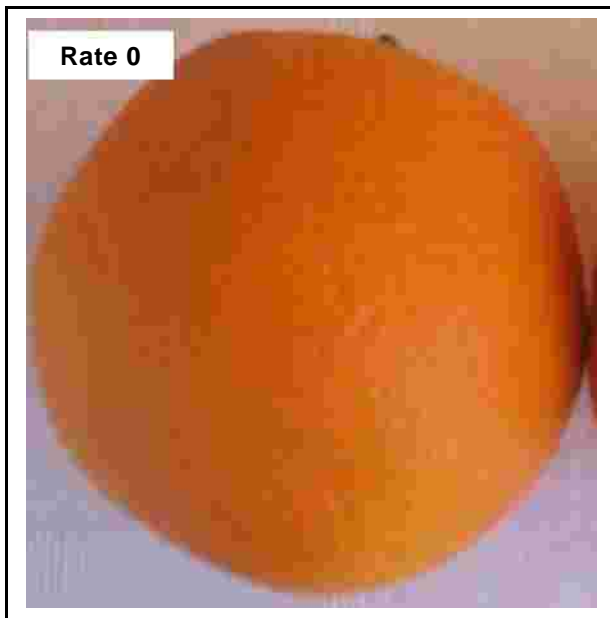


PLATE 2:

Photographs illustrating the rating scale for rind pitting disorder.

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CHAPTER 4: GENERAL DISCUSSION

In this study, it was not possible to establish a direct relationship between fruit mineral concentration and the incidence and intensity of rind physiological disorders of mango (lenticel damage) and citrus (rind pitting). However, in both cases, mango and citrus, fruit receiving additional nitrogen were more susceptible to rind physiological disorders than the control fruit. Nevertheless, results indicate that there is a tendency (but not strong) for nitrogen to have an influence on the development of these disorders, although this does not seem to be direct, nor clearly via mineral nutrition.

On all tested fruit, the correlation between fruit nitrogen concentration and rind physiological disorder was not apparent. It was, however, clear that fruit which received additional nitrogen were more susceptible to the disorder than control fruit. Of the four treatments on 'Tommy Atkins' at Bavaria, the LAN treatment had the highest incidence of lenticel damage, but the pulp and skin nitrogen contents of this treatment were comparable to the other treatments. While mango fruit receiving nitrogen in the form of LAN had higher lenticel damage than other formulations, this was not the case with citrus. In citrus, fruit that received a slow release formulation of nitrogen had the highest incidence of rind pitting disorder.

It would seem that, although the mineral concentration is the primary physiological factor, the direct effect of nitrogen on the development of both disorders was minimal. The most important effect of nitrogen on these disorders appear to be through partitioning of minerals and assimilates to actively growing and transpiring vegetative plant parts at the expense of the fruit. In view of the fact that nitrogen is the most important nutritional element associated with the balance between reproductive and vegetative development (Shear and Faust, 1980; Stassen *et al.*, 1981a; 1981b; 1997), its partitioning in tree crops is largely affected by application time (Sanchez *et al.*, 1992; Khemira *et al.*, 1998; Toselli *et al.*, 2000). Considering that vegetative and reproductive growth components are largely antagonists, results obtained in this study suggest that nitrogen applied in the soil late in the season would either result in high tree nitrogen concentration,

which would in turn enhance vegetative growth at the expense of fruit (Calvert, 1970; Weir and Cresswell, 1995) or nitrogen was not available to the fruit and the tree. Since fruit are weaker sinks for minerals than leaves and shoots (Witney *et al.*, 1990), it would seem that applied nitrogen was directed to vegetative parts at the expense of the fruit.

Further, it has been reported that carbohydrate distribution within a plant is affected by nitrogen supply, which strongly influences the processes of carbon assimilation, allocation and partitioning (Kaiser, 1997). It would seem that sink/source relations may have changed in response to late nitrogen application, resulting from enhanced vegetative growth which could have established an alternative sink competing with fruit for assimilates and mineral elements. This would in turn create a cascade of events, such as partitioning of carbohydrates and minerals to actively growing and transpiring shoots (Shear and Faust, 1980; Stassen *et al.*, 1981a; 1981b; 1997). This would explain why citrus fruit from trees receiving additional nitrogen were smaller than the control.

The lack of direct correlation between fruit nitrogen concentration and occurrence of the disorders served to indicate that a proportion of these disorders can be ascribed to other factors. When comparing the lenticel damage of fruit from two locations with different orchard management practices and climatic conditions, it is shown that control fruit from the Numbi orchard had a higher incidence of lenticel damage than the orchard at Bavaria. It is noteworthy that fruit from the Numbi orchard, which had the highest incidence of lenticel damage, also had the highest rind and pulp nitrogen and manganese concentrations. In terms of mineral association with the disorder, there is not enough evidence to support that fruit N and Mn concentrations are important in the development of the disorder. The lack of this evidence highlighted the importance of fruit origin rather than fruit mineral concentration on the development of lenticel damage. It therefore seems that the role played by nitrogen on lenticel damage is not as significant as that played by orchard location. These findings are in agreement with Kaiser *et al.* (2004) who reported that fruit rind nitrogen concentration does not play a direct role in the development lenticel discolouration. It is therefore

suggested that, in addition to nitrogen, other factors such as climatic conditions and perhaps orchard management practices play a significant role in development of the disorder.

The severity of disorders varied with sampling date, with relatively lower incidences of lenticel damage and rind pitting at the start of the sampling period. The incidence of mango lenticel damage increased threefold on fruit harvested after the first rain and then either remained high or decreased on fruit harvested towards the end of the observation period. On 'Keitt' fruit, the skin moisture percentage was found to increase and then decrease in a pattern that closely resembled the lenticel damage pattern. This strengthens the hypothesis made by Kruger and Fraser (2004), that fluctuations in fruit moisture content are significantly influenced by the rainfall pattern. This concurs with the suggestion that the risk of lenticel damage in mangoes is considerably higher after rain due to increased turgor in the rind (Oosthyse, 1998b; Cronje, 2005b; 2006). Cronje (2005b) also reported that the incidence of lenticel damage was reduced as the soil dried off. LAN treated fruit were an exception to this trend. Instead of dropping back to the initial score, it was only reduced to two times this value. Similarly, the incidence of rind pitting was low at the beginning of the sampling period, followed by a peak and a decline towards the end of the sampling period. However, the occurrence of rind pitting was not dependent on rainfall as was the case in mango lenticel damage.

With regard to citrus fruit position in a canopy, the severity of the disorder was not affected by fruit position. These results disagree with the findings reported by Almela *et al.* (1992), where the incidence of rind pitting was higher in fruit exposed to the sun than non-exposed fruits, and higher on the exposed than the non-exposed side of individual fruits. These authors also reported that fruit oriented to the North-West (Northern hemisphere) of the canopy were most affected by the disorder. A similar situation was found with peteca disorder on lemon fruit, where fruit hanging on the east side of trees are found to be more susceptible than fruit on the west side (Southern hemisphere) (Wild, 1991). It was also observed that the difference in rind pitting between LAN and Horticote[®]

treated fruit was more pronounced on fruit harvested from the western side than those from the eastern sides of the trees.

Mineral analysis results of different position within the canopy obtained in this study were similar to those reported by Kruger *et al.* (2005). Kruger *et al.* (2005) reported a significant difference between the rind mineral concentrations of fruit from different positions of the tree. In this study, fruit from the eastern side of the tree were found to contain significantly lower concentrations of more mobile N, P and K than western fruit. In contrast to mobile elements, less mobile Ca, Mg, Cu, and B were found to be consistently lower in fruit positioned on the western side than fruit on the eastern side of the canopy. However, with other less mobile elements such as zinc, manganese, copper and iron, an opposite trend was noticed in that rind of fruit from the eastern side contained lower concentrations of these elements than the western side. It is suggested that this may be as a result of the light and heat dependent transport of these less mobile elements that fruit on the eastern side of the tree had more of these elements (Curie *et al.*, 2000; Curie and Briat, 2003). For instance, tissue nitrogen concentration per dry weight decreases with increasing irradiance (Niinemets, 1996). In a study conducted on grapes (*Vitis vinifera*), light limitation reduced tissue concentrations of phosphorus, potassium, and magnesium in the xylem sap by about 50% (Rodriguez-Lovelle and Gaudillere, 2002). It is probably the result of the light and heat dependent transport of these mobile elements that fruit on the eastern side of the tree had higher concentrations of immobile elements. Because a considerable fraction of leaf and fruit magnesium is contained in chlorophyll, decreasing leaf magnesium content with increasing irradiance, and a linear relationship between contents of leaf nitrogen and magnesium, provide evidence for the partitioning of foliar nitrogen towards enhanced light capture with decreasing irradiance (Boardman, 1977; Dorenstouter *et al.*, 1985).

Kruger *et al.* (2003a) reported that exposure to radiation heat improves uptake of immobile elements and the daily distribution pattern of the heat units may possibly play a role. Fruit from the hotter western side (Barry *et al.*, 2003) of the tree would be expected to be more mature and have higher immobile elements

(Kruger *et al.*, 2003a; 2005). However, high afternoon temperatures to which the western side was exposed to, nearly always occur during periods of high water stress that may lead to the cessation of transpiration by stomatal closure (Fitter and Hay, 1987). Gernama and Sardo (1996) reported a correlation between xylem water potential and net photosynthesis in orange trees. Stomatal closure would in turn decrease the transpiration stream, reducing water flow and subsequently reducing translocation of relatively immobile elements into the fruit. Kamota *et al.* (1974) found higher water use in 'Satsuma' in the early morning and afternoon as compared to midday. The effects of water stress during this period may be linked to net carbohydrate accumulation resulting from photosynthesis in adjacent leaves and fruit (Blanke and Lenz, 1989). Over a day the cooler eastern side is less subject to stress, thus transpiration and photosynthesis are higher due to stomata staying open. In this case, the cooler eastern side of the tree was arguably subject to higher transpiration and photosynthesis, hence it had more mature fruit and higher immobile elements such as calcium, magnesium and boron than the western side of the tree.

From these findings it is evident that all minerals that move through the transpiration stream were higher in the less stressed eastern side than the western side. Furthermore, fruit position within a canopy affected the severity of rind pitting disorder, with fruit located on the western side of the tree being more susceptible to rind pitting than fruit on the eastern side. These results support findings reported by Almela *et al.* (1992), where the incidence of rind pitting was higher in fruit exposed to the sun than non-exposed fruit, and higher on exposed than the non-exposed side of individual fruit. Meidera *et al.* (1999) maintained that symptoms of rind pitting appear on the regions of the rind most exposed to external factors such as radiation. Both authors also reported that fruit oriented to the western side of the canopy were most affected by the disorder. Light has been shown to be necessary for efficient iron uptake and transport between organs and tissues (Curie and Briat, 2003). In agreement with this finding is the light-dependent transport of iron to the mesophyll cells reported by Curie *et al.* (2000). It is probably the result of this light dependent transport of these elements that fruit on the eastern side of the tree had more immobile elements. In addition,

sun-exposed fruit accumulate minerals such as calcium through the transpiration system.

When comparing the rind mineral concentration of fruit with rind pitting (pitted) to those without rind pitting (non pitted), the mean concentration of less mobile elements in pitted fruit was higher than that of non pitted fruit. Irrespective of being affected, the control fruit had a higher concentration of immobile elements than treated fruit. The mean elemental ratio of pitted/non pitted fruit shows that all indices were around 1 except for calcium, magnesium and boron that were exceptionally higher than 1. Indices greater than 1 imply that the concentrations of these elements were significantly higher in pitting-affected fruit than in unaffected fruit. In many fruits, low rather than high calcium concentration has been implicated in the incidence of physiological disorders (Poovaiah *et al.*, 1988). However, gold speck of tomato fruit is an example of a disorder associated with excess calcium (De Kreij *et al.*, 1992). In peteca, a lemon rind disorder, Khalidy (1969) and Storey and Treeby (2002) reported an increase in calcium levels in peteca-affected regions. Storey and Treeby (2002) suggested that high levels calcium on lemon rinds causes lemon rind pitting.

Results observed in this study, where nitrogen treatments accumulated more calcium and had high incidence of the disorder, suggest that the disorder could have resulted from the accumulation of high calcium levels. The physiology of this occurrence could be explained as follows. The first signs of cellular damage in pitted fruit are associated with internal membrane disorganisation of the plastids, followed by vesiculation of the cytoplasm and degradation of the cytoplasm (Vercher *et al.*, 1994; Obeland *et al.*, 1997; Medeira *et al.*, 1999). Internally, the damage caused by peel pitting is manifested by flattened and collapsed parenchyma cells immediately above the oil glands. Obeland *et al.* (1997) verified the collapse of parenchyma cells above oil glands. These authors are further of the opinion that this causes the oil bodies to rupture, which in turn could release oil into the surrounding cells, causing injury to lemons. Khalidy *et al.*, (1969) report that cells surrounding oil bodies of affected fruit were broken and had an abundance of calcium oxalate. Citrus fruit contain considerable

quantities of organic acids and oxalic acid predominates in the peel. High calcium content in affected regions could have arisen from the formation of calcium oxalate as a result of rind pitting as was suggested on lemons by Storey and Treeby (2002).

Conversely to less mobile elements, the mobile mineral concentration in pitted fruit was significantly lower ($P < 0.001$) than that of non pitted fruit. It is also important to note that nitrogen and zinc were the only two elements with indices equal to 1. In contrast to current observations, Kruger *et al.* (2005) found non pitted fruit to have higher rind nitrogen concentration than affected fruit. It is, however, important to note that these authors only analysed holdback samples from export consignments with fruit divided into pitted and non pitted groups. In addition, potassium and phosphorus concentrations were significantly ($P < 0.005$) lower in pitting affected fruit compared with unaffected fruit. Hence phosphorus and potassium indices were below 1. Similar results were observed for lemon peteca by Storey and Treeby (2002) and rind breakdown on clementines by Kruger *et al.* (2005). Storey and Treeby (2002) explained that lower potassium in cells might be indicative of loss of cell integrity and ion leakage as potassium is, generally, the most abundant solute of plant cells. This may imply that rind pitting damage resulted in cell leakage, thus reducing potassium in affected tissues.

Regarding lenticel damage on mangoes, the overall deduction is that nitrogen seems to have some influence in lenticel damage disorder, in that the damage seems to be higher than the control. However, when considering the lack of evidence that nitrogen plays a direct role, due to the lack of significant differences in the fruit, it would seem that nitrogen applications shortly before harvest may influence some unknown factors that perhaps change the fruit reaction to available soil water content. Therefore, the strongest evidence explaining lenticel damage relates to water relations, where high water contents as may occur after rain or irrigation enhance damage. There seems to be some evidence that nitrogen applications within a month of harvest may modify this relationship, but by what means is unknown, as there is no evidence of fruit nitrogen being changed, and only one other element was modified, which may

not be meaningful. Overall, the cause of lenticel susceptibility to damage seems more complex than expected, but the best evidence thus far available points to tree and fruit water relations at the time of harvest, area or climatic differences may be related in the same manner.

In citrus rind pitting, it appears that while some trends were evident, there is not sufficient evidence to demonstrate the effects of nitrogen application on rind pitting, nor the mechanism or involvement of other mineral elements, and thus results are inconclusive as to the effect of late nitrogen application on the development of rind pitting and lenticel damage. It is concluded that while there is a tendency for late nitrogen applications to enhance the incidence and intensity of these disorders, although the mechanism may be indirect. It was therefore impossible to draw any clear conclusions regarding the relationship between nitrogen and rind pitting disorder. Since nitrogen partitioning in fruit trees is largely affected by application time and, preferentially, to the vegetative parts (Stassen *et al.*, 1981a; 1981b; Khemira *et al.*, 1998; Sanchez *et al.*, 1992), it would seem that late nitrogen application may have promoted vegetative growth, hence partitioning minerals and assimilates to actively growing and transpiring shoots at the expense of the fruit. However, whether any of these elements played a direct role in development of disorders, or whether other unknown factors were influences and were the primary drivers, is not known. In the citrus trial, of the two nitrogen treatments, fruit that received slow release fertilizer tended towards highest incidence of the disorder, implying that the residual effect of the once off LAN treatment was shorter than that of continuous supply provided by the slow release Horticote[®] treatment. The study further demonstrated that the incidence and severity of rind pitting disorder varied with sampling dates, with relatively low incidence at the beginning of the sampling period followed by a peak and then a drop towards the end of the period. The reason for this is unknown, and may relate to some fruit maturity factor or environmental conditions. Nevertheless, despite the uncertain effect of nitrogen application on rind pitting, the demonstrated tendency to enhance the disorder should indicate caution, and in the absence of other clear causative evidence, it

is suggested that late (near harvest) nitrogen applications should be avoided. In the case of mango, picking immediately after rain should also be avoided.

CHAPTER 5 : REFERENCES

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