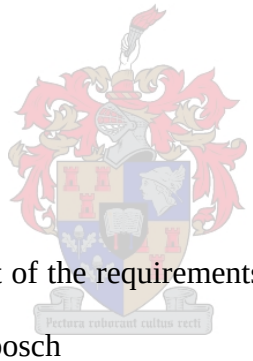
# ECOPHYSIOLOGICAL RESPONSES OF *CITRUS* TREES AND SUGAR ACCUMULATION OF FRUIT IN RESPONSE TO ALTERED PLANT WATER RELATIONS

**JOHAN ANDRIES PRINSLOO**




Thesis presented in partial fulfilment of the requirements for the degree of Master of Science in Agriculture, University of Stellenbosch

Supervisor: Dr. G.H. Barry (Department of Horticultural Science)

Co-supervisor: Prof. E. Rabe (Department of Horticultural Science)

March 2007

DECLARATION

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




…… ……..

Signature Date

## ACKNOWLEDGEMENTS

I gratefully acknowledge the following institutions and individuals:

The Foundation for Research and Development and Citrus Research International for their financial support.

Dr. G.H. Barry for his guidance, advice, encouragement and patience during the long duration of the study.

The lecturers and staff of the Department of Horticultural Science, University of Stellenbosch, for their assistance during the study.

All personnel at Backsberg and Greendale for their time and effort in helping to prepare the orchards for the project.

My family and friends for their love, support and encouragement throughout my studies.

## SUMMARY

This study was undertaken to quantify some of the effects of daily fertigation on ecophysiological responses in citrus trees (*Citrus* spp.). Initial research was conducted to optimise and standardise the sampling procedure to quantify stem water potential ($\psi_{stem}$) in citrus trees. To reliably determine the plant water status of citrus trees, the following conditions are required to minimise unwanted variation in $\psi_{stem}$ measurements. Bagging of leaves with black polyethylene envelopes covered with aluminium foil 3 to 4 hours prior to measuring $\psi_{stem}$ allows the plant water status in those leaves to equilibrate with whole-tree plant water status, thereby providing a realistic measurement of the current water status. The use of aluminium foil to cover the bagged leaves, reduces unwanted heat stress by reflecting sunlight, and dramatically reduced variation in $\psi_{stem}$. The time of day at which $\psi_{stem}$ measurements are made is important to ensure consistency in comparisons among treatments and interpretation of irrigation treatment effects. "Physiological midday" is the preferred time of day to measure $\psi_{stem}$, i.e. 1100 HR. Transpiring leaves with open stomata would be in sun-exposed positions on the east side of trees and should be used for making $\psi_{stem}$ measurements. Under similar experimental conditions as those used here, only three leaves per replicate are required to detect a difference of 0.05 MPa in $\psi_{stem}$ between treatment means. Plant water status categories were developed which may have useful practical applications, i.e. >-1.0 MPa = no water deficit; -1.0 to -1.2 MPa = low water deficit; -1.2 to -1.4 MPa = moderate water deficit; <-1.4 to -1.6 MPa = high water deficit; and <-1.6 MPa = severe water deficit.

Attempts are being made to develop systems that improve crop management and enhance citrus fruit production through efficient and timeous application of water and mineral nutrients which has led to the use of daily drip fertigation or the open hydroponics system (OHS). However, the perceived benefits are not necessarily supported by facts. Fruit size

and yield are apparently enhanced, but possible negative aspects of the system have not been quantified. Fruit produced on trees grown under daily drip fertigation generally have a lower total soluble solids concentration than on trees under micro-sprinkler irrigation. This is mainly due to a dilution effect that is caused by the greater availability of water and the uptake thereof. Sugar accumulation can be optimised by controlling the amount of water that the plant receives at different developmental stages. Therefore, it is essential to quantify the ecophysiological responses and benefits of OHS/daily fertigation, as well as the effects of this technology on fruit quality. 'Nules Clementine' mandarin (*C. reticulata* Blanco) trees in two commercial orchards in Simondium, Western Cape province, South Africa, received differential irrigation treatments. The treatments were applied at the end of stage I (± mid December) of fruit development. Stem water potential, fruit size and internal fruit quality were determined. Water-deficit stress enhanced sugar accumulation of 'Nules Clementine' mandarin by 0.3 to 0.6 °Brix under certain conditions. These conditions require that the difference in $\psi_{stem}$ should be of a sufficient intensity of between 0.16 and 0.3 MPa, and this difference should be maintained for a sufficient duration of between 4 and 6 weeks. Furthermore, deficit irrigation should be applied relatively early in fruit development, namely during the sugar accumulation stage which starts within 4 weeks of the end of the fruit drop period and continues until harvest.

## OPSOMMING

Dié studie is onderneem om van die effekte van daaglikse sproeibemesting op die ekofisiologiese veranderinge in sitrus bome (*Citrus* spp.) te bepaal. Aanvanklike navorsing is gedoen om die proses waarmee stamwaterpotensiaal ($\psi_{stam}$) in sitrus bome bepaal word te optimaliseer en te standaardiseer. Om betroubare waardes vir die water status van die plant te bepaal, word die volgende voorwaardes vereis om ongewenste wisseling in $\psi_{stam}$ te minimaliseer. Blare is 3 tot 4 uur voor die meet van $\psi_{stam}$ in swart poli-etileen sakkies geplaas sodat die plant se water status in die spesifieke blare in ewewig gebring kan word met die water status van die algehele plant. ‘n Realistiese maatstaf van die huidige water status word hiermee verkry. Die sakkies word omhul met aluminium foelie om ongewenste hitte stres weens sonlig te verminder en sodoende variasie in $\psi_{stam}$ ook drasties te verminder. Die tyd gedurende die dag waartydens $\psi_{stam}$ bepaal word is van belang om te verseker dat konsekwente vergelykings getref kan word tussen verskillende besproeiing behandelings. “Fisiologiese middag” is die gewenste tyd van die dag om $\psi_{stam}$ te bepaal, nl. om 1100 HR. Transpirerende blare met oop stomata wat aan sonlig blootgestel is aan die oostelike kant van die boom moet gebruik word om $\psi_{stam}$ te bepaal. Onder soortgelyke eksperimentele toestande, is slegs drie blare per herhaling nodig om ’n verskil van 0.05 MPa in $\psi_{stam}$ tussen behandelings te bepaal. Plantwaterstatus kategorië is ontwikkel om `n praktiese manier van waterstres te bepaal, m.a.w. >-1.0 MPa = geen watertekort; -1.0 to -1.2 MPa = lae watertekort; -1.2 tot -1.4 MPa = gematigde watertekort; -1.4 MPa tot -1.6 MPa = hoë watertekort; <-1.6 MPa = ernstige watertekort.

Die ontwikkeling van sisteme om oesbestuur te verbeter en om sitrus opbrengste te verhoog deur effektiewe en tydige toediening van water en minerale voedingelemente het gelei tot die gebruik van daaglikse drip bemesting of die oop hidroponiese stelsel (OHS). Ongelukkig is

die waargenome voordele nie ondersteun deur feite nie. Vruggrootte en opbrengs word skynbaar verbeter, maar die negatiewe aspekte van die stelsel is nog nie gekwantifiseer nie. Vrugte van bome wat daaglikse drip bemesting gekry het, het in die algemeen ’n laer totale oplosbare stowwe konsentrasie gehad as bome wat mikro-besproeiing gekry het. Dit kan grootliks toegeskryf word aan die verdunningseffek wat plaasvind weens die groter beskikbaarheid van water aan die bome en die verhoogde opname daarvan. Die optimalisering van suiker akkumulasie kan bereik word deur die hoeveelheid water wat toegedien word gedurende verskillende groeistadiums van die plant te beheer. Dit is dus van belang om die ekofisiologiese veranderinge en die voordele van OHS/daaglikse besproeiing asook die effek van hierdie tegnologie op vrugkwaliteit te bepaal. ‘Nules Clementine’ mandaryn (*C. reticulata* Blanco) bome in twee kommersiële boorde in Simondium, Wes-Kaap provinsie, Suid-Afrika, het differensiële besproeiing behandelings ontvang. Die behandelings is toegepas aan die einde van groeifase I (± middel Desember) van vrugontwikkeling. Stamwaterpotensiaal, vruggrootte en interne vrugkwaliteit is bepaal. Stres a.g.v. water onthouding het gelei tot verhoogde suiker akkumulasie van ‘Nules Clementine’ mandaryn van 0.3 tot 0.6 °Brix onder sekere toestande. Hierdie toestande vereis dat ‘n voldoende verskil in $\psi_{stam}$ van tussen 0.16 en 0.3 MPa waargeneem word en dat hierdie verskil gehandhaaf moet word vir ’n tydperk van tussen 4 en 6 weke. Differensiële besproeiing moet ook relatief vroeg tydens vrugontwikkeling toegedien word, nl. tydens die suiker akkumulasie stadium wat begin 4 weke voor die einde van die vrugval stadium en strek tot oestyd.

## TABLE OF CONTENTS

Language and style used in this thesis are in accordance with the requirements of the scientific journals of the *American Society for Horticultural Science.* This thesis presents a compilation of manuscripts where each chapter is an individual entity and some repetition between chapters has, therefore, been unavoidable.

# CHAPTER 1

# INTRODUCTION

The water potential of a plant is an indication of the plant's water status as water potential accounts for the integrated effect of soil, plant and atmospheric conditions on water availability within the plant (McCutchan and Shackel, 1992). The potentials and resistances to water flow within a plant could not be measured accurately until the development of the pressure chamber simplified the measurement of water potentials in fields and orchards (Scholander et al., 1964; 1965). By measuring the water potential of sun-exposed (transpiring) leaves, leaf water potential could be estimated.

Leaf water potential is highly variable due to prevailing environmental conditions (McCutchan and Shackel, 1992). Variability due to changing environmental conditions can be reduced by using predawn water potential measurements. These measurements indicate overnight recovery in water potential through equilibration with soil water potential, rather than the water potential experienced under midday conditions when a plant's stomata are open and water demand is highest due to large vapour pressure deficit.

In contrast, stem water potential is closely related to plant water use and stomatal conductance, and is thus a more appropriate measure of plant water stress (McCutchan and Shackel, 1992; Shackel et al., 1997). Stem water potential is less influenced by environmental variability than transpiring leaf water potential. However, the technique of stem water potential as a reliable measure of plant water status has not been extensively used in citrus trees (*Citrus* spp.) (Goldhamer and Salinas, 2000; Barry et al., 2004b).

Fertigation is the application of water-soluble fertilisers typically with drip irrigation to control water and nutrient supplies to crops (Bar-Yosef, 1999). The main differences between drip irrigation and micro-sprinkler irrigation are that with drip irrigation water is generally applied to a restricted root zone of the plant and at more frequent intervals than micro-sprinkler irrigation (Elfving, 1982). By applying fertilisers through the drip irrigation system the fertilisation efficiency is increased since nutrients are applied to a restricted root zone (Bar-Yosef, 1999) and the roots are capable of much faster uptake of nutrients (Bar-Yosef, 1988).

The open hydroponics system (OHS) is a sensitive nutrient and moisture management system with a high degree of control over the development of the crop (Stassen et al., 1999). Specific water and nutrient requirements for different phenological stages of the plant are applied through daily fertigation. A balanced nutrient solution with controlled pH and EC is applied using a drip irrigation system.



At the beginning of the 1990's, Professor Rafael Martinez Valero of Spain brought all the concepts of hydroponics together to develop the commercial application of the open hydroponics system and later called it Martinez Open Hydroponics Technologies (MOHT) (Martinez and Fernandez, 2004; Falivene et al., 2005). Since the implementation of OHS in horticultural practices all over the world, many variations of the concept have developed. The variation in OHS will be referred to as Daily Drip Fertigation (DDF). Daily drip fertigation involves drip fertigation of plants on a daily basis during daylight hours (Stassen et al., 1999; Pijl, 2001; Schoeman, 2002). DDF results in increased yield and fruit size (Kruger et al. 2000a; 2000b; Kuperus et al., 2002; Martinez and Fernandez, 2004), but there is no evidence that DDF does not adversely affect sugar accumulation. Anecdotal evidence from several

citrus producers in South Africa suggests that the sugar content of fruit produced under OHS tends to be lower than that of fruit produced under conventional micro-sprinkler irrigation (G.H. Barry, personal communication).

The reported reduction in sugar content under OHS conditions could be explained as follows: DDF results in a dilution effect as the amount of water given by the continuous/daily irrigation dilutes the sugars which accumulate in the fruit. It is well-known that rootstocks of differing vigour have varying affects on the dilution of accumulated sugars (Harding and Lewis, 1941; Miller, 1990; Barry et al., 2004b). Managed drought stress is known to increase sugar accumulation in *Citrus* (Yakushiji et al., 1996; 1998). Meyer and Boyer (1981), Yakushiji et al. (1996; 1998) and Barry et al. (2004b) observed more sugar accumulation in plants under water stress than in unstressed plants.

The timing of water deficit stress seems to be important in enhancing juice quality in citrus. Barry et al. (2004b) found that when deficit irrigation was applied late in fruit development (stage III) of 'Valencia' sweet orange [*C. sinensis* (L.) Osbeck], no increase in soluble solids concentration (SSC) was achieved. However, when deficit irrigation was applied during the major sugar accumulation period (stage II) of fruit development, increases in SSC occurred.

The objectives of this study were to optimise the sampling procedure to reliably determine the plant water status of citrus trees, and to quantify the effects of water deficit stress on SSC of 'Nules Clementine' mandarin (*C. reticulata* Blanco) fruit grown under OHS conditions.

# CHAPTER 2

# REVIEW OF LITERATURE

## 2.1 Fruit growth and development

The mature citrus (*Citrus* spp.) fruit is the end product of a complex set of events that starts with the formation of the reproductive structures, or flowers. The ovary develops into a mature fruit by the processes of cell division, cell differentiation and cell growth. Citrus fruit have a single sigmoid growth curve and are classified as nonclimacteric fruit (Coombe, 1976).

Bain (1958) described the three stages of fruit development for 'Valencia' orange [*C. sinensis* (L.) Osb.]. During stage I, there is slow volume growth, but intense cell division. This period is approximately 9 weeks in duration. Holtzhausen (1969) found the same results and growth patterns for 'Navel' orange. Cell division occurs only in the outer layers of the rind and in juice sacs (Fig. 2.1). Most of the volume growth that occurs is due to the growth in the rind (Lowell et al., 1989). Bain (1958) found that at the end of stage I, the rind represents 95% of the fruit volume. The growth of the juice sacs is primarily due to cell division. This cell division is mainly at the head of developing juice sacs.

Stage II of fruit development is characterised by very rapid fruit growth, and is due to cell enlargement and cell differentiation. Cell division stops at the beginning of stage II, except for the outer layers of the flavedo and the tips of the juice sacs. During this stage, the rind becomes thinner as the pulp segments undergo rapid growth due to cell enlargement. Although the rind becomes thinner, the albedo cells continue to enlarge. This is due to the albedo cells enlarging in a tangential direction which results in spongy tissue in which the cell layers are fewer than in the rind at the end of stage I. The same spongy tissue development

that develops in the albedo, develops in the central axis and in the septum tissue. Most of the increase in size during stage II is due to growth of the pulp segments (Lowell et al., 1989). According to Bain (1958), the central axis and the pulp account for 5% of the volume of the developing fruit at the end of stage I, but at the end of stage II, the central axis and the pulp account for 58% of the volume and 67% of the fresh weight of the fruit. Most of this is due to pulp segments. Juice sacs become larger and juice content increases in the enlarging cells of the juice sacs. The majority of sugar accumulation takes place during stage II of fruit development (Bain 1958).

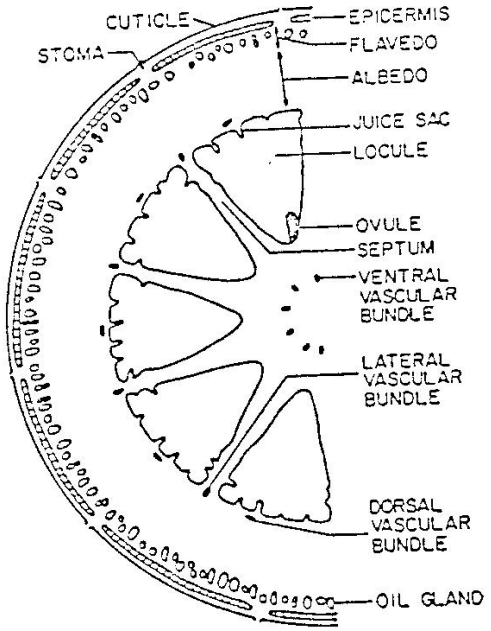



Fig. 2.1. Diagrammatic cross-section of a citrus fruit (Holtzhausen, 1969).

Stage III of fruit development is the maturation period. Although volume growth still continues, the rate of growth is much lower than in stage II. Chlorophyll pigments disappear from the flavedo, with the subsequent carotenoid pigment development. Carotenoids increase significantly and are converted into highly coloured pigments during and after the loss of chlorophyll.

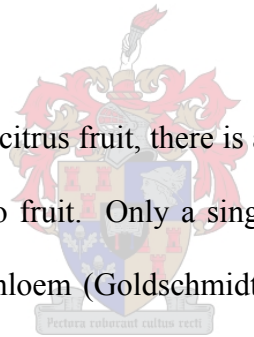
## 2.2 Sugar metabolism

Citrus leaves begin exporting photosynthates when they are fully expanded (Kriedemann, 1969a; 1969b). Schaffer et al. (1987) found that this expansion can take 2 months or more to complete. Several leaves are produced at a time on a new shoot and expand together during elongation of the shoot. This results in a significant intensity and duration of photosynthate import into young leaves until the leaves develop export capabilities. Thus, new leaves can compete strongly for photosynthates with other sinks (Schaffer et al., 1987; Goldschmidt and Koch, 1996). Older leaves export more photosynthates and at a more rapid rate than younger leaves (Kriedemann, 1969a; 1969b). Canny et al. (1968) described citrus trees as "slow paced organisms" with regards to the rate of photo-assimilate translocation. Wallerstein et al. (1978) found that export from source leaves only started 19 hours after labelling. Fruit located at the apical end of a shoot, derive their photo-assimilates acropetally from the leaves of the same growth cycle, whereas leaves of lower vegetative laterals revealed basipetal, root-directed transport (Kriedemann, 1970). Leaves from previous growth cycles initially provide photosynthates for the new developing leaves and flowers, but ultimately their export is destined to the roots (Goldschmidt and Koch, 1996).

Photo-assimilates are transported through the phloem from source leaves into the albedo of fruit. As the amount of photosynthates increase during development, the diameter of the phloem tissue also increases. This structural change helps the transport into growing fruit (Koch and Avigne, 1990). The leaf-to-fruit transfer of photosynthates can be affected by the xylem backflow of water from the fruit. Net water movement from fruit to leaves proceeds in the opposite direction than that of phloem transfer. Elfving and Kaufmann (1972) indicated that water outflow from fruit to transpiring leaves may occur nearly continuously from mid-

morning until sundown. Mass flow into fruit via phloem requires a turgor gradient opposite that of the total water potential drawing xylem water from the fruit to the leaves. Phloem loading in the leaf with a reduced turgor at the sink can provide the necessary gradient. Low turgor may be difficult to achieve in a sink where water loss is extremely limited (Huang et al., 1992) and high pressures are present in internal tissues (Kaufmann, 1971).

Vascular bundle strands become amphicribral as they enter fruit, thus the xylem is completely surrounded by phloem (Lowell, 1986; Tomlinson et al., 1991). This can influence the phloem turgor by reducing the turgor in the fruit end of the translocation path. It may also maximise the capacity of phloem to reload any solutes moving toward the xylem during xylem backflow (Lowell, 1986).



As the vascular bundle strands enter citrus fruit, there is a reduction in the xylem content with distance from the point of entry into fruit. Only a single vessel member can remain inside encircling primary and secondary phloem (Goldschmidt and Koch, 1996). This contributes further to the xylem backflow.

According to Huang et al. (1992), phloem can supply all the water and carbon needed for fruit maturation. Relatively little water is required via xylem, although the amount can change during diurnal influx and efflux. Mantell et al. (1980) indicated that both directions of flow occur throughout the development of fruit. But the amount of water that can be withdrawn from juice sacs decreases during growth due to the extension of long threadlike, nonvascular stalks that increases the distance between the juice sacs and vascular bundle strands (Huang et al., 1992). Most of the water loss that takes place is from the rind of the fruit (Mantell et al. 1980; Huang et al., 1992).

Entry of photo-assimilates into the juice sacs occurs via three vascular bundles located at the segment epidermis of each segment (Koch, 1984; Lowell et al., 1989). One of the bundles is located on the dorsal side with the remaining two along the lateral septa of the segment walls (Fig. 2.2).

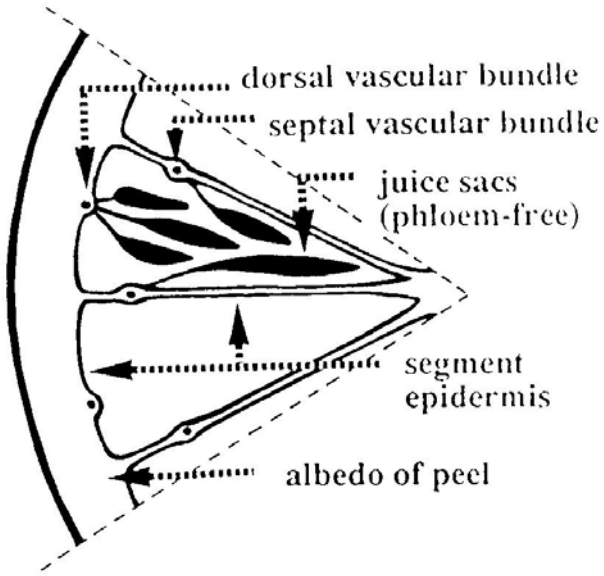



Fig. 2.2. Diagram of vascular and non-vascular areas of developing citrus fruit (Lowell et al., 1989).

Nonvascular transport takes place between vascular bundles and the heads of juice sacs as no phloem is present in juice sections of the fruit (Koch, 1984; Koch and Avigne, 1990). Photosynthates that were delivered via the three vascular bundles mentioned earlier, are transported along the unusually long (1-16 mm) nonvascular path before reaching the base of the juice sac head (Koch and Avigne, 1990). Vascular bundles are surrounded by parenchyma which extends through the center of each hairlike stalk of a juice sac. Koch and Avigne (1990) indicated that minimal sucrose cleavage of the photo-assimilates entering juice sacs occurred during the nonvascular transfer. They also found that the transfer is very slow

with photo-assimilates taking 24 hours to reach the end of vascular bundles and a further 3 days are required for the assimilates to reach the juice sac heads. The final intended location for photo-assimilate in the juice sac heads is in the vacuoles with different amounts and types of sugars differing from sac to sac (Ting, 1969). The process of vascular compartmentalisation is very important to the final import capacity in an expansion sink. When juice sacs expand, the rind is stretched to half its original thickness (Bain, 1958). The expansion process is driven by a high internal turgor and negative osmotic potentials in the juice sac heads (Kaufmann, 1970), combined with an ascending concentration of sugars along the post-phloem transport path (Koch and Avigne, 1990). Yakushiji et al. (1996; 1998) indicated that water plays a direct role in the osmotic adjustment and this causes differences in sink strength which in turn effects the assimilate translocation and accumulation.

Sucrose is the main form of photo-assimilate for translocation and phloem unloading in citrus fruit (Kriedemann, 1969a; 1969b; Garcia-Luis et al., 1991). Sucrose acts as an energy source in photosynthetic cells. Synthesis of sucrose occurs in the cytosol of leaves and is then translocated through the phloem to growing tissues. Sucrose (Fig. 2.3) is synthesised by combining the phosphorylated forms of glucose and fructose sugars (Bean, 1960). This process requires energy which is provided by uridine triphosphate (UTP). UTP reacts with the glucose-1-phosphate to form a molecule called uridine diphosphate glucose (UDPG). The glucose in UDPG can then be transferred to an acceptor molecule such as fructose-6-phosphate to form sucrose and UDP (energy) (Salisbury and Ross, 1991).

The enzymes of sucrose metabolism are most active in juice sac heads prior to the period of maximum translocation into the juice sac heads (Lowell et al., 1989). Sucrose synthase and invertase were most active during the cell division stage. During the cell expansion period,

minimal activity of soluble invertase was detected (Kato and Kubota, 1978). Some alkaline invertase and sucrose synthase could be detected, but sucrose phosphate synthase was most active (Koch and Avigne, 1990).

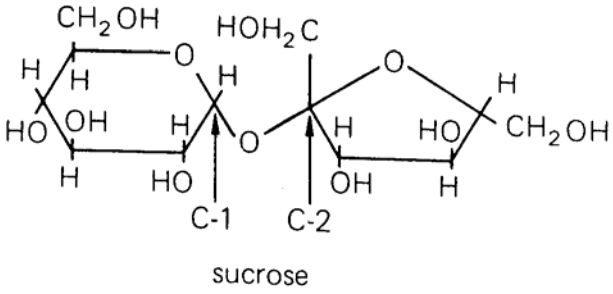



Fig. 2.3. The structure of sucrose, a molecule made of a glucose unit (left) and a fructose unit (right) connected between carbons 1 and 2, as shown (Salisbury and Ross, 1991).

## 2.3 Factors affecting sugar accumulation

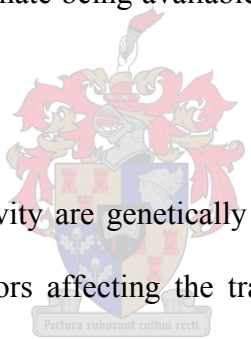
All plant organs act as sinks or receivers of photoassimilates at some stage of development. The ability of a sink organ to import photoassimilates is defined as the sink strength (Ho, 1988). The import rate for a sink can be altered by changing the sink strength or changing the strength of the competing sink.

Competition for photosynthates can be among different sinks in a tree, e.g. shoots and fruit, or it can be among the same type of sinks, e.g. fruit and fruit. As mentioned earlier, new leaves can compete strongly for photosynthates with other sinks (Schaffer et al., 1987; Goldschmidt and Koch, 1996), until leaves have fully expanded and start to export photosynthates (Kriedemann, 1969a; 1969b). The competition between fruit has been linked to the carbohydrate status of the tree due to the reduction in fruit numbers during early fruit development through fruitlet abscission (Schaffer et al., 1985). This is also linked to the

inverse relationship between the number of fruit and fruit size. Processes like girdling and fruit thinning alter the source-sink relationship (Goldschmidt and Koch, 1996).

Girdling is the process of blocking the transport of photoassimilates by removing a ring of bark from the trunk or scaffold branches. The effect of girdling depends on the time period when girdling is done. Girdling in autumn enhances flowering (Goldschmidt and Golomb, 1982), girdling at full bloom improves fruit set (Monselise et al., 1972) and girdling in summer enhances fruit size (Fishler et al., 1983).

Fruit thinning is the removal of some fruit, with the result that the same leaf area supports less fruit. This results in more photosynthate being available for each fruit and thus increases the fruit size (Fishler et al., 1983).



The potential sink strength and activity are genetically determined (Ho, 1988). The actual sink strength is determined by factors affecting the transport rate within the sinks during development, e.g. low yields due to drought or high temperatures. Yields can thus be increased by manipulating growing conditions to optimise rate-limiting processes.

Genetics also influence the ability of different rootstocks to absorb and translocate water while also affecting sugar accumulation. Scions budded on invigorating rootstocks, e.g. rough lemon (*C. jambhiri* Lush.), produce large fruit with low sugars, while scions budded on less invigorating rootstocks, e.g. trifoliate [*Poncirus trifoliata* (L.) Raf.] or citrange (*C. sinensis* x *P. trifoliata* ), produce smaller fruit with higher sugars (Harding and Lewis, 1941; Miller, 1990; Barry et al., 2004a; 2004b). Rough lemon rootstock has a more extensive root system than citrange rootstocks, which allows the roots to absorb more water from a larger

soil volume. Rough lemon rootstock is also more effective at water absorption due to differences measured in plant water status between these rootstocks. Fruit harvested from scions on citrange rootstock had 30% higher sugars than fruit from rough lemon trees (Barry et al., 2004b).

Sink strength is also determined by the ability of the sink to metabolise sucrose and the storage thereof (Ho, 1988; Sung, 1989). There are two specific enzymes capable of cleaving sucrose. The first is invertase, which yields fructose and glucose through a catalytic action. The second is sucrose synthase which uses sucrose and UDP to form UDP-glucose and fructose (Hockema and Echeverria, 2000). The isolation of sucrose in the vacuole allows the sink to maintain a sucrose gradient which in turn permits continuous transport of sucrose into the sink. Song et al. (1998) found that the activity of sucrose synthase in the juice cells in the stylar-end of fruit is significantly higher than in the stem-end of fruit. A low vacuolar pH may be involved in sink strength as sucrose cleavage takes place at a low pH (Hockema and Echeverria, 2000). This would allow acid hydrolysis of sucrose without the necessary enzymes of sucrose metabolism (Echeverria and Burns, 1989).

Drought stress influences sugar accumulation in *Citrus* (Yakushiji et al., 1996; 1998; Barry et al., 2004b). Although Syvertsen and Albrigo (1980) found no evidence of osmotic adjustment in citrus trees to water stress, the water stress conditions that they applied was not severe enough. Meyer and Boyer (1981), Yakushiji et al. (1996; 1998) and Barry et al. (2004b) observed more sugar accumulation in plants under water stress than unstressed plants. Osmotic adjustment is a physiological function that takes place under water stress conditions (Meyer and Boyer, 1981). This process involves enough solute accumulation in cells to decrease the cell osmotic potential when cell water potential decreases at low water potential.

Water can then be absorbed from a water source by cells without losing cell turgor or decreasing cell volume, while solutes accumulate in juice vesicles (Morgan, 1984). During water stress conditions, the cell size and turgor will be maintained due to solute accumulation in cells at low water potentials. Yakushiji et al. (1996; 1998) found that 'Satsuma' mandarin (*C. unshiu* Marc.) fruit underwent osmotic adjustment as a mechanism of accumulating sugars under low water potentials when trees were moderately stressed by mulching or withholding irrigation. The sugar accumulation was not due to dehydration. The water stress increased concentrations of sucrose, glucose and fructose (Yakushiji et al., 1998). Barry et al. 2004b) also demonstrated this phenomenon. Hockema and Etxeberria (2001) explained the importance of sucrose hydrolysing enzymes in this process.

The timing of water deficit stress seems to be important in enhancing juice quality in citrus. Barry et al. (2004b) found that when deficit irrigation was applied late in fruit development (stage III) on 'Valencia' sweet orange [*C. sinensis* (L.) Osbeck], no increase in soluble solids concentration (SSC) was achieved. However, when the deficit irrigation was applied during the major sugar accumulation period (stage II) of fruit development, increases in SSC occurred. This is in contrast to what Ginestar and Castel (1996) and Gonzalez-Altozano and Castel (1999) found on 'Nules Clementine' mandarin (*C. reticulata* Blanco). SSC was increased when deficit irrigation was applied during stage III and not during stage II, but fruit size was smaller for treatments applied in stage III. Applying regulated deficit irrigation early in the season did not decrease fruit size at harvest due to accelerated fruit growth that occurred following the reintroduction of full irrigation (Goldhamer and Arpaia, 1998; Goldhamer and Salinas, 2000).

## 2.4 Water potential

Water movement in the soil-plant-atmosphere continuum is due to differences in the free energy content (capacity to do work) of water in different parts of the continuum. The free energy change is called the chemical potential (Spanner, 1964; Slatyer, 1967). The free energy content of the water in a well-watered plant decreases progressively as it moves from the soil through the plant to the atmosphere. A diffusing solute tends to move from a region of high chemical potential to a region of low chemical potential. The result is that water flows from the soil through the plant to the air in response to the gradient in free energy (Fitter and Hay, 1981).

### 2.4.1 Definition of water potential

Water potential ($\psi$) is defined as the chemical potential of water in a system compared to the chemical potential of pure water at atmospheric pressure and at the same temperature (Slatyer and Taylor, 1960; Slatyer, 1967). Further, the chemical potential of the reference pure water is set at zero (Fitter and Hay, 1981). This definition can be expressed with the following relationship (Slatyer and Taylor, 1960):

$$\psi = (\mu_w - \mu_w^* )/V_w$$

Where

$\psi$ = water potential

$\mu_w$ = chemical potential of water in the system under consideration

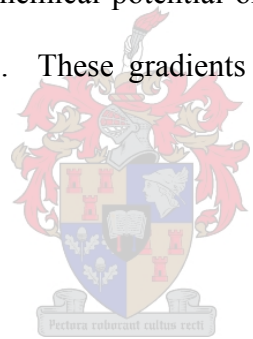
$\mu_w^*$ = chemical potential of pure water at atmospheric pressure and at the same temperature as the system under consideration

$V_w$ = partial molar volume of water (18 $cm^3 \cdot mol^{-1}$).

If the chemical potential of the water being considered is less than that of pure water, its water potential will have a negative value. Water potential is nearly always negative in all parts of the soil-plant-atmosphere continuum. It is usually most negative in the atmosphere and least negative in the soil. The result is a net movement of water from the soil towards regions with more negative values, i.e. the atmosphere. The formation of dew is an example of water flow from the atmosphere to the plant (Fitter and Hay, 1981).

### 2.4.2 Key factors affecting water potential gradients

The water potentials that are within plants are functions of the availability of water from the soil, atmospheric demand for water and the resistance of water movement within the plant (Kramer, 1983). The gradients in chemical potential or water potential produce the driving force for diffusion (Spanner, 1964). These gradients are produced by the following five factors.




#### *2.4.2.1 Concentration*

The effective concentration of the solution is the most important factor in establishing the chemical-potential gradients that drive diffusion. Solute particles in plants (minerals, sugars, etc.) diffuse from a region with a high concentration to a region of low concentration (Salisbury and Ross, 1991).

#### *2.4.2.2 Temperature*

Temperature differences are normally ignored because the thermodynamic equations assume that temperature is constant throughout the system and its surroundings (Spanner, 1964). But it is important to consider temperature effects because temperature gradients may exit. When temperatures at soil surfaces decrease at night, water vapour diffuses from deeper in the soil

to the surface (Kramer, 1934; Kramer, 1940). As temperatures increase during the day, water vapour will diffuse deeper into the soils. The same applies to plants growing in the arctic. The roots of these plants are in soil that is close to freezing point while their leaves can be warmed during the day to above 20 °C (Salisbury and Ross, 1991).

#### *2.4.2.3 Pressure*

Increasing pressure increases the free energy and hence the chemical potential in a system is increased. The contents of most plant cells are under pressure when compared to the surroundings. Fluids in xylem can be under tension (negative pressure) when not enough water is available in the soil. This will result in water moving from the cell to the xylem (Slatyer, 1967).

#### *2.4.2.4 Effect of solutes*

Solute particles decrease the chemical potential of the solvent (Slatyer, 1967). This is due to the mole fraction, which is the number of solvent particles compared with the total number of particles in the solution. The mole fraction is calculated by using the following equation (Salisbury and Ross, 1991):

$$\text{mole fraction of solvent} = \frac{\text{moles of solvent}}{\text{moles of solute + moles of solvent.}}$$

In a closed container with pure water on one side of a membrane and a solution on the other side, a water potential gradient will be present (Slatyer, 1967). The water potential will be lower on the solution side. Water will diffuse from the pure water side through the membrane into the solution. This case of diffusion is called osmosis (Slatyer and Taylor, 1960). In

plants, osmosis is the process whereby water moves from the soil into the plant and then from cell to cell through the membranes.

#### *2.4.2.5 Matrix potential*

A matrix is a material with surfaces that bind water (Salisbury and Ross, 1991). Negatively charged surfaces (i.e. clay particles in soil, proteins) attract water by binding with the positive side of the polar water molecules. This process is called hydration. Hydration and gravity are the main causes of water flow in soils.

### 2.4.3 Components of water potential

Slatyer (1967) stated that the total water potential of any system is the sum of various effects or components including solute or osmotic potential ($\psi_s$), gravitational potential ($\psi_g$), matric potential ($\psi_m$) and pressure potential ($\psi_p$). Water potential is a pressure and is measured in megapascal (MPa), using the following equation:

$$\psi = \psi_p + \psi_s + (\psi_g + \psi_m).$$

Pressure potential is caused by additional pressure and is equal to the real pressure in the part of the system being considered. Osmotic potential is caused by the presence of solute particles in the water. The gravitational component of the total water potential is only 0.01 $MPa \cdot m^{-1}$ and can thus be ignored except in very tall trees (Kramer, 1983). However, in wet soils, gravity may have a significant effect on water flow (Turner, 1981). Matric potential is a measure at atmospheric pressure of the tendency for the matrix to adsorb additional water molecules. In the cytosol the matric component of the final water potential is small and could

also be neglected (Wiebe, 1966). This reduces the equation to the two most contributing factors of water potential, pressure potential and osmotic potential:

$$\psi = \psi_p + \psi_s.$$

Pressure potential can have any value and can be positive, negative or zero (i.e. at atmospheric pressure). Positive pressure is the result of an increase in pressure while tension (sucking or pulling) results in a negative pressure. Osmotic potential is always negative, or zero in pure water. The osmotic potential is negative because the solvent water in a solution can only do less work than pure water. Water potential can be negative, zero or positive due to pressure that can be positive and very high while osmotic potential can be zero or negative (Salisbury and Ross, 1991).

## 2.5 Techniques to determine water potential and osmotic potential

### 2.5.1 Water potential

The most useful single measurement in the soil-plant-atmosphere continuum is water potential (Kramer, 1983). Water potential can be measured by Chardakov's dye method, tissue volume equilibration, thermocouple psychrometers or the pressure chamber.

#### *2.5.1.1 Chardakov's dye method*

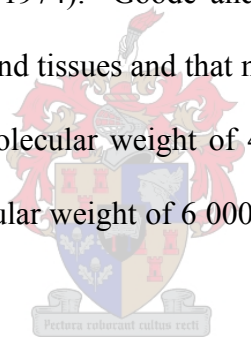
Test tubes, with solutions with known but different concentrations, are coloured by adding a crystal of dye such as methylene blue to the solution (Knipling, 1967; Slavik, 1974). Samples of plant tissue are placed in test tubes with similar concentrated solutions but with no dye. Sufficient time for the possibility of water exchange to take place is allowed. After the removal of the tissue, a small drop of the equivalent coloured solution is added to the test tube. If the coloured drop rises, then the solution in which the tissue was held became denser. This means that the tissue has absorbed water and that the tissue had a lower (more negative)

water potential than the solution. If the drop sinks, the solution has absorbed water from the tissue and became less dense. The solution had a lower water potential than the tissue. If the drop diffuses evenly without rising or sinking, then there was no change in concentration and the water potential of the solution equals that of the tissue.

Knipling and Kramer (1967) found differences of 1 to 5 bar in water potential measurements determined by the dye and psychrometric methods.

*2.5.1.2 Tissue volume / liquid equilibration method*

Solutions of varying concentrations are made by using sucrose, sorbitol, mannitol or polyethylene glycol (PEG) (Slavik, 1974). Goode and Hegarty (1965) found that sucrose penetrates into the intact plant cells and tissues and that mannitol is absorbed by higher plants. Jackson (1962) used PEG with a molecular weight of 400 to 20 000, but Lagerwerff et al. (1961) found that PEG with a molecular weight of 6 000 was toxic and recommends a weight of 20 000.




A sample of plant tissue which has been measured (length or weight) is placed in the series of solutions (Slavik, 1974). After sufficient time has been allowed for the possibility of water exchange to take place, the tissues are measured again. A tissue that measures the same before and after being in the solution had no gain or loss of water. This is an indication that the tissue and solution is in equilibrium. The osmotic potential of the solution is theoretically equal to the water potential of the tissue. Using the method of measuring tissue length is only feasible for tissue composed of thin-walled cells with no major vascular bundles. The gravimetric method is only suited for large masses of tissue and errors occur from failing to dry tissue uniformly and loss of weight during handling (Kramer, 1983).

To avoid some of the problems with the gravimetric method, the sucrose solution can be measured instead of the plant tissue (Kramer, 1983). The tissue gains water from solutions with a higher potential which results in a higher concentrated solution. If the solution has a lower potential, water will diffuse from the tissue and dilute the solution. Changes in concentration of the sucrose solutions can be measured with a refractometer or by the Chardakov dye method (Knipling, 1967; Knipling and Kramer, 1967).

*2.5.1.3 Thermocouple psychrometer*

Tissue samples and thermocouples are enclosed in small containers and immersed in a water bath which is kept at a constant temperature (Boyer and Knipling, 1965). The sample will generate a relative humidity in the container that is equivalent to the total water potential of the sample at the time of sealing of the containers (Turner, 1981). By measuring the relative humidity with a thermocouple psychrometer, the water potential can be determined by the following equation (Turner, 1981):

$$\psi = \frac{RT}{V} \ln e/e^0$$

Where

$\psi$ = water potential

$R$ = the gas constant (0.0831 kg·barsmol$^{-1}$K$^{-1}$)

$T$ = the absolute temperature (K) = ˚C + 273

$V$ = the partial molar volume of water (18 cm$^3$·mol$^{-1}$)

e/e$^0$ = the relative humidity.

A problem with this method is measuring the relative humidity in the containers (Slavik, 1974; Turner, 1981). A psychrometer design suggested by Spanner (1951) uses the Peltier

effect to condense water on a thermocouple junction (Fig. 2.4). The rate of cooling depends on the humidity of the air in the chamber and the current generated during the cooling is measured. It was suggested that a drop of water is placed on the junction and the current generated by evaporative cooling is measured (Richards and Otaga, 1958). Both types of thermocouple psychrometers must be calibrated over a range of solutions with known osmotic potentials. By modifying the Richards psychrometer with a removable thermocouple and obtaining equilibrium readings with drops of solution of three or four different concentrations on the junction, the isopiestic method was developed. This eliminates the need for calibration and can be used for tissues with very low water potentials (Boyer and Knipling, 1965).

Various changes have been made to thermocouple psychrometers for monitoring soil water potentials, the water potential in tree trunks, attached roots and attached shoots (Turner, 1981; Kramer, 1983).

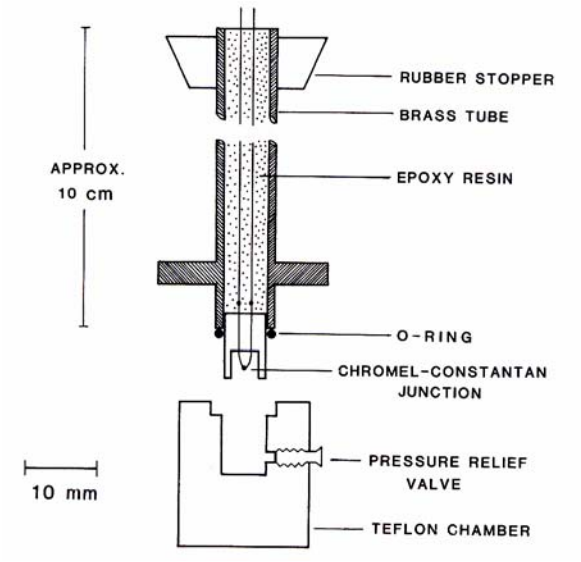



Fig. 2.4. A Peltier thermocouple psychrometer that is used for measuring water potential of leaf tissue. To prevent a pressure build-up in the cup, the relief valve is left open until the cap and cup are joined together (Kramer, 1983).

*2.5.1.4 Pressure chamber*

A sun-exposed (transpiring) leaf is removed from the tree with a razor blade and fitted through the lid of a pressure chamber. The cut end is sealed air-tight through a rubber compression gland with the petiole extending outside the chamber and which is exposed to atmospheric pressure (Fig. 2.5). Pressure is slowly increased in the chamber by compressed air from a cylinder until xylem sap appears from the cut end of the petiole. The pressure at which the liquid just wets the surface is noted. The pressure is released and the sample is removed. The pressure necessary to force water out of the leaf cells represents negative pressure existing in the intact stem (Scholander et al., 1964; 1965).

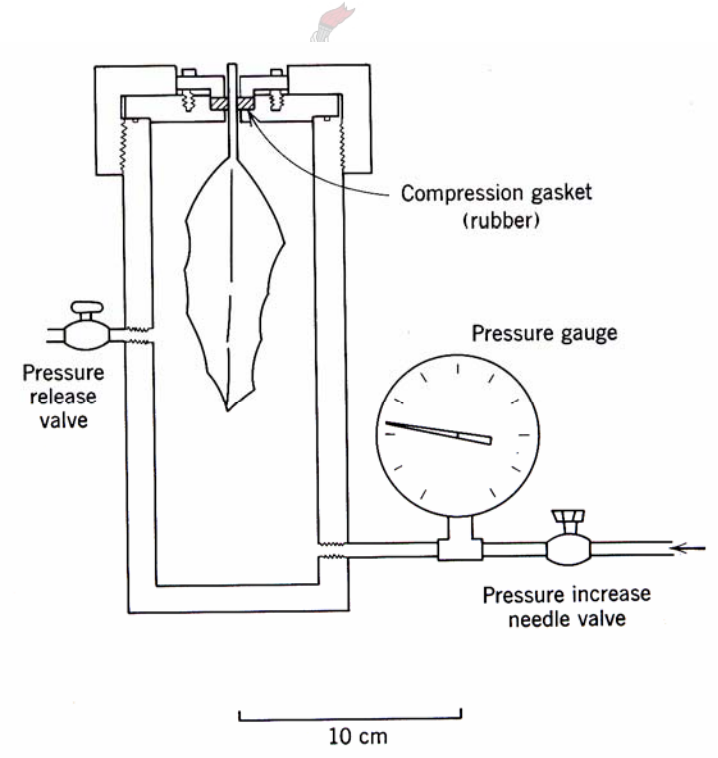



Fig. 2.5. Diagram of a pressure chamber for the measurement of leaf water potential (Kauffman and Kramer, 1967).

Leaf water potential is highly variable due to environmental conditions (McCutchan and Shackel, 1992). Variability due to changing environmental conditions can be reduced by predawn water potential measurements. These measurements indicate overnight recovery in water potential through equilibration with soil water potential, rather than the water potential experienced under midday conditions when a plant's stomata are open and water demand is highest due to large vapour pressure deficit. However, leaf water potential has not been clearly related to plant water status (McCutchan and Shackel, 1992) as Jones (1985) found that apples (*Malus domestica* Borkh.) grown under water stress conditions showed symptoms of premature leaf senescence when compared to well irrigated trees, while both had similar water potential measurements.

In contrast, stem water potential is closely related to plant water use and stomatal conductance, and is thus a more appropriate measure of plant water stress (McCutchan and Shackel, 1992; Schackel et al., 1997). To measure stem water potential, a leaf should be enclosed in a black polyethylene bag and covered with aluminium foil 4 to 5 hours prior to measurement (Begg and Turner, 1970; McCutchan and Shackel, 1992; Shackel et al., 1997; Barry et al., 2004b). Bagging of a leaf prevents evaporation from the enclosed leaf and eliminates within-leaf water potential gradients (Begg and Turner, 1970; Garnier and Berger, 1985). When bagged leaves remain attached to the plant, leaf water potential would be able to reach equilibrium with the water potential of the stem and thus the plant (McCutchan and Shackel, 1992).

Stem water potential is less influenced by environmental variability than transpiring leaf water potential. Garnier and Berger (1985) found no difference in leaf water potential of stressed vs. control trees, whereas there was a significant difference in stem water potential in

leaves of trees exposed to different water stress levels. The influence of stomatal conductance and leaf transpiration on the water potential gradient between the leaf and the stem may be responsible for the difference in stem and leaf water potentials (McCutchan and Shackel, 1992). During transpiration, leaf water potential is lower than stem water potential and the difference between these two potentials will represent a water potential gradient. If this gradient is large, then stomatal responses could cause any reduction in stem water potential to be counterbalanced by a reduction in transpiration and the size of the water potential gradient. If both reductions are equivalent, there could be a reduction in stomatal conductance with decreases in stem water potential, but no change in leaf water potential.

However, the technique of stem water potential as a reliable measure of plant water status has not been extensively used in citrus trees (*Citrus* spp.) (Goldhamer and Salinas, 2000; Barry et al., 2004b). Previous studies that used this technique were done on tobacco (*Nicotiana tabacum* L.) (Begg and Turner, 1970), prunes (*Prunus domestica* L.) (McCutchan and Shackel, 1992), almonds (*P. dulcis* (Miller) D. A. Webb), cherries (*P. cerasus* L.) and pears (*Pyrus communis* L. ) (Shackel et al., 1997), nectarines (*Prunus persica* (L.) Batsch) (Naor et al., 1999) and apples (Naor and Cohen, 2003). The number and the position of leaves used for measuring $\psi_{stem}$ differed in each of the studies. For example, a single leaf per tree from four trees per treatment was used by Goldhamer and Salinas (2000). Barry et al. (2004b) measured $\psi_{stem}$ from two terminal, sun-exposed leaves per tree that were situated in the southwest upper canopy from two trees per treatment. McCutchan and Shackel (1992) used two to three leaves that were located near the main scaffold branch of the tree, whereas Naor et al. (1999) and Naor and Cohen (2003) used two leaves situated in the inner part of the tree canopy.

The pressure chamber provides approximate measurements of water potential, but there is likely more variability than using a psychrometer (Boyer, 1967).

### 2.5.2 Osmotic potential

The absolute value of osmotic potential is equal to the real pressure in pure water at equilibrium (Salisbury and Ross, 1991). Osmotic potential varies with changes in solute concentrations and is thus a less satisfactory measurement of water potential (Kramer, 1983). The osmotic potential of solutions can be measured by using the cryoscopic / freezing-point method, plasmolysis, refractomeric method, thermocouple psychrometers and the pressure chamber.

#### *2.5.2.1 Cryoscopic / freezing-point method*

Properties of solutions that are functions of the mole fraction are called colligative properties (Salisbury and Ross, 1991), and include freezing point, boiling point, vapour pressure and osmotic potential. Osmotic potential can be calculated by using any of the other values. Measuring the freezing point to calculate the osmotic potential is called cryoscopic or freezing-point method and is a very accurate method of measuring the freezing point of solutions (Slavik, 1974). Mercury thermometers and thermocouples are used for accurately determining the freezing points of solutions. Osmotic potential can then be calculated using the following equation (Salisbury and Ross, 1991):

$$\psi_s \text{ (in MPa)} = 1.22 \text{ x freezing point } (^{\circ}C).$$

Obtaining a plant sap solution is problematic, with different methods resulting in different values for $\psi_s$ from the same plant tissue (Slavik, 1974; Turner, 1981).

*2.5.2.2 Plasmolysis*

Tissue samples are placed in a series of solutions with known osmotic potentials (Slatyer, 1967). After an equilibration period, the tissue is examined under a microscope. Plasmolysis occurs when half the cells in the tissue begin to plasmolyse (protoplasts begin to pull away from cell walls). This represents an internal pressure of zero. The osmotic potential of the solution that caused plamolysis is then the same as the osmotic potential within the cells (Slavik, 1974).

*2.5.2.3 Refractomeric method*

In the refractomeric method, a drop of sap is placed on the prism of a refractometer to determine the reading (Slavik, 1974). The relationship between the refractive index and the osmotic potential is influenced by the concentration of solutes as well as the components of the solution. Sugars have a larger effect than electrolytes. As the concentration of sugars increases, so does the effect on the refractive index. Results will be more reliable if the temperature of the measuring and illuminating prisms is kept constant. Readings are easily made, but prisms must be cleaned and dried after each measurement (Turner, 1981).



*2.5.2.4 Thermocouple psychrometer*

Osmotic potential can also be measured with a psychrometer (Slavik, 1974). After the water potential is measured, the tissue is killed by freezing with liquid nitrogen (Turner, 1981). The equilibrium vapour pressure is measured again. The turgor pressure has been eliminated by killing of the tissue, the new measurement represents the osmotic potential. The main problem with this method is that the vascular sap is diluted by cell wall sap after the disruption of the cell membranes by freezing. This may create matric potentials which are not present in the unfrozen tissue (Kramer, 1983).

*2.5.2.5 Pressure chamber*

The pressure chamber can be used to estimate osmotic potential (Tyree and Hammel, 1972). In the pressure chamber the turgor pressure is reduced to zero (Turner, 1981). This is not done by killing the tissue, but by applying pressure to the leaves and determining the osmotic potential from the pressure-volume relation of the cells (Scholander et al., 1965; Tyree and Hammel, 1972).

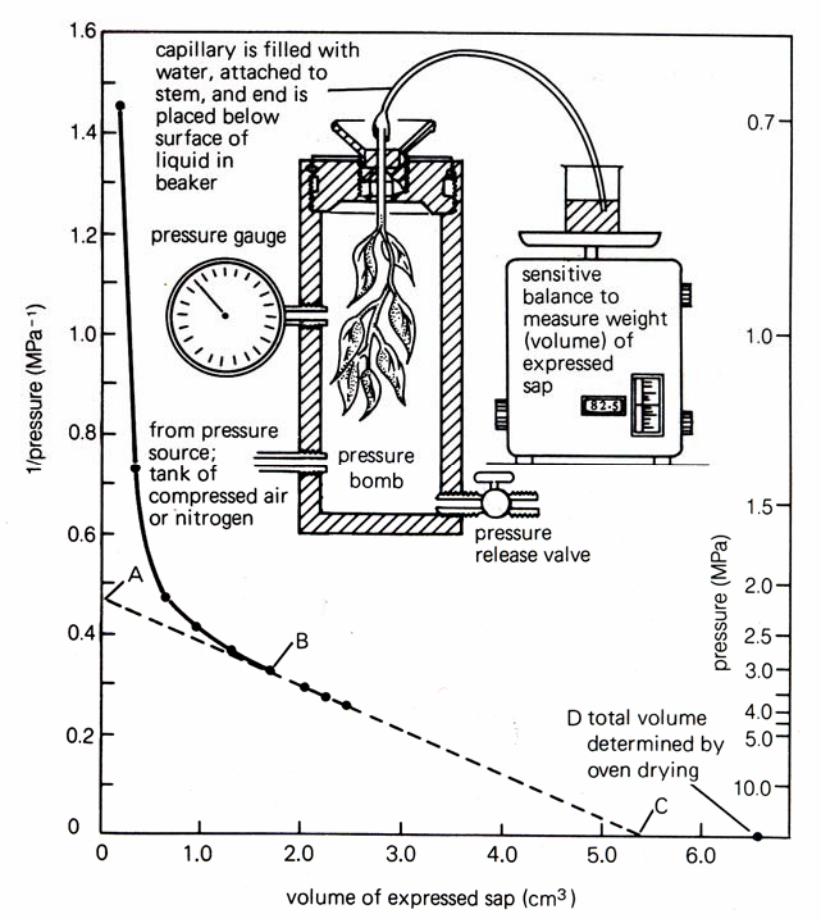



Fig. 2.6. Using the pressure chamber for measuring several parameters important to the water relations of plants. Point A: negative value of the pressure at this point equals the volume average of the osmotic potential of hydrated tissue. Point B: the turgor loss point (comparable to incipient plasmolysis). Point C: volume of free water in hydrated tissue. Point D: total

volume of tissue water. Bound water (including some apoplastic water) equals D minus C (Salisbury and Ross, 1991).

A leaf is hydrated by placing the cut end in pure water for several hours and then fitted through the lid of a pressure chamber (Salisbury and Ross, 1991). The cut end is sealed air-tight through a rubber compression gland with the petiole extending outside the chamber (Scholander et al., 1965). Pressure is slowly increased in the chamber until xylem sap appears from the cut end of the petiole. As mentioned before, this pressure is an indication of the leaf water potential. The leaf is then over-pressurised and the water which is forced from the leaf is collected and weighed (Tyree and Hammel, 1972). From these data a pressure-volume curve can be plotted (Fig. 2.6) which indicates the original osmotic potential and the volume of apoplastic water (Tyree and Hammel, 1972; Turner, 1981).



## 2.6 Fertigation

Fertigation is the application of water-soluble fertilisers typically with drip or micro-sprinkler irrigation systems to control water and nutrient supplies to crops (Bar-Yosef, 1999). The main differences between drip irrigation and micro-sprinkler irrigation are that with drip irrigation water is generally applied to a restricted root zone of the plant and at more frequent intervals than micro-sprinkler irrigation (Elfving, 1982). By applying fertilisers through the drip irrigation system the fertilisation efficiency is increased since nutrients are applied to a restricted root zone (Bar-Yosef, 1999) and the roots are capable of much faster uptake of nutrients (Bar-Yosef, 1988).

There are several advantages of fertigation. By modifying the factors affecting the changes in the phenological stages of plant growth and development, e.g. change from reproductive to

vegetative growth, helps the development of trees during the first year after planting to form a structure on which to bear fruit the following year (Richards, 1986).

Bravdo and Poebstring (1993) found that by using fertigation better control over the quality aspects of fruit can be achieved. The best fruit quality is obtained when fruit ripens under conditions of sufficient leaf area and minimal vegetative growth. Robinson and Stiles (1993) found that fertigation resulted in larger apple trees with greater bearing capacity. In addition to increasing the total yield, fertigation also increased fruit size. Boman (1996) found that fertigation increased yield and total soluble solids for grapefruit (*C. paradisi* Macf.) when compared to conventional broadcast application of nutrients. Minute concentrations of minor elements can easily be applied by drip fertigation with less runoff than micro-sprinkler irrigation (Bar-Yosef, 1999).

By comparing different fertigation methods, Tolmay et al. (2002) found that double drip fertigation line treatments, each fertigated four and eight times per day, had the highest yield. This was followed by the micro-sprinkler fertigation treatments, also fertigated four and eight times per day. These treatments were better than the single drip fertigation treatment. Kruger et al. (2000a) also found that a double drip line with higher fertigation frequencies had the highest yields. This was also followed by micro-sprinkler irrigation with a single drip line having significantly lower yields than other treatments. Lombaard (1994) found that both double and single drip line fertigation were better than micro-sprinkler fertigation for young citrus trees. Syvertsen and Sax (1999) found no difference in canopy growth, tree water usage, fruit yield or growth when they compared different micro-sprinkler fertigation treatments.

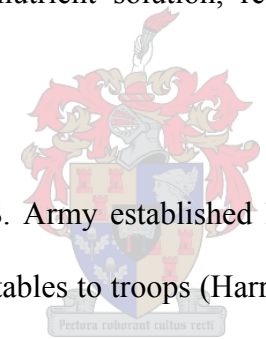
## 2.7 Open Hydroponics System (OHS)

Hydroponics is the technique of growing plants without soil (Mason, 1990). By using hydroponics, the biosphere is altered by changing the growth medium and thereby eliminating the dependence of plants on soil. Under normal conditions, soils function as support for the plant and as a source of water and nutrients (Schwarz, 1995). The plant roots in a hydroponics system grow either in air, water or in some solid, non-soil medium. This medium does not provide or store nutrients. The water around the roots contains a balanced mixture of nutrients which provides the food for the plant (Mason, 1990). Therefore, hydroponics is the science of growing plants in a medium, using mixtures of essential plant nutrient elements dissolved in water (Harris, 1971).

Hydroponic systems can be classified as either being open or closed. In open systems, the surplus amounts of nutrient solutions are not recovered, whereas in closed systems the surplus nutrient solution is recovered and re-used. The recovered solution is analysed for nutrients, and nutrients that have been used by the plants can then be replaced before returning the solution to the plants (Lippert, 1993; Stanghellini and Rasmussen 1994; Jones, 1997; Jensen, 1999; Venter, 1999).

The word hydroponics is derived from two Greek words: “hydro” meaning water and “ponos” meaning labour (Bentley, 1959; Harris, 1971; Harris, 1987; Mason, 1990; Schwarz, 1995; Jones, 1997). The term was first used in 1929 by Dr. W.F. Gericke, a Californian professor who began to develop a laboratory technique into a commercial means of growing plants (Bentley, 1959; Harris, 1971; Harris, 1987; Mason, 1990; Jones, 1997).

The concept of hydroponics has been practiced for centuries, for example, in the ancient Hanging Gardens of Babylon and the floating gardens of the Aztecs in Mexico (Jones, 1990). The basic concept was established by plant scientists investigating how plants grow. In 1600, Jan Van Helmont of Belgium conducted an experiment that showed that plants obtain their necessary substances from water (Schwarz, 1995). In 1699, John Woodward from England grew plants in water to which he added different types of soil and found that certain substances derived from soil, rather than water, were responsible for plant growth (Harris, 1971; Schwarz, 1995). German scientists, Sachs in 1860 and Knop in 1861, made synthetic solutions of essential plant nutrients in water (Harris, 1971; Mason 1990). These formulations combined with many other experiments, provided Dr. Gericke with the knowledge to make an effective nutrient solution, realising the commercial potential of hydroponics.



In the Second World War, the U.S. Army established large hydroponic gardens on several Pacific islands to supply fresh vegetables to troops (Harris, 1971; Mason, 1990; Jones, 1997). The development of a system known as Nutrient Film Technique (NFT) by Dr. A. Cooper from the United Kingdom in the 1970's made the hydroponic growing of a wide range of plants commercially viable (Mason, 1990; Cooper, 1996).

Open hydroponics adapts the principles of soil-less hydroponics to the production of fruit trees in a soil medium. The open hydroponics system (OHS) is a sensitive nutrient and moisture management system with a high degree of control over the development of the crop (Stassen et al., 1999). Specific water and nutrient requirements for different phenological stages of the plant are applied through daily fertigation. A balanced nutrient solution with controlled pH and EC is applied using a drip irrigation system. This concept includes

reducing the influence of soil as a water and nutrient storage medium, and using the soil to anchor the plant and to deliver nutrients to the roots (Stassen et al., 1999). The principles of these adaptations are to reduce the size of the root zone, by reducing the wetted soil volume, and the continuous application of a pH-buffered nutrient solution. At the beginning of the 1990's, Professor Rafael Martinez Valero of Spain brought all the concepts of hydroponics together to develop the commercial application of the open hydroponics system and later called it Martinez Open Hydroponics Technologies (MOHT) (Martinez and Fernandez, 2004; Falivene et al., 2005).

### 2.7.1 Advantages of OHS

The advantages of using OHS include: crops can be grown in areas where soils are not suitable for cultivation (Mason, 1990; Jones, 1997); less labour is required as fertilisers are applied through the irrigation system and not by hand (Schwarz, 1995); water and nutrients are conserved as both are applied in correct quantities and close to the plant with less waste (Mason, 1990; Schwarz, 1995; Jones, 1997), which also leads to less weed growth (Harris, 1971; Mason, 1990); yields are increased with increases in fruit size (Harris, 1971; Schwarz, 1995); and producers have better control over the system to ensure that the correct amounts of nutrients are applied at the different physiological stages of plant development to optimise growth (Harris, 1971).

Reducing the wetted soil volume by reducing the amount of drippers per tree restricts the root zone. This principle makes it possible to practice OHS in arid climates (Falivene et al., 2005). Studies done on restricting root zones found a decrease in fruit yields. Boland et al. (2000) found a decrease in yield and growth of peach trees. Bar-Yosef et al. (1988) found that restricting root zones decreased yield, total dry matter production and water uptake rates, but

found an increase in soluble solids in fruit. These declines coincided with a decrease in apple tree growth. It is possible that under OHS conditions, roots grow more densely in a smaller soil volume, but that this volume is sufficient to support root growth and a productive tree (Falivene et al., 2005). The smaller tree makes harvesting easier (Coetzee, 1998).

As a nutrient solution moves through the soil to the roots, the soil will buffer and change the nutrient solution. In a restricted root zone, the applied nutrients move through a smaller amount of soil to reach the roots (Falivene et al., 2005). Water and nutrients are conserved as both are applied in correct quantities and close to the plant with less waste (Mason, 1990; Schwarz, 1995; Jones, 1997).

Different nutrient solutions are used at different physiological growth stages, but any nutrient can be added at any stage (Woods, 1999). A computer system is necessary for injecting the nutrients in the correct amounts as programmed before entering the irrigation system (Van Rooyen, 2000).



OHS attempts to maintain soil moisture near field capacity whereas in the MOHT system soil moisture is maintained above field capacity. Martinez and Fernandez (2004) suggests that roots are able to take up more water and nutrients at soil moisture levels above field capacity. Martinez and Fernandez (2004) claim that this allows stomata to stay open longer during peak water demand periods. Leaves will be able to photosynthesise longer, producing more carbohydrates which increases the productivity of the tree. To optimise this productivity, Woods (1999) suggests that irrigation should be applied at 1300 HR. However, Schoeman (2002) suggested that morning conditions should be optimised by early irrigation so that plants can perform optimally at the time of highest photosynthetic activity.

Water from the drippers form separate “onion-shaped” wetting patterns in the soil with 96% of roots found in the wetted areas. Drippers should be spaced to ensure that the wetted areas do not touch (Woods, 1999). The roots are always well aerated as flooding of the whole root system does not occur.

Trees can easily be manipulated by using OHS. By applying water stress selectively, fruit quality can be manipulated (Woods, 1999) and shoot growth can be controlled (Woods, 1999; Groenewald, 2000).

Martinez and Fernandez (2004) claimed to have eliminated alternate bearing and increased yields with citrus fruit quality being better than the export thresholds by using the MOHT system. Kuperus et al. (2002) found an increase in fruit yield for ‘Valencia’ orange on OHS when compared to conventional systems. Kruger et al. (2000b) found an increase in yield in ‘Clementine’ mandarin, but fruit size was smaller than conventional treatment. They also found an increase in yield and fruit size in ‘Valencia’ orange.



### 2.7.2 Disadvantages of OHS

The disadvantages of using OHS are mainly related to costs and control. The initial costs of construction and implementation of OHS equipment is high (Harris, 1971, Mason, 1990, Jones, 1997), and converting from a conventional system to OHS is expensive (Woods, 1999).

To successfully use the open hydroponic system, knowledge is required to operate the system and of the principles of plant physiology and plant nutrition (Harris, 1971; Mason, 1990;

Jones, 1997; Schwarz, 1997). As the root zone is more restricted, a higher level of control over the irrigation and nutrient solution is required (Falivene et al., 2005).

OHS requires a constant supply of water. The shut down of the irrigation system has major implications for OHS with a restricted root zone whereas the implication for a conventional system is relatively minor (Falivene et al., 2005). By using OHS, the advantages of receiving rainfall are neutralised (Woods, 1999).

An intensive fertigation system increases the possibility of soil acidification and salt accumulation on the edges of the wetted zone. The opposite is also possible with maintaining soil moisture close to field capacity, the risk of nutrient leaching is increased, although leaching can be a problem in all irrigation systems.


## 2.8 Daily Drip Fertigation (DDF)

Since the implementation of MOHT in horticultural practices all over the world, many variations of the concept have developed. The variation in OHS will be referred to as Daily Drip Fertigation (DDF). Daily drip fertigation involves drip fertigation of plants on a daily basis during daylight hours (Stassen et al., 1999; Pijl, 2001; Schoeman, 2002).

The main difference between OHS and DDF is that DDF uses a larger conventional root zone volume with irrigation being applied more frequently and only during daylight hours compared with OHS (Falivene et al., 2005). The implication hereof is that the characteristics of the soil, and its function as a storage medium is better utilised. This reduces the application rate of certain nutrients, and the cost-benefit should also be taken into account.

The larger wetted volume contains more water and nutrients that is available for a longer period of time for the roots. Irrigation is applied in numerous "pulses" using the DDF system.

The highest concentration of roots is found under drippers where the most nutrients and water are available (Falivene et al., 2005). Pijl (2001) found that root development under DDF was excellent, but limited to the strip next to and between the wetted zones. Roots of plants under conventional (micro-sprinkler) irrigation were well developed and much more scattered than DDF.

Pre-dawn xylem water potential measurements indicated that plants under DDF performed optimally early in the morning compared to plants under micro-sprinkler irrigation (Schoeman, 2002). However, the plants were also under stress since midday depression occurred with little recovery in the afternoon. In general, plants under DDF have excellent water conditions in rooting volume and therefore experience minimal levels of stress. The limited root volume does not supply water to the same extent as a larger root volume would in response to high atmospheric demand. A larger root volume enhances recovery from midday depression (Schoeman, 2002).

# CHAPTER 3

# STEM WATER POTENTIAL AS A MEASURE OF PLANT WATER STATUS IN CITRUS TREES (*CITRUS* SPP.)


## Abstract

This study was undertaken to quantify the effects of daily fertigation on ecophysiological responses in citrus trees (*Citrus* spp.) as part of a larger study. Initial research was conducted to optimise and standardise the sampling procedure to quantify stem water potential ($\psi_{stem}$) in citrus trees. To reliably determine the plant water status of citrus trees, the following conditions are required to minimise unwanted variation in $\psi_{stem}$ measurements. Bagging of leaves with black polyethylene envelopes covered with aluminium foil 3 to 4 hours prior to measuring $\psi_{stem}$ allows the plant water status in those leaves to equilibrate with whole-tree plant water status, thereby providing a realistic measurement of the current water status. The use of aluminium foil to cover the bagged leaves, reduces unwanted heat stress by reflecting sunlight, and dramatically reduced variation in $\psi_{stem}$. On average, the difference between measurements of bagged leaves without aluminium foil and bagged leaves covered with aluminium foil was 0.19 MPa in the 2002-03 season and 0.13 MPa in the 2003-04 season. The time of day at which $\psi_{stem}$ measurements are made is important to ensure consistency in comparisons among treatments and interpretation of irrigation treatment effects. "Physiological midday" is the preferred time of day to measure $\psi_{stem}$, i.e. 1100 HR. Transpiring leaves with open stomata would be in sun-exposed positions on the east side of trees and should be used for making $\psi_{stem}$ measurements. On average, the difference in $\psi_{stem}$ on the east and west side of trees was 0.17 MPa and 0.22 MPa in the 2002-03 and 2003-04 seasons, respectively. Under similar experimental conditions as those used here, only three leaves per replicate are required to detect a difference of 0.05 MPa in $\psi_{stem}$ between treatment

means. Plant water status categories were developed which may have useful practical applications, i.e. >-1.0 MPa = no water deficit; -1.0 to -1.2 MPa = low water deficit; -1.2 to -1.4 MPa = moderate water deficit; <-1.4 to -1.6 MPa = high water deficit; and <-1.6 MPa = severe water deficit.

**Introduction**

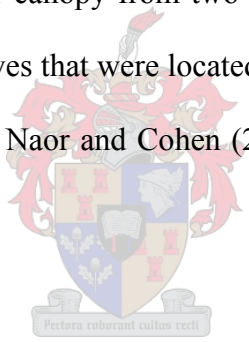
The water potential of a plant is an indication of the plant's water status as water potential accounts for the integrated effect of soil, plant and atmospheric conditions on water availability within the plant (McCutchan and Shackel, 1992). The potentials and resistances to water flow within a plant could not be measured accurately until the development of the pressure chamber simplified the measurement of water potentials in fields and orchards (Scholander et al., 1964; 1965). By measuring the water potential of sun-exposed (transpiring) leaves, leaf water potential could be estimated.

Leaf water potential is highly variable due to prevailing environmental conditions (McCutchan and Shackel, 1992). Variability due to changing environmental conditions can be reduced by using predawn water potential measurements. These measurements indicate overnight recovery in water potential through equilibration with soil water potential, rather than the water potential experienced under midday conditions when a plant's stomata are open and water demand is highest due to large vapour pressure deficit. However, leaf water potential has not been clearly related to plant water status (McCutchan and Shackel, 1992) as Jones (1985) found that apples (*Malus domestica* Borkh.) grown under water stress conditions showed symptoms of premature leaf senescence when compared to well irrigated trees, while both had similar water potential measurements.

In contrast, stem water potential is closely related to plant water use and stomatal conductance, and is thus a more appropriate measure of plant water stress (McCutchan and Shackel, 1992; Shackel et al., 1997). To measure stem water potential, a leaf is enclosed in a black polyethylene bag and covered with aluminium foil 4 to 5 hours prior to measurement (Begg and Turner, 1970; McCutchan and Shackel, 1992; Shackel et al., 1997; Barry et al., 2004b). Bagging of a leaf prevents transpiration from the enclosed leaf and eliminates within-leaf water potential gradients (Begg and Turner, 1970; Garnier and Berger, 1985). When bagged leaves remain attached to the plant, leaf water potential would be able to reach equilibrium with the water potential of the stem and thus the plant (McCutchan and Shackel, 1992).

Stem water potential is less influenced by environmental variability than transpiring leaf water potential. Garnier and Berger (1985) found no difference in leaf water potential of stressed vs. control trees, whereas there was a significant difference in stem water potential in leaves of trees exposed to different water stress levels. The influence of stomatal conductance and leaf transpiration on the water potential gradient between the leaf and the stem may be responsible for the difference in stem and leaf water potentials (McCutchan and Shackel, 1992). During transpiration, leaf water potential is lower than stem water potential and the difference between these two potentials will represent a water potential gradient. If this gradient is large, then stomatal responses could cause any reduction in stem water potential to be counterbalanced by a reduction in transpiration and the size of the water potential gradient. If both reductions are equivalent, there could be a reduction in stomatal conductance with decreases in stem water potential, but no change in leaf water potential.

However, the technique of stem water potential as a reliable measure of plant water status has not been extensively used in citrus trees (*Citrus* spp.) (Goldhamer and Salinas, 2000; Barry et al., 2004b). Previous studies that used this technique were done on tobacco (*Nicotiana tabacum* L.) (Begg and Turner, 1970), prunes (*Prunus domestica* L.) (McCutchan and Shackel, 1992), almonds [*P. dulcis* (Miller) D.A. Webb], cherries (*P. cerasus* L.) and pears (*Pyrus communis* L.) (Shackel et al., 1997), nectarines (*P. persica* (L.) Batsch) (Naor et al., 1999) and apples (Naor and Cohen, 2003). The number and the position of leaves used for measuring stem water potential differed in each of the studies. For example, a single leaf per tree from four trees per treatment was used by Goldhamer and Salinas (2000). Barry et al. (2004b) measured stem water potential from two terminal, sun-exposed leaves per tree that were situated in the southwest upper canopy from two trees per treatment. McCutchan and Shackel (1992) used two to three leaves that were located near the main scaffold branch of the tree, whereas Naor et al. (1999) and Naor and Cohen (2003) used two leaves situated in the inner part of the tree canopy.




Therefore, the purpose of this study was to optimise the sampling procedure to reliably determine the plant water status of citrus trees. To optimise this sampling procedure, leaf and stem water potentials were quantified under varying conditions to minimise the variation in water potential measurements and thereby develop a reliable sampling procedure to determine plant water status.

## Materials and Methods

*Site and plant material.* This experiment was conducted at Welgevallen Experimental Farm, Stellenbosch University, Stellenbosch, Western Cape province, South Africa (33°57'S, 18°53'E; 75 m elev.). 'Nules Clementine' mandarin trees (*C. reticulata* Blanco) on 'Troyer'

citrange rootstock [*Poncirus trifoliata* (L.) Raf x *C. sinensis* (L.) Osb.] planted in 1992 in a N-S row orientation, were used during the late summer and autumn of 2002-03 and 2003-04.

*Data collection.* To determine the importance of minimising heat build-up of bagged leaves when measuring stem water potential ($\psi_{stem}$), 20 mature, healthy leaves were selected on the east side of five trees. Leaves were bagged with small, black polyethylene envelopes at 0800 HR, and half of the bagged leaves were immediately covered with aluminium foil and the other half were left uncovered. Stem water potential was measured with a PMS 600 pressure chamber (PMS Instruments, Albany, Ore., USA) at 1100 HR following procedures outlined by McCutchan and Shackel (1992) and Barry et al. (2004b).

To determine whether canopy position on which leaves are borne affects $\psi_{stem}$, 10 mature, healthy leaves were randomly selected from each of the east and west sides of five trees. Leaves were bagged and covered with aluminium foil, as described above, at 0800 HR. Stem water potential was measured at 1100 HR.

To quantify the diurnal changes in leaf water potential ($\psi_{leaf}$) to determine optimal sampling time for $\psi_{stem}$ measurements, 10 mature, healthy leaves were selected on the east side of five trees. Leaf water potential of unbagged leaves was measured at 0630 HR, 0800 HR, 1045 HR and 1400 HR.

*Statistical analysis.* Data were subjected to analysis of variance (ANOVA) using the GLM procedure of SAS (SAS Inc., Cary, N.C., USA). Means were separated by Fisher's least significant difference (LSD).

To determine sample size, a standard sample size equation (Steel and Torrie, 1980) was used to calculate sample size estimates for the number of leaves per sample or trees (replicates) required to detect a difference between two means, $n = (2 \cdot t_{\alpha}^2 \cdot S^2)/d^2$, where *n* is the number of leaves or trees, *t* is Student's *t*-value for the degrees of freedom associated with $S^2$ at $P \leq 0.05$ (when $\alpha = 0.05$ and df = 15 to 27, then $t \approx 2.1$), $S^2$ is the sample variance for leaves or trees, and *d* is the desired degree of precision, or the difference to be detected between treatment means. The numbers of leaves per sample and replications required to detect differences between two treatment means for $\psi_{stem}$, at a desired degree of precision and at $P \leq 0.05$, was plotted to generate Fig. 3.7.

**Results**

*Effect of reducing heat build-up on $\psi_{stem}$.* During both seasons, $\psi_{stem}$ measurements of bagged leaves without aluminium foil were significantly ($P<0.05$) more negative than that of bagged leaves covered with aluminium foil (Figs. 3.1 and 3.2). On average, over all sampling dates, this difference was 0.19 MPa in the 2002-03 season and 0.13 MPa in the 2003-04 season. In both seasons, sample variance was greatest in this component of the overall study where the black polyethylene bags were either covered with aluminium foil or left uncovered (Table 3.1).

*Effect of canopy position on $\psi_{stem}$.* Stem water potential at 1100 HR was significantly ($P<0.05$) more negative on the east than the west side of trees in both the 2002-03 (Fig. 3.3) and the 2003-04 (Fig. 3.4) seasons. The difference in $\psi_{stem}$ between the east and west side of trees was consistent over nine sampling dates during the two seasons. On average, this difference in $\psi_{stem}$ was 0.17 MPa and 0.22 MPa in the 2002-03 and 2003-04 seasons, respectively.

Where $\psi_{stem}$ measurements were taken from two canopy positions, sample variance was relatively large (0.01008; Table 3.1).

*Diurnal changes in $\psi_{leaf}$.* Leaf water potential was highest (least negative) at dawn and decreased through the day until stomatal closure at physiological midday (1045 HR), at which time $\psi_{leaf}$ was most negative. This response was consistent over both the 2002-03 (Fig. 3.5) and 2003-04 seasons (Fig. 3.6). During 2002-03, $\psi_{leaf}$ at 1400 HR was slightly less negative than at 0800 HR, until 13 Jan. 2003 after which time the 1400 HR $\psi_{leaf}$ measurements were slightly more negative. There was no significant difference in $\psi_{leaf}$ on 13 Jan. 2003 and 4 Feb. 2003. During the 2003-04 season, the 1400 HR $\psi_{leaf}$ measurements were more negative except on 16 Jan. 2004 and 5 Feb. 2004. Leaf water potential measurements were significantly ($P$<0.05) different on 5 Dec. 2003 and 12 Mar. 2004. The average differences in $\psi_{leaf}$ between dawn and midday measurements were 0.41 MPa in 2002-03 and 0.58 MPa in 2003-04. Sample variance was 0.00738 during the 2003-04 season when $\psi_{leaf}$ was measured at different times of the day (Table 3.1).

*Determining optimal sample size.* The relationship between the number of leaves or replicates and the difference to be detected between treatment means (degree of precision) are represented by an asymptotic line (Fig. 3.7). The difference to be detected, or the degree of precision, becomes smaller as the number of leaves increases, and the slope of the line decreases with the increasing sample size. To detect a difference in $\psi_{stem}$ between treatment means of 0.05 MPa, three leaves would be required to take $\psi_{stem}$ measurements.

**Discussion and Conclusions**

Stem water potential of leaves that were bagged in black polyethylene envelopes, but not covered with aluminium foil, was more negative than leaves that were bagged and covered. The uncovered black polyethylene envelopes have the ability to absorb more sunlight than envelopes covered by aluminium foil which probably results in an increase in temperature, although this was not measured. The rise in temperature will affect humidity in the envelope, and changes in humidity will adversely effect stomatal functioning. Whereas aluminium foil has the ability to reflect more sunlight than the black envelopes, the micro-climate in the covered envelopes is less affected. Furthermore, potential variance in $\psi_{stem}$ measurements was dramatically reduced when the black polyethylene envelopes were covered with aluminium foil.

In rows planted in a north-south orientation, which is considered to be the most efficient row orientation for the interception of available sunlight for photosynthesis, the east side of trees in the row is exposed to morning sun, while the west side of trees is partly shaded during the morning when the rate of photosynthesis is highest and stomata are open. Stem water potential was more negative on the east side of trees during both the 2002-03 and the 2003-04 seasons, which is the side that is more representative of transpiring trees prior to midday depression (1100 HR). By sampling leaves from a fixed canopy position, variation in $\psi_{stem}$ measurements was reduced. The difference in $\psi_{stem}$ between the east and west sides of trees was relatively consistent within a season and between seasons. Although it appears not to be critical which side of the tree is used for $\psi_{stem}$ measurements, it is imperative that leaves be sampled from only one canopy position, and sampling from sun-exposed leaves provides a more accurate measurement of plant water status.

Where water potential of unbagged leaves was measured, the typical diurnal changes in $\psi_{leaf}$ were observed; leaf water potential was highest at dawn and lowest at physiological midday due to depression of photosynthesis, with recovery thereafter resulting in less negative values of $\psi_{leaf}$ at 1400 HR.

Seasonal changes in $\psi_{stem}$ were observed in both seasons. More negative $\psi_{stem}$ measurements at certain times of the year were likely due to hotter, drier conditions experienced during those times. Direct comparisons of $\psi_{stem}$ between sampling dates or between seasons cannot be meaningfully made unless soil water status is also taken into account. However, the development of plant water status categories may have useful practical applications, i.e. >-1.0 MPa = no water deficit; -1.0 to -1.2 MPa = low water deficit; -1.2 to -1.4 MPa = moderate water deficit; <-1.4 to -1.6 MPa = high water deficit; and <-1.6 MPa = severe water deficit.

To optimise and standardise the sampling procedure to reliably determine the plant water status of citrus trees, the following conditions are required to minimise unwanted variation in $\psi_{stem}$ measurements. Bagging of leaves with black polyethylene envelopes covered with aluminium foil 3 to 4 hours prior to measuring $\psi_{stem}$ allows the plant water status in those leaves to equilibrate with whole-tree plant water status, thereby providing a realistic measurement of the current water status. The use of aluminium foil to cover the bagged leaves reduces heat build-up by reflecting sunlight and dramatically reduces variation in $\psi_{stem}$. The time of day at which $\psi_{stem}$ measurements are made is important to ensure consistency in comparisons among treatments. The preferred time of day to measure $\psi_{stem}$ is prior to midday depression in photosynthesis (1100 HR). The east side of trees is more representative of transpiring leaves with open stomata and should be used for making $\psi_{stem}$ measurements. When a standardised sampling procedure was used to determine $\psi_{stem}$, sample variance was

relatively low (0.00317; Table 3.1), and under similar experimental conditions, only three leaves per replicate would be required to detect a difference in $\psi_{stem}$ between treatment means of 0.05 MPa.

Table 3.1. Summary of sample variances during the 2003-04 season for various experiments.

| Experiment | Sample variance |
|---|---|
| Cover with aluminium foil | 0.01526 |
| Canopy position | 0.01008 |
| Time of day | 0.00738 |
| Backsberg (5 Mar 2004) | 0.00317 |

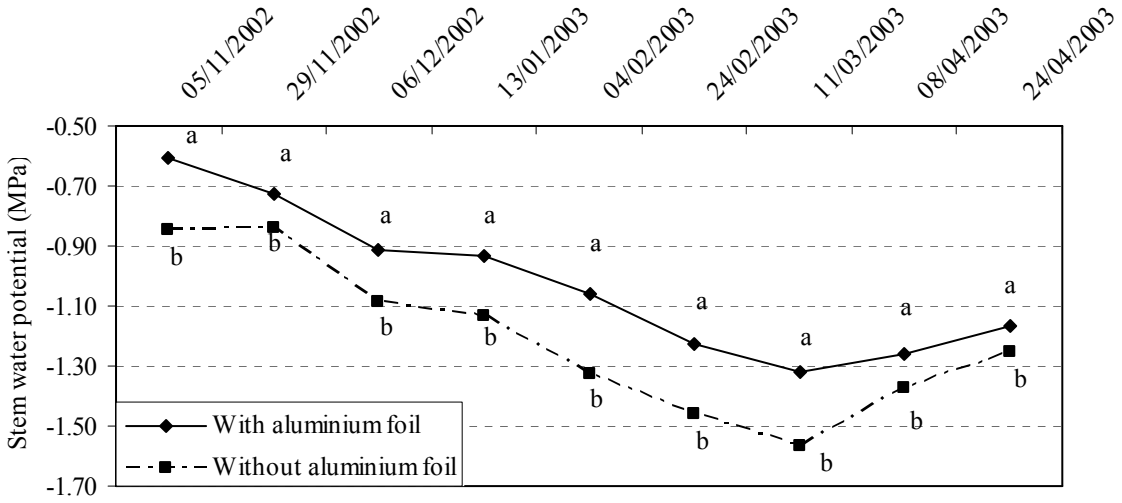



Fig. 3.1. Effect of aluminium foil on stem water potential (MPa) of 'Nules Clementine' mandarin sampled at Welgevallen Experimental Farm, Stellenbosch, during the 2002-03 season. Leaves from the east side of the trees were bagged and either covered with aluminium foil or left uncovered at 0800 HR and sampled at 1100 HR. There were 10 leaves per replicate, and five replicates. Data points, within sampling dates, with different letters are significantly different ($P$<0.05).

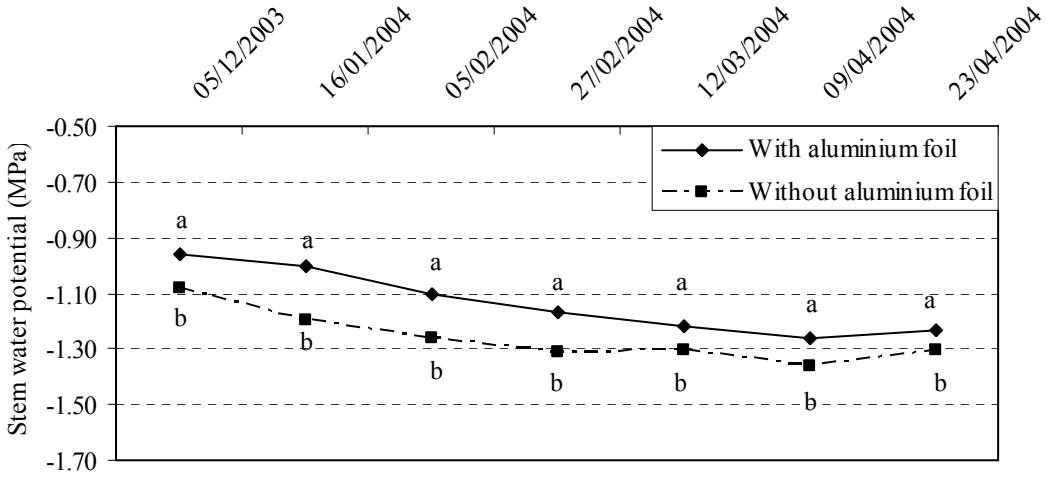



Fig. 3.2. Effect of aluminium foil on stem water potential (MPa) of 'Nules Clementine' mandarin sampled at Welgevallen Experimental Farm, Stellenbosch, during the 2003-04 season. Leaves from the east side of the trees were bagged and either covered with aluminium foil or left uncovered at 0800 HR and sampled at 1100 HR. There were 10 leaves per replicate, and five replicates. Data points, within sampling dates, with different letters are significantly different ($P$<0.05).

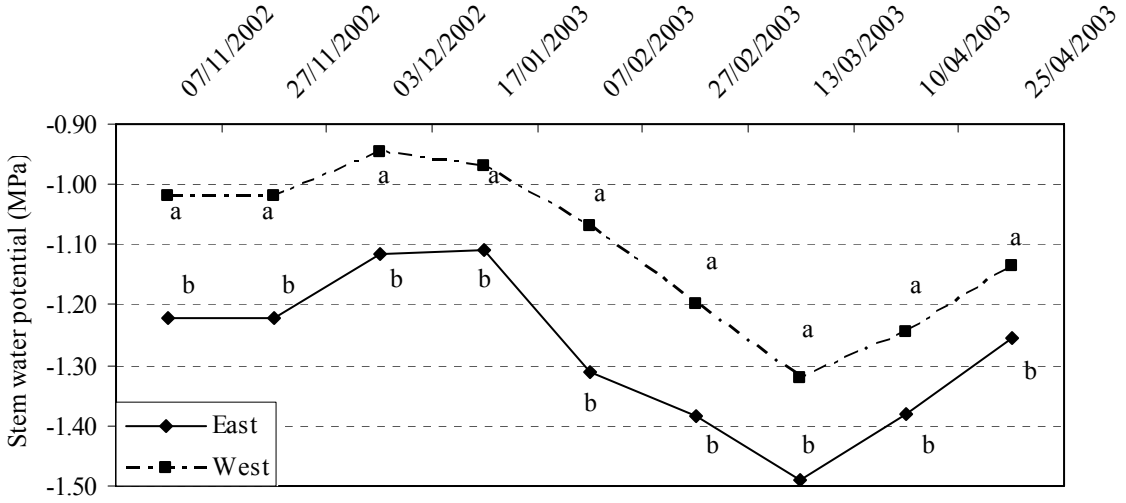



Fig. 3.3. Effect of canopy position on stem water potential (MPa) of 'Nules Clementine' mandarin sampled at Welgevallen Experimental Farm, Stellenbosch, during the 2002-03 season. Leaves from the east and west sides of the trees were bagged and covered with aluminium foil at 0700 HR and sampled at 1100 HR. There were 10 leaves per replicate, and five replicates. Data points, within sampling dates, with different letters are significantly different ($P$<0.05).

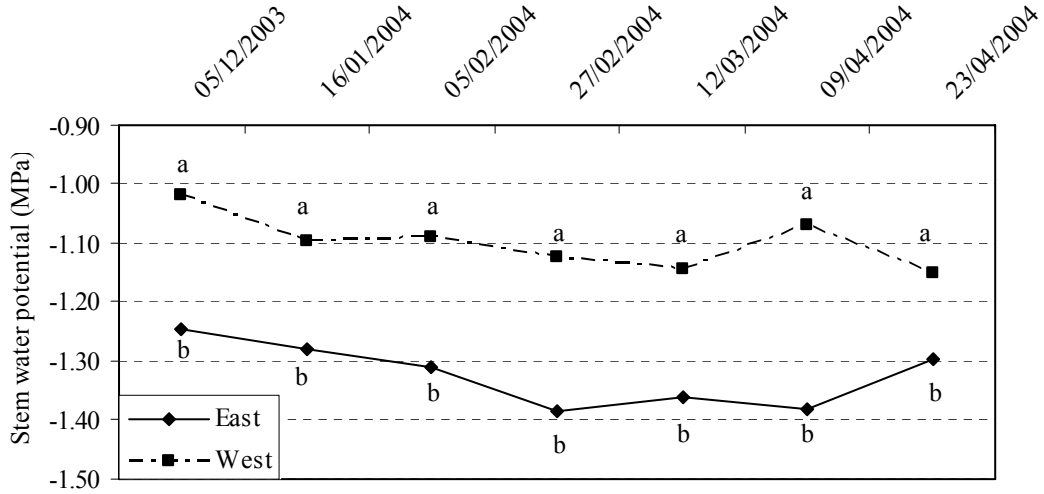
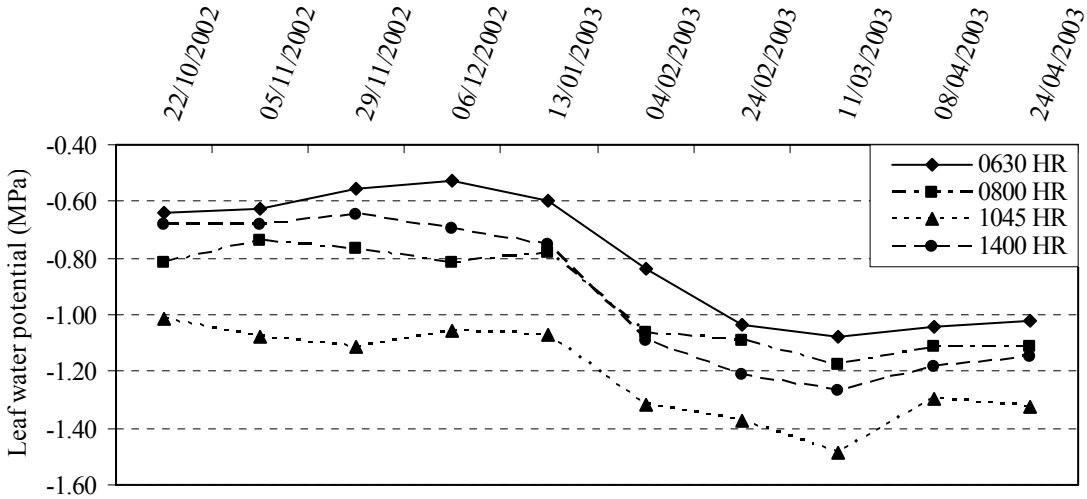


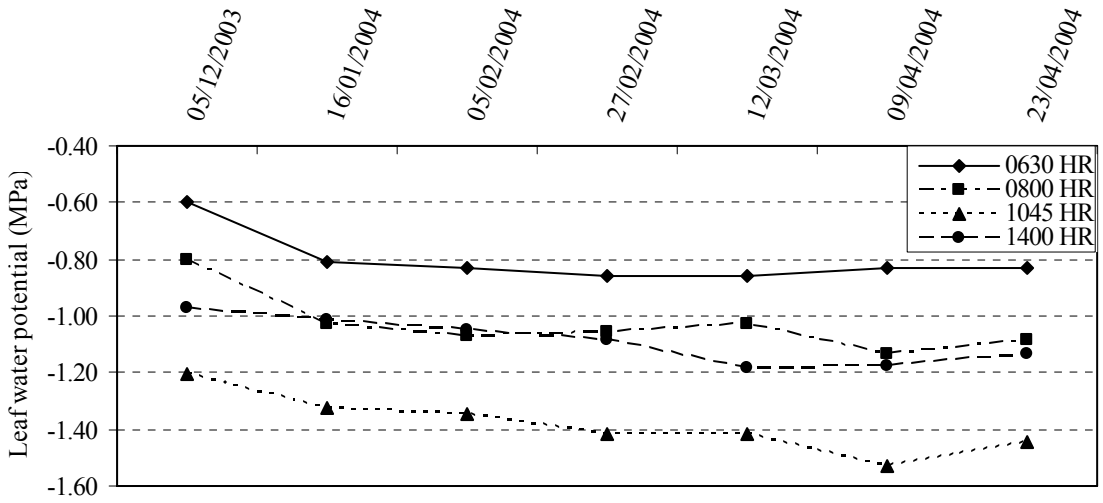

Fig. 3.4. Effect of canopy position on stem water potential (MPa) of 'Nules Clementine' mandarin sampled at Welgevallen Experimental Farm, Stellenbosch, during the 2003-04 season. Leaves from the east and west sides of the trees were bagged and covered with aluminium foil at 0700 HR and sampled at 1100 HR. There were 10 leaves per replicate, and five replicates. Data points, within sampling dates, with different letters are significantly different ($P$<0.05).




| Time | 22/10/2002 | 05/11/2002 | 29/11/2002 | 06/12/2002 | 13/01/2003 |
|---|---|---|---|---|---|
| 0630 HR | -0.64 a | -0.62 a | -0.56 a | -0.53 a | -0.60 a |
| 0800 HR | -0.82 b | -0.74 b | -0.77 b | -0.82 c | -0.78 b |
| 1045 HR | -1.02 c | -1.08 c | -1.11 c | -1.06 d | -1.07 c |
| 1400 HR | -0.69 a | -0.68 ab | -0.65 ab | -0.70 b | -0.76 b |
| P-value | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 |
| lsd | 0.118 | 0.111 | 0.119 | 0.102 | 0.099 |

| Time | 04/02/2003 | 24/02/2003 | 11/03/2003 | 08/04/2003 | 24/04/2003 |
|---|---|---|---|---|---|
| 0630 HR | -0.84 a | -1.04 a | -1.08 a | -1.05 a | -1.02 a |
| 0800 HR | -1.06 b | -1.09 a | -1.18 b | -1.12 b | -1.11 b |
| 1045 HR | -1.32 c | -1.38 c | -1.49 d | -1.30 d | -1.33 c |
| 1400 HR | -1.09 b | -1.22 b | -1.27 c | -1.19 c | -1.15 b |
| P-value | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 |
| lsd | 0.083 | 0.078 | 0.080 | 0.068 | 0.065 |

Fig. 3.5. Effect of time of day on leaf water potential (MPa) of 'Nules Clementine' mandarin sampled at Welgevallen Experimental Farm, Stellenbosch, during the 2002-03 season. Unbagged leaves from the east side of the trees were sampled. There were 10 leaves per replicate and five replicates. Data are presented both in graphical and in tabular format to assist with interpretation.




| Time | 05/12/2003 | 16/01/2004 | 05/02/2004 | 27/02/2004 | 12/03/2004 | 09/04/2004 | 23/04/2004 |
|---|---|---|---|---|---|---|---|
| 0630 HR | -0.60 a | -0.81 a | -0.83 a | -0.86 a | -0.86 a | -0.83 a | -0.83 a |
| 0800 HR | -0.81 b | -1.03 b | -1.07 b | -1.06 b | -1.03 b | -1.14 b | -1.09 b |
| 1045 HR | -1.21 d | -1.33 c | -1.35 c | -1.42 c | -1.42 d | -1.53 c | -1.45 c |
| 1400 HR | -0.97 c | -1.02 b | -1.05 b | -1.09 b | -1.19 c | -1.18 b | -1.14 b |
| P-value | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 |
| lsd | 0.078 | 0.080 | 0.093 | 0.102 | 0.086 | 0.100 | 0.100 |

Fig. 3.6. Effect of time of day on leaf water potential (MPa) of 'Nules Clementine' mandarin sampled at Welgevallen Experimental Farm, Stellenbosch, during the 2003-04 season. Unbagged leaves from the east side of the trees were sampled. There were 10 leaves per replicate and five replicates. Data are presented both in graphical and in tabular format to assist with interpretation.

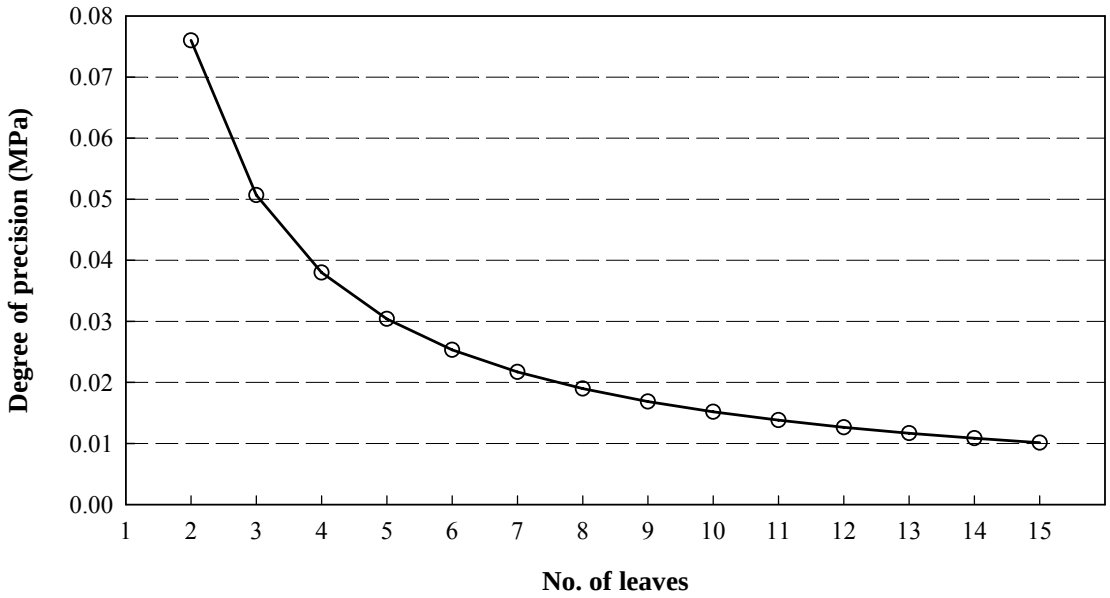



Fig. 3.7. Number of leaves required per sample to estimate the difference between two means for stem water potential for a given degree of precision at $P \leq 0.05$. The degree of precision refers to the difference in stem water potential of the tree.

# CHAPTER 4

# QUANTIFICATION OF DAILY DRIP FERTIGATION ON PLANT WATER STATUS AND SUGAR ACCUMULATION IN CITRUS TREES (*CITRUS* SPP.)

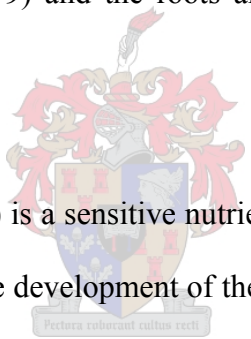

## Abstract

Attempts are being made to develop systems that improve crop management and enhance citrus fruit (*Citrus* spp.) production through efficient and timeous application of water and mineral nutrients which has led to the use of daily drip fertigation or the open hydroponics system (OHS). However, these perceived benefits are not necessarily supported by facts. Fruit size and yield are apparently enhanced, but possible negative aspects of the system have not been quantified. Fruit produced on trees grown under daily drip fertigation generally have a lower total soluble solids concentration. This is mainly due to a dilution effect that is caused by the greater availability of water and the uptake thereof. Sugar accumulation can be optimised by controlling the amount of water that the plant receives at different developmental stages. Therefore, it is essential to quantify the ecophysiological responses and benefits of OHS/daily fertigation, as well as the effects of this technology on fruit quality. 'Nules Clementine' mandarin (*C. reticulata* Blanco) trees in two commercial orchards in Simondium, Western Cape province, South Africa, received differential irrigation treatments. The treatments were applied at the end of stage I (± mid December) of fruit development. Stem water potential, fruit size and internal fruit quality were determined. Water-deficit stress enhanced sugar accumulation of 'Nules Clementine' mandarin by 0.3 to 0.6 °Brix under certain conditions. These conditions require that the difference in $\psi_{stem}$ should be of a sufficient intensity of between 0.16 and 0.3 MPa, and this difference should be maintained for a sufficient duration of between 4 and 6 weeks. Furthermore, deficit irrigation should be

applied relatively early in fruit development, namely during the sugar accumulation stage which starts within 4 weeks of the end of the fruit drop period and continues until harvest.

**Introduction**

Fertigation is the application of water-soluble fertilisers typically with drip or micro-sprinkler irrigation systems to control water and nutrient supplies to crops (Bar-Yosef, 1999). The main differences between drip irrigation and micro-sprinkler irrigation are that with drip irrigation water is generally applied to a restricted root zone of the plant and at more frequent intervals than micro-sprinkler irrigation (Elfving, 1982). By applying fertilisers through the drip irrigation system the fertilisation efficiency is increased since nutrients are applied to a restricted root zone (Bar-Yosef, 1999) and the roots are capable of much faster uptake of nutrients (Bar-Yosef, 1988).




The open hydroponics system (OHS) is a sensitive nutrient and moisture management system with a high degree of control over the development of the crop (Stassen et al., 1999). Specific water and nutrient requirements for different phenological stages of the plant are applied through daily fertigation. A balanced nutrient solution with controlled pH and EC is applied using a drip irrigation system.

At the beginning of the 1990's, Professor Rafael Martinez Valero of Spain brought all the concepts of hydroponics together to develop the commercial application of the open hydroponics system and later called it Martinez Open Hydroponics Technologies (MOHT) (Martinez and Fernandez, 2004; Falivene et al., 2005). Since the implementation of MOHT in horticultural practices all over the world, many variations of the concept have developed. The variation in OHS will be referred to as Daily Drip Fertigation (DDF). Daily drip

fertigation involves drip fertigation of plants on a daily basis during daylight hours (Stassen et al., 1999; Pijl, 2001; Schoeman, 2002).

DDF results in increased yield and fruit size (Kruger et al. 2000a; 2000b; Kuperus et al., 2002; Martinez and Fernandez, 2004), but there is no evidence that DDF does not adversely affect sugar accumulation. Anecdotal evidence from several citrus producers in South Africa suggests that the sugar content of fruit produced under OHS tends to be lower than that of fruit produced under conventional micro-sprinkler irrigation (G.H. Barry, personal communication). The reported reduction in sugar content under OHS conditions could be explained as follows: DDF results in a dilution effect as the amount of water given by the pulse/daily irrigation dilutes the sugars which accumulate in the fruit. It is well-known that rootstocks of differing vigour have varying affects on the dilution of accumulated sugars (Harding and Lewis, 1941; Miller, 1990; Barry et al., 2004b). Scions budded on invigorating rootstocks, e.g. rough lemon (*C. jambhiri* Lush.), produce large fruit with low sugars, while scions budded on less invigorating rootstocks, e.g. trifoliate [*Poncirus trifoliata* (L.) Raf.] or citrange (*C. sinensis* x *P. trifoliata*), produce smaller fruit with higher sugars (Harding and Lewis, 1941; Miller, 1990; Barry et al., 2004a; 2004b). Rough lemon rootstock has a more extensive root system than citrange rootstocks, which allows the roots to absorb more water from a larger soil volume. Rough lemon rootstock is also more effective at water absorption due to differences measured in plant water status between these rootstocks. Fruit harvested from scions on citrange rootstock had 30% higher sugars than fruit from rough lemon trees (Barry et al., 2004b).

Managed drought stress is known to increase sugar accumulation in *Citrus* (Yakushiji et al., 1996; 1998; Barry et al., 2004b). Although Syvertsen and Albrigo (1980) found no evidence

of osmotic adjustment in citrus trees to water stress, the water stress conditions that they applied was not severe enough. Meyer and Boyer (1981), Yakushiji et al. (1996; 1998) and Barry et al. (2004b) observed more sugar accumulation in plants under water stress than in unstressed plants. Osmotic adjustment is a physiological function that takes place under water stress conditions (Meyer and Boyer, 1981). This process involves enough solute accumulation in cells to decrease the cell osmotic potential when cell water potential decreases at low water potential. Water can then be absorbed from a water source by cells without losing cell turgor or decreasing cell volume, while solutes accumulate in juice vesicles (Morgan, 1984). During water stress conditions, the cell size and turgor will be maintained due to solute accumulation in cells at low water potentials. Yakushiji et al. (1996; 1998) found that 'Satsuma' mandarin (*C. unshiu* Marc.) fruit underwent osmotic adjustment as a mechanism of accumulating sugars under low water potentials when trees were moderately stressed by mulching or withholding irrigation. The sugar accumulation was not due to dehydration. The water stress increased concentrations of sucrose, glucose and fructose (Yakushiji et al., 1998). Barry et al. (2004b) also demonstrated this phenomenon.

The timing of water deficit stress seems to be important in enhancing juice quality in citrus. Barry et al. (2004b) found that when deficit irrigation was applied late in fruit development (stage III) of 'Valencia' sweet orange [*C. sinensis* (L.) Osbeck], no increase in soluble solids concentration (SSC) was achieved. However, when the deficit irrigation was applied during the major sugar accumulation period (stage II) of fruit development, increases in SSC occurred. This is in contrast to what Ginestar and Castel (1996) and Gonzalez-Altozano and Castel (1999) found on 'Nules Clementine' mandarin (*C. reticulata* Blanco). SSC was increased when deficit irrigation was applied during stage III and not during stage II, but fruit size was smaller for treatments applied in stage III. Applying regulated deficit irrigation early

in the season did not decrease fruit size at harvest due to accelerated fruit growth that occurred following the reintroduction of full irrigation (Goldhamer and Arpaia, 1998; Goldhamer and Salinas, 2000).

Therefore, the objective of this study was to quantify the effects of water deficit stress on SSC of 'Nules Clementine' mandarin (*C. reticulata* Blanco) fruit grown under OHS conditions.

## Materials and Methods

*Sites and plant material.* This study was conducted on two commercial citrus farms (Backsberg and Greendale) near Simondium, Western Cape province, South Africa (33°50'S, 18°54'E; 300 m elev.). 'Nules Clementine' mandarin trees (*C. reticulata* Blanco) on 'Troyer' citrange rootstock (*Poncirus trifoliata* (L.) Raf x *C. sinensis* (L.) Osb.) were used at both sites during the 2002-03 and 2003-04 seasons. At Backsberg, trees were planted in 1991 with a spacing of 5 m x 3 m. The trees were planted in 1992 at Greendale with a spacing of 4.5 m x 2.5 m. Healthy, mature trees were selected within the orchards which were subjected to standard cultural practices.



*Treatments and experimental design.* Differential irrigation treatment consisting of 2X, X and 1/2X was applied at the end of stage I (± mid Dec.) of physiological fruit development. For the X treatment the existing drip irrigation line was used with a delivery rate of 3.5 $L \cdot h^{-1}$. Emitters were 1 m apart and every third emitter was closed. This means that each tree had two emitters that delivered 7 $L \cdot h^{-1}$ in total. The 2X treatment was applied by inserting an additional irrigation line with the same delivery volume as the existing drip irrigation line. For the 1/2X treatment, the emitters of the existing drip irrigation line were closed and a new drip irrigation line with a delivery volume of 1.6 $L \cdot h^{-1}$ was inserted. Six replicates of six trees

per replicate were selected in a completely randomised experimental layout. Sampling was conducted on the middle four trees in each replicate.

*Data collection.* Soil water content. Soil samples were taken from between two trees in the row, between the drip-emitters at a depth of 15 cm using a 7-cm diameter soil auger. Samples were weighed, baked in an oven for 30 minutes at 140 °C and then reweighed, from which gravimetric soil water content was calculated.

Stem water potential. Two healthy, fully developed leaves were randomly selected on the west side of the trees. Leaves were bagged with small, black polyethylene envelopes and immediately wrapped with aluminium foil before sunrise. Stem water potential measurements were taken at 1100 HR at Greendale and at 1200 HR at Backsberg with a PMS 600 pressure chamber (PMS Instruments, Albany, Ore., USA).

Fruit size and juice quality. Five fruit were randomly selected and tagged on both sides of the trees when the treatments were applied. Fruit size measurements were taken two-weekly by using an electronic caliper integrated with a data logger (Güss Manufacturing, Strand, South Africa). At harvest, the tagged fruit were analysed for fruit quality variables. An additional five fruit were randomly harvested every 2 weeks from both sides of the trees for fruit analysis. Fruit samples were weighed, fruit diameter was measured, and fruit were cut in half to extract juice for juice weight and percentage calculations. Brix was measured with an electronic temperature-compensating refractometer (Atago, Tokyo, Japan). Titratable acid was determined by titrating 10 ml juice sample against 0.1N NaOH to end-point pH 8.2 using a 719 S Metrohm titrino autotrator (Metrohm, Herisau, Switzerland). The ratio of Brix-to-TA was calculated.

*Statistical analysis.* Data were subjected to analysis of variance (ANOVA) using the GLM procedure of SAS (SAS Inc., Cary, N.C., USA). Means were separated by least significant difference (LSD).

**Results**

*Soil water content (SWC) 2002-03 season.* At Backsberg, there was no significant difference in SWC at the beginning of differential irrigation treatments in mid Dec. (Fig. 4.1). Significant differences ($P$<0.05) in SWC were recorded throughout the rest of the season except on 16 and 30 Apr. 2003. The 2X treatment had a consistently higher SWC during the season with the highest value of 12.6% on 3 Apr. 2003. The 1/2X treatment had the lowest SWC throughout the season with the lowest value (9.9%) on 5 Mar. 2003.

At Greendale, there were no significant differences in SWC during the season except on 20 Mar. 2003 (Fig. 4.2). On this day, the 2X treatment had the highest SWC (6.3%) compared to the lowest (5.2%) of the 1/2X treatment. Soil water content for all treatments during the season ranged between 4.6% and 7.5%.

*Soil water content (SWC) 2003-04 season.* On all sampling dates at Backsberg, there were significant ($P$<0.05) differences in SWC (Fig. 4.3). The 2X treatment had the highest SWC during the season except on 6 Feb. 2004 when the SWC of the X treatment was higher. Similar to the situation in 2002-03, the 1/2X treatment had the lowest SWC throughout the season. Soil water content for all treatments during the season ranged between 2.0% and 5.0%.

On all sampling dates at Greendale, there were significant differences in SWC (Fig. 4.4). Once again the 1/2X treatment had the lowest SWC throughout the season with the lowest value (1.6%) on 9 Jan. 2004. The 2X treatment had the highest SWC throughout the season with the highest value (4.1%) on 30 April 2004. Soil water content for all treatments during the season ranged between 1.6% and 4.1%.

The SWC in the 2003-04 season at both Backsberg and Greendale was much lower than in the 2002-03 season, i.e. the soil was drier. The average SWC for all treatments at Greendale was 6.1% in 2002-03 and 2.6% in 2003-04. The difference at Backsberg was even greater, with the average for the 2002-03 season being 12.0% and 3.5% for 2003-04.

*Stem water potential ($\psi_{stem}$) 2002-03 season.* At the start of the differential irrigation treatments at Backsberg, there were no differences in $\psi_{stem}$ among treatments (Fig. 4.5). Statistical differences ($P<0.05$) in $\psi_{stem}$ were measured from 22 Jan. 2003 until fruit maturity. From 19 Feb. 2003, 2X had the highest (least negative) $\psi_{stem}$, while 1/2X had the lowest (most negative) $\psi_{stem}$. The average $\psi_{stem}$ for all treatments fluctuated between -0.95 MPa and -1.55 MPa. A difference of 0.2 MPa between the 1/2X and 2X treatments was only reached after 3 months at Backsberg on 20 Mar. 2003. During the remainder of the 2002-03 season the difference in $\psi_{stem}$ was <0.2 MPa.

At Greendale, the 2X treatment had the highest $\psi_{stem}$ throughout the experiment, except on 19 Feb. 2003 when it was the lowest (Fig. 4.6). The average $\psi_{stem}$ for all treatments fluctuated between -0.87 MPa and -1.30 MPa. The 1/2X treatment had the lowest $\psi_{stem}$ throughout the season, except on 19 Feb. 2003 and on 3 Apr. 2003. A difference of 0.28 MPa was reached

within 3 weeks at Greendale in the 2002-03 season. This difference decreased to 0.16 MPa during the next 4 weeks.

*Stem water potential ($\psi_{stem}$) 2003-04 season.* Throughout the 2003-04 season at Backsberg, the 2X treatment had significantly ($P$<0.05) higher $\psi_{stem}$, except on 23 Jan. 2004 when there was no significant difference (Fig. 4.7). The X treatment had the lowest $\psi_{stem}$ on 6 Feb. 2004 and 20 Mar. 2004, whereas the 1/2X treatment was lower on all other sampling dates. It took 8 weeks to reach a difference of 0.16 MPa between treatments. This difference lasted more than 2 weeks, after which time this difference decreased. The average $\psi_{stem}$ for all treatments fluctuated between -0.80 MPa and -1.40 MPa.

All treatments at Greendale were significantly different ($P$<0.05) during 2003-04 season (Fig. 4.8). Once again, the $\psi_{stem}$ of 2X was higher except on 6 Feb. 2004. The 1/2X treatment had the lowest $\psi_{stem}$ during the season with the exception being on 2 Apr. 2004. The average $\psi_{stem}$ for all treatments fluctuated between -0.93 MPa and -1.35 MPa. During the 2003-04 season, there was a 0.2 MPa difference only after 2 months, but this difference did not even last for 2 weeks.

*Fruit size of tagged fruit during the 2002-03 season*. Fruit size at the start of experiment at Backsberg differed ($P$<0.05) among treatments (Fig. 4.9), when average fruit size was 26.0 mm. The X treatment had the largest fruit at harvesting (58.8mm). There was no difference in weekly average growth rate (≈1.9 mm·week$^{-1}$) among treatments.

At Greendale, fruit size of the X treatments was significantly smaller ($P$<0.05) at the beginning of the experiment than the other treatments (Fig. 4.10). At harvesting, fruit from

the X treatment were significantly smaller than fruit from the 1/2X treatment although the X treatment had an average weekly growth rate of 2.1 mm·week$^{-1}$ compared with 1.9 mm·week$^{-1}$ for the 1/2X and 2X treatments. The average fruit size at harvesting was smaller (56.4 mm) than at Backsberg.

*Fruit size of tagged fruit during the 2003-04 season*. At Backsberg, fruit size among treatments did not differ throughout the measurement period (Fig. 4.11), and the average fruit size of 52.4 mm at harvest was smaller than in the 2002-03 season. The average weekly growth rate was 2.0 mm·week$^{-1}$ for the 1/2X treatment and 1.9 mm·week$^{-1}$ for both the X and the 2X treatment.

The fruit size at Greendale was only significantly different ($P$<0.05) at harvesting (Fig. 4.12). The X treatment had the largest fruit and the 1/2X treatment had the smallest fruit. At harvesting, the average fruit size was 51.8 mm. There was no difference in the weekly growth rate of the X and 2X treatments (2.0 mm·week$^{-1}$), whereas the 1/2X treatment had a lower weekly growth rate (1.8 mm·week$^{-1}$).

*Quality analysis of tagged fruit during the 2002-03 season.* There were no significant differences in all fruit quality variables, except fruit size at Backsberg (Table 1). The X treatment had significantly ($P$<0.05) larger fruit (58.8mm) than other treatments. Average fruit weight at harvest was 77.1 g, when fruit juice content was 54.6%. Soluble solid content and acid values were 10.2 °Brix and 0.82%, respectively, and the Brix-to-acid ratio was 12.5.

At Greendale, there were no significant differences in all fruit quality variables, except SSC (Table 4.2). Average fruit weight was 68.7 g and juice content was 53.5%. The 1/2X

treatment had significantly ($P$<0.05) higher SSC (11.2 °Brix) than the 2X treatment (10.4 °Brix). The average acidity was 0.86 and the ratio was 12.6.

*Quality analysis of tagged fruit during the 2003-04 season.* At Backsberg, significant ($P$<0.05) differences in treatments were measured for SSC and juice content (Table 4.3). Fruit weight averaged 67.8 g. The highest juice content was measured for the X treatment (58.0%) and the 2X treatment had the lowest juice content (51.3%). The 1/2X treatment had the highest SSC (10.8 °Brix) compared with the 2X treatment which had the lowest SSC (10.5 °Brix). Average acidity was 1.22 and ratio was 9.3.

There were no significant differences in fruit quality, except fruit size (Table 4.4) at Greendale. The X treatment had the largest fruit (53.3mm) and 1/2X had the smallest fruit. The average fruit weight was lower (66.4 g) than in 2002-03. Juice content was higher (57.5%) than in the previous season. Soluble solids content averaged 10.7 °Brix, acidity was 1.08, and ratio was 10.1, which was lower than in the 2002-03 season.



*Quality analysis of fruit harvested on two-weekly basis during the 2002-03 season.* Although fruit diameter differed among treatments on 5 and 10 Mar. 2003 when the X treatment had the smallest fruit, fruit diameter did not differ among treatments thereafter (Table 4.5). Fruit weight, juice content, SSC, acidity and ratio (Table 4.5) of fruit sampled at Backberg on 7 May 2003 did not differ among treatments throughout the sample period.

At Greendale, fruit diameter, fruit weight, juice content, SSC, acidity and ratio (Table 4.6) did not differ among treatments throughout the sampling period, except on 5 Mar. 2003 when fruit diameter of the 1/2X treatment was larger than that of the 2X treatment.

*Quality analysis of fruit harvested on two-weekly basis during the 2003-04 season.* At Backsberg, fruit diameter and fruit weight differed among treatments on 2 and 16 Apr. 2004 when the 2X treatment had the largest and heaviest fruit and the 1/2X treatment had the smallest fruit (Table 4.7). Juice content and SSC did not differ among treatments. Acidity was significantly ($P$<0.09) higher for the 1/2X treatment (1.27%) than the 2X treatment (1.05%) resulting in a marginally ($P$<0.14) higher ratio (10.4:1) for the 2X treatment than the 1/2X treatment (8.9:1).

At Greendale, the average fruit diameter was larger (Table 4.8) than for the tagged fruit (Table 4.4), but there was no difference in fruit diameter among treatments. There was also no significant difference among treatments in fruit weight, juice content, SSC, acidity and ratio (Table 4.8).


**Discussion**

At both sites and in both seasons, SWC was highest in the 2X treatment and lowest in the 1/2X treatment, according to the experimental plan. The only exceptions were on 6 Feb. 2004 at Backsberg and on 16 Apr. 2003 at Greendale. This is probably due to rain that fell during the week before SWC was measured. The 1/2X treatment had the lowest SWC during the experiment with the exception of 16 Apr. 2003 at Greendale. The soils at Greendale are compact and the surface appears to be sealed as irrigation water tending to run off the ridges. The trees at Backsberg are larger than at Greendale and these trees are probably capable of obtaining water from greater depths due to a generally better developed root system.

As a result of these differences in SWC among treatments, 'Nules Clementine' mandarin trees in the 2X treatment had less negative $\psi_{stem}$ throughout the duration of the experiment, with a difference of 0.2 MPa between 1/2X and 2X treatments only being reached 8 to 12 weeks after the treatments were applied.

On average, the tagged fruit were larger at harvest during the 2002-03 season than during the 2003-04 season, despite a smaller crop load in the 2003-04 season. The tagged fruit were harvested on 7 May 2003 in the first season, but on 30 Apr. 2004 during the second season of the experiment. The reason for the earlier harvesting was that the second season was an "off" year for production. This meant that the yield was lower and to be cost effective, the picking of fruit had to be more severe, i.e. two harvests instead of three. Fruit that were relatively large at the start of the differential irrigation treatments tended to remain relatively large as found by Koch (1995), irrespective of irrigation treatment. However, the average growth rate (1.9 mm·week$^{-1}$) over a 3- to 4-month period was similar among treatments. Fruit harvested on a two-weekly basis were larger than the tagged fruit during the 2002-03 season at both Greendale and Backsberg. There were more fruit on the trees during 2002-03 than in 2003-04. As these fruit matured, they increased in weight and this made the branches bend down, in turn causing the tagged fruit to be closer to the bottom of the tree and to be over-shadowed. To prevent this in the 2003-04 season, the tagged fruit were randomly selected 20 cm higher than the previous season.

Although SSC at Backsberg was not significantly different ($P$=0.43) during the 2002-03 season, the X and 2X treatments had a lower SSC (10.1 °Brix) than the 1/2X treatment (10.5 °Brix). However, during the 2003-04 season, SSC was significantly higher for the 1/2X treatment (10.8 °Brix) than for the 2X treatment (10.5 °Brix).

During the 2002-03 season, the 1/2X treatment at Greendale had the largest fruit, probably as a result of too much water being applied to the X and 2X treatments. The X treatment had the largest fruit during the second season at Greendale, and average fruit growth rate did not differ among treatments. There was a significant difference in SSC during 2002-03, with the 1/2X treatment having the highest value (11.2 °Brix) and the 2X treatment the lowest (10.4 °Brix). In 2003-04, SSC did not differ significantly among treatments. Apparently, a general over-irrigation occurred in both the X and 2X treatments during both the 2002-03 and 2003-04 seasons at Backsberg and Greendale.

In conclusion, withholding water by applying differential irrigation increased SSC by 0.3 to 0.6 °Brix under certain conditions. For example, SSC increased by 0.6 °Brix at Greendale in the 2002-03 season when irrigation volume was reduced resulting in a difference in $\psi_{stem}$ of 0.28 MPa between the 1/2X and 2X treatments within 3 weeks of applying differential irrigation, although this difference decreased to 0.16 MPa over a period of 4 weeks. SSC also increased at Backsberg by 0.3 °Brix in the 2003-04 season when a difference in $\psi_{stem}$ of 0.16 MPa was achieved within 8 weeks of applying the differential irrigation treatments, but this differential only lasted for 2 weeks. However, where a $\psi_{stem}$ differential of 0.2 MPa could only be achieved after more than 2 months, e.g. Greendale in the 2003-04 season and Backsberg in the 2002-03 season, SSC was not increased.

These data are consistent with previous work. Barry and co-workers (2004b) suggested that the severity of the differential irrigation should be between 0.15 MPa and 0.20 MPa for 'Valencia' sweet orange. This difference in $\psi_{stem}$ was sufficient to cause a difference in the SSC. No mention was made about the duration of the difference in $\psi_{stem}$. However, where

over-irrigation may have occurred, no difference in fruit size occurred between the relatively wet and relatively dry treatments. In such cases, SSC did not differ between these treatments.

Therefore, certain water-deficit stress conditions must be met to enhance sugar accumulation of 'Nules Clementine' mandarin. These conditions require that the difference in $\psi_{stem}$ should be of a sufficient intensity of between 0.16 and 0.3 MPa, and this difference should be maintained for a sufficient duration of between 4 and 6 weeks. Furthermore, deficit irrigation should be applied relatively early in fruit development (Barry et al., 2004b), namely during the sugar accumulation stage which starts within 4 weeks of the end of the fruit drop period and continues until harvest.

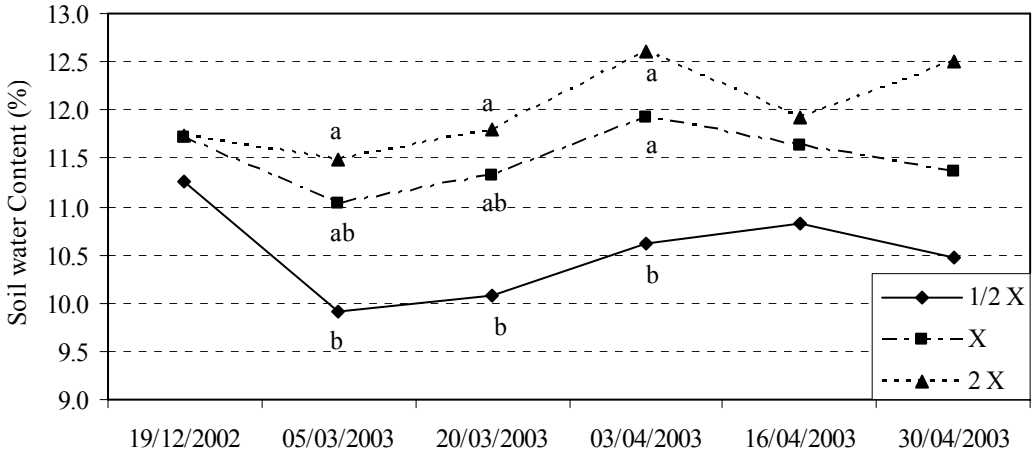



Fig. 4.1. Soil water content (%) of 'Nules Clementine' mandarin over a period of 5 months at Backsberg during the 2002-03 season. Soil samples were taken between trees. One soil sample was taken for each of the six trees in a replicate (n=6).


Fig. 4.2. Soil water content (%) of 'Nules Clementine' mandarin over a period of 5 months at Greendale during the 2002-03 season. Soil samples were taken between trees. One soil sample was taken for each of the six trees in a replicate (n=6).

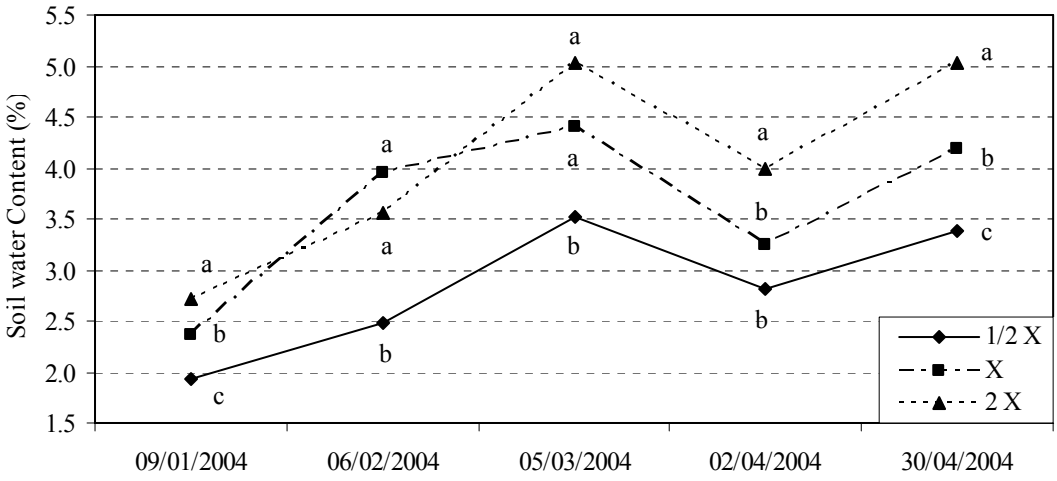



Fig. 4.3. Soil water content (%) of 'Nules Clementine' mandarin over a period of 4 months at Backsberg during the 2003-04 season. Soil samples were taken between trees. One soil sample was taken for each of the six trees in a replicate (n=6).

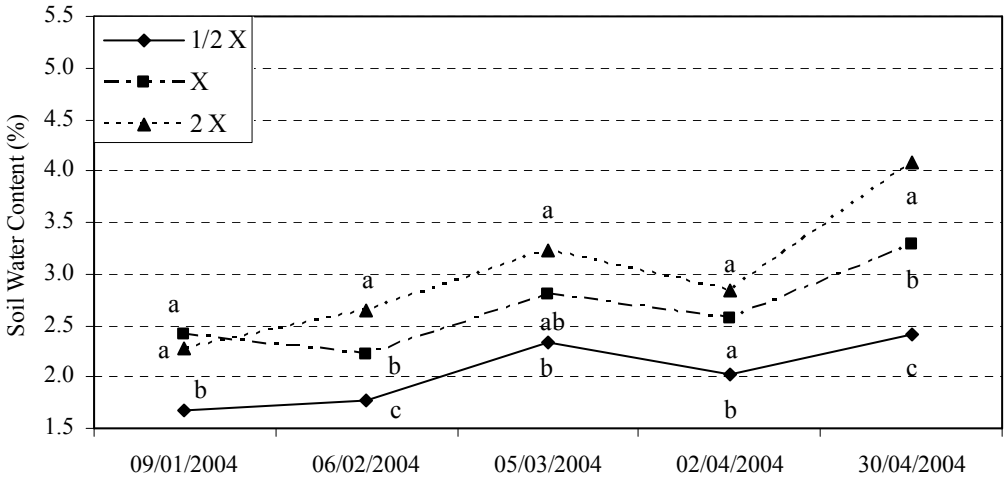



Fig. 4.4. Soil water content (%) of 'Nules Clementine' mandarin over a period of 4 months at Greendale during the 2003-04 season. Soil samples were taken between trees. One soil sample was taken for each of the six trees in a replicate (n=6).

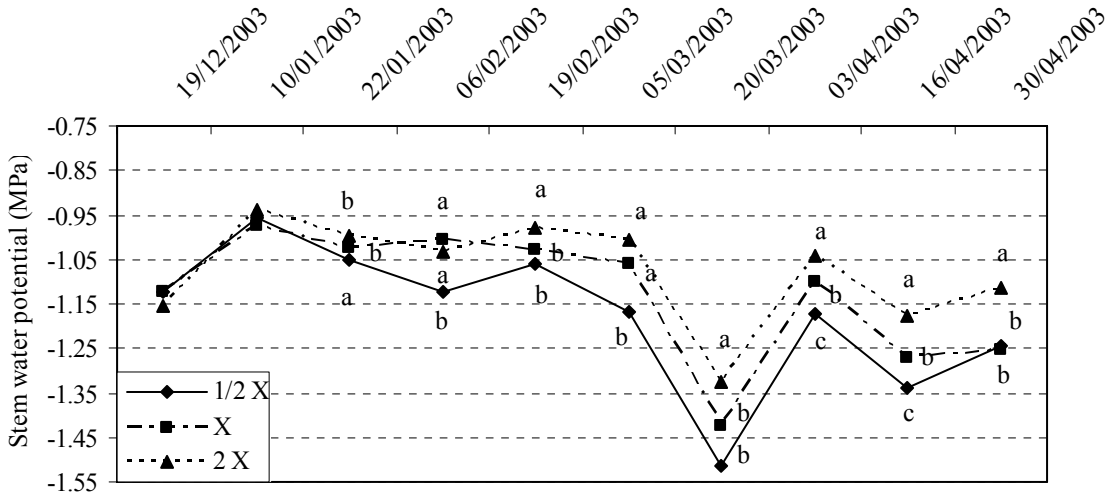



Fig. 4.5. Stem water potential (MPa) of 'Nules Clementine' mandarin over a period of 5 months at Backsberg during the 2002-03 season. Leaves were bagged on the west side of trees before sunrise. Measurements were taken from 1200 HR. There were six replicates with two leaves per replicate.

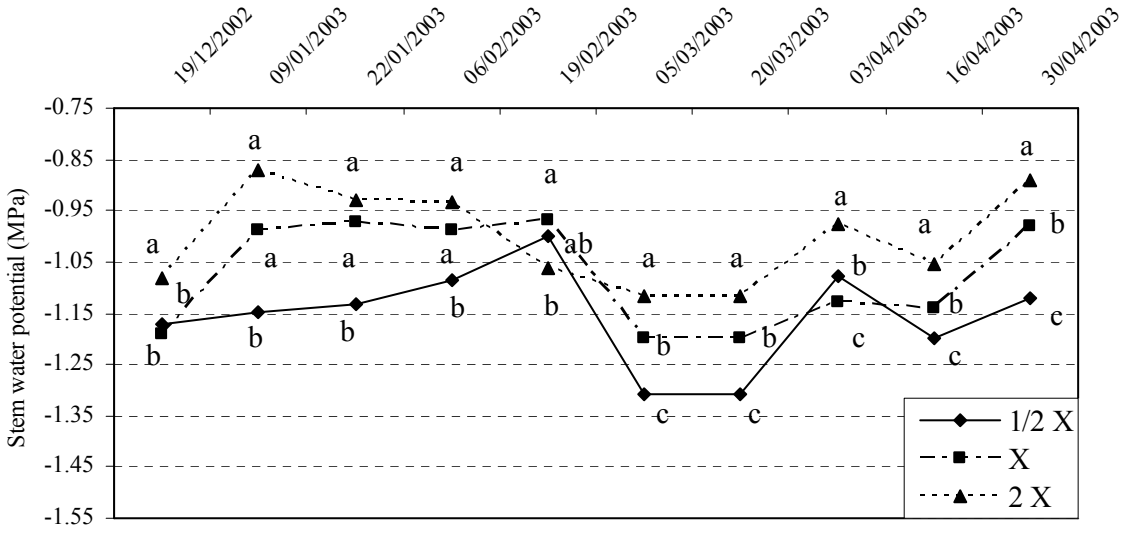



Fig. 4.6. Stem water potential (MPa) of 'Nules Clementine' mandarin over a period of 5 months at Greendale during the 2002-03 season. Leaves were bagged on the west side of trees before sunrise. Measurements were taken from 1100 HR. There were six replicates with two leaves per replicate.

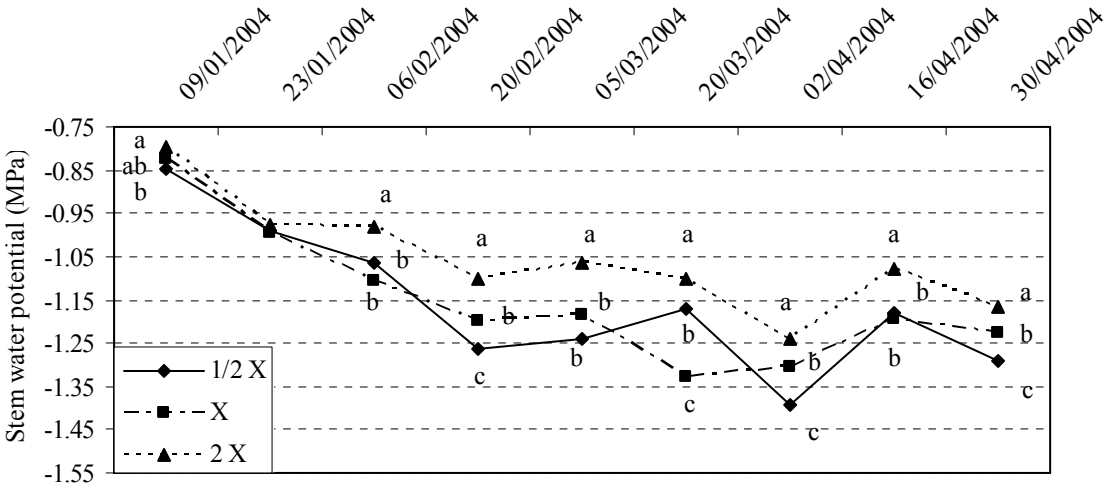



Fig. 4.7. Stem water potential (MPa) of 'Nules Clementine' mandarin over a period of 4 months at Backsberg during the 2003-04 season. Leaves were bagged on the west side of trees before sunrise. Measurements were taken from 1200 HR. There were six replicates with two leaves per replicate.

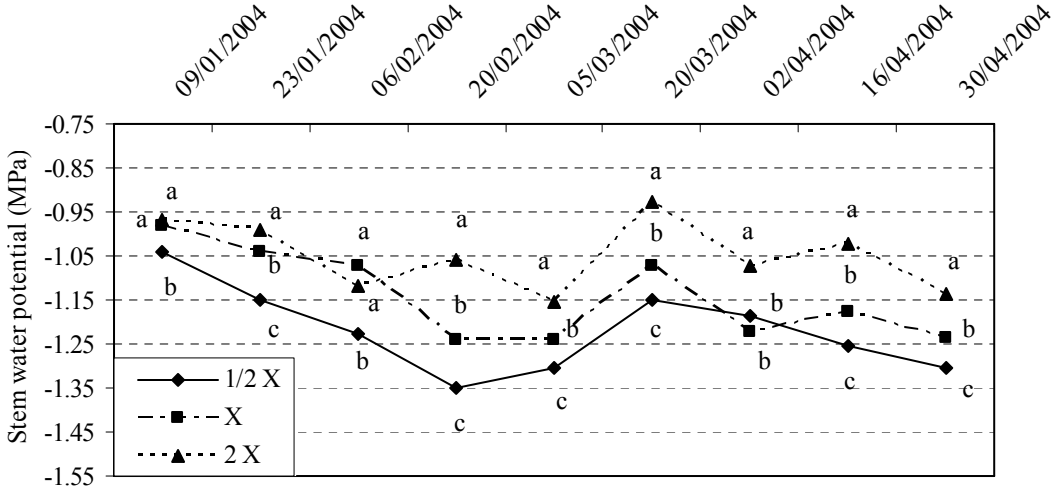



Fig. 4.8. Stem water potential (MPa) of 'Nules Clementine' mandarin over a period of 4 months at Greendale during the 2003-04 season. Leaves were bagged on the west side of trees before sunrise. Measurements were taken from 1100 HR. There were six replicates with two leaves per replicate.

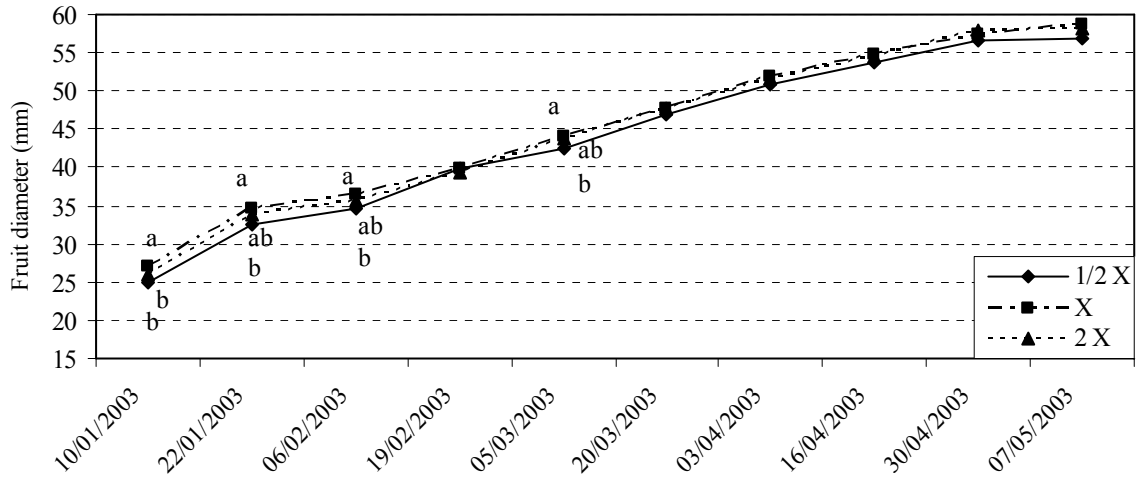



Fig. 4.9. Fruit diameter measurements of tagged fruit of 'Nules Clementine' mandarin over a period of 5 months at Backsberg during the 2002-03 season. There were six replicates with ten tagged fruit per replicate.

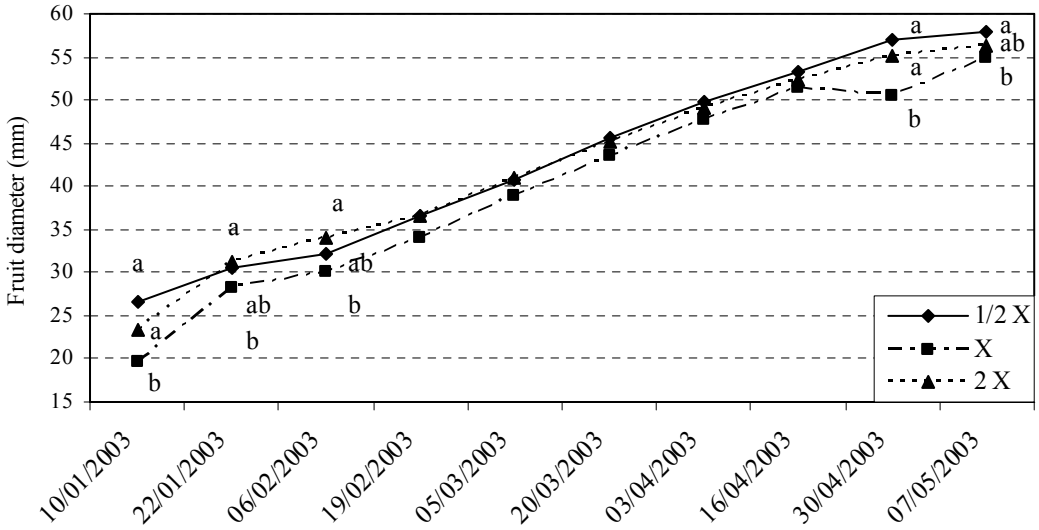



Fig. 4.10. Fruit diameter measurements of tagged fruit of 'Nules Clementine' mandarin over a period of 5 months at Greendale during the 2002-03 season. There were six replicates with ten tagged fruit per replicate.


Fig. 4.11. Fruit diameter measurements of tagged fruit of 'Nules Clementine' mandarin over a period of 4 months at Backsberg during the 2003-04 season. There were six replicates with ten tagged fruit per replicate.

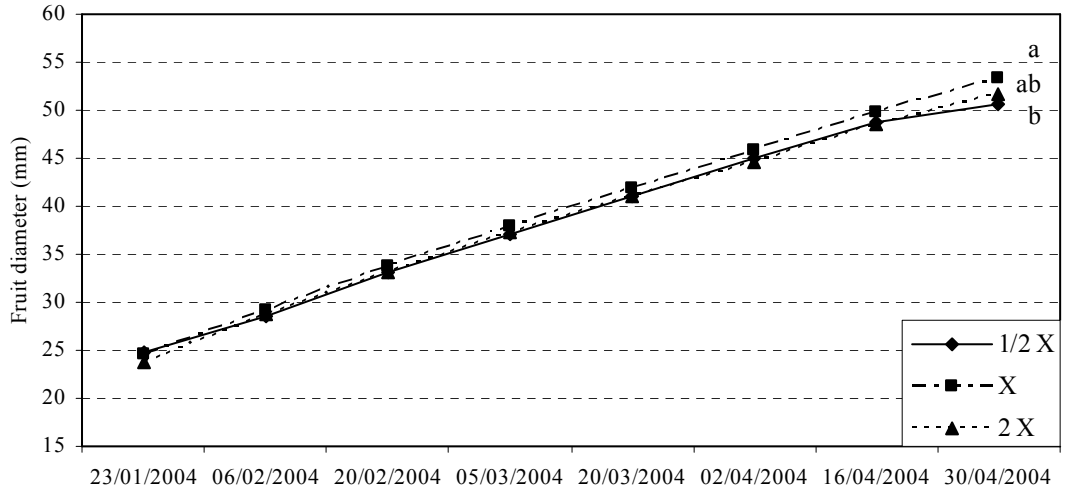



Fig. 4.12. Fruit diameter measurements of tagged fruit of 'Nules Clementine' mandarin over a period of 4 months at Greendale during the 2003-04 season. There were six replicates with ten tagged fruit per replicate.

Table 4.1. Fruit quality of tagged 'Nules Clementine' mandarin fruit harvested on 7 May 2003 at Backsberg.

| Treatment | Fruit size (mm) | Fruit weight (g) | Juice content (%) | SSC (°Brix) | Acid (%) | Ratio |
|---|---|---|---|---|---|---|
| 1/2 X | 56.8 | 69.3 | 54.5 | 10.5 | 0.81 | 12.9 |
| X | 58.8 | 82.3 | 52.8 | 10.1 | 0.81 | 12.5 |
| 2 X | 58.3 | 79.8 | 56.5 | 10.1 | 0.84 | 12.1 |
| P-value | 0.1364 | 0.1473 | 0.3195 | 0.4287 | 0.7633 | 0.4716 |
| lsd | 1.92 | 14.07 | 5.09 | 0.74 | 0.08 | 1.34 |

Table 4.2. Fruit quality of tagged 'Nules Clementine' mandarin fruit harvested on 7 May 2003 at Greendale.

| Treatment | Fruit size (mm) | Fruit weight (g) | Juice content (%) | SSC (°Brix) | Acid (%) | Ratio |
|---|---|---|---|---|---|---|
| 1/2 X | 58.0 a | 70.0 | 52.4 | 11.2 a | 0.91 | 12.6 |
| X | 54.8 b | 69.2 | 53.2 | 10.6 ab | 0.87 | 12.3 |
| 2 X | 56.3 ab | 66.0 | 55.0 | 10.4 b | 0.81 | 13.0 |
| P-value | 0.0595 | 0.9784 | 0.2688 | 0.0331 | 0.4044 | 0.8262 |
| lsd | 2.57 | 37.21 | 3.78 | 0.61 | 0.18 | 2.34 |

Table 4.3. Fruit quality of tagged 'Nules Clementine' mandarin fruit harvested on 30 April 2004 at Backsberg.

| Treatment | Fruit size (mm) | Fruit weight (g) | Juice content (%) | SSC (°Brix) | Acid (%) | Ratio |
|---|---|---|---|---|---|---|
| 1/2 X | 52.7 | 69.2 | 55.7 ab | 10.8 a | 1.20 | 9.3 |
| X | 53.1 | 68.7 | 58.0 a | 10.7 ab | 1.10 | 9.9 |
| 2 X | 51.4 | 65.6 | 51.3 b | 10.5 b | 1.35 | 8.5 |
| P-value | 0.4540 | 0.7486 | 0.0466 | 0.0561 | 0.4384 | 0.3914 |
| lsd | 2.77 | 11.09 | 5.28 | 0.28 | 0.41 | 2.02 |

Table 4.4. Fruit quality of tagged 'Nules Clementine' mandarin fruit harvested on 30 April 2004 at Greendale.

| Treatment | Fruit size (mm) | Fruit weight (g) | Juice content (%) | SSC (°Brix) | Acid (%) | Ratio |
|---|---|---|---|---|---|---|
| 1/2 X | 50.6 b | 61.7 | 57.4 | 10.7 | 1.15 | 9.7 |
| X | 53.3 a | 71.3 | 57.6 | 10.8 | 1.01 | 10.8 |
| 2 X | 51.6 ab | 66.4 | 57.5 | 10.6 | 1.09 | 9.8 |
| p-value | 0.0080 | 0.2706 | 0.9912 | 0.7005 | 0.3849 | 0.2659 |
| lsd | 1.70 | 12.09 | 4.03 | 0.52 | 0.21 | 1.59 |

Table 4.5. Fruit quality of 'Nules Clementine' mandarin fruit harvested on a two-weekly basis at Backsberg during the 2002-03 season. There were six replicates with ten fruit per replicate.

| | Sampling date | | | | |
|---|---|---|---|---|---|
| Treatment | 05/03/2003 | 20/03/2003 | 03/04/2003 | 16/04/2003 | 30/04/2003 |
| | Fruit diameter (mm) | | | | |
| 1/2 X | 46.4 a | 50.0 ab | 53.0 | 57.2 | 61.3 |
| X | 44.7 b | 49.1 b | 53.1 | 56.5 | 61.2 |
| 2 X | 46.2 a | 50.8 a | 53.2 | 56.6 | 60.3 |
| P-value | 0.0540 | 0.0517 | 0.9371 | 0.5849 | 0.3633 |
| lsd | 1.50 | 1.37 | 1.38 | 1.51 | 1.44 |
| | Fruit weight (g) | | | | |
| 1/2 X | 52.7 | 63.3 | 72.3 | 86.1 | 101.5 |
| X | 47.7 | 60.0 | 73.0 | 83.3 | 101.9 |
| 2 X | 52.0 | 64.8 | 72.2 | 83.9 | 96.9 |
| P-value | 0.3407 | 0.4052 | 0.9469 | 0.7101 | 0.5221 |
| lsd | 7.58 | 7.50 | 5.78 | 7.56 | 10.03 |
| | Juice content (%) | | | | |
| 1/2 X | 41.0 | 47.4 | 50.2 | 52.1 | 52.2 |
| X | 41.0 | 48.0 | 50.5 | 51.9 | 52.3 |
| 2 X | 42.0 | 48.5 | 50.2 | 51.6 | 52.3 |
| P-value | 0.8733 | 0.5269 | 0.8339 | 0.6719 | 0.9325 |
| lsd | 4.59 | 2.06 | 1.36 | 1.12 | 0.94 |
| | SSC (°Brix) | | | | |
| 1/2 X | 10.9 | 10.4 | 10.9 | 10.9 | 11.4 |
| X | 10.6 | 10.4 | 10.5 | 10.7 | 11.1 |
| 2 X | 10.8 | 10.3 | 10.7 | 10.7 | 11.4 |
| P-value | 0.4694 | 0.9893 | 0.2460 | 0.6668 | 0.3798 |
| lsd | 0.44 | 0.74 | 0.53 | 0.53 | 0.47 |
| | Acidity (%) | | | | |
| 1/2 X | 2.64 | 1.99 | 1.42 | 1.16 | 0.94 |
| X | 2.72 | 1.89 | 1.43 | 1.14 | 0.93 |
| 2 X | 2.64 | 1.88 | 1.44 | 1.09 | 0.92 |
| P-value | 0.8412 | 0.6080 | 0.9337 | 0.3075 | 0.6201 |
| lsd | 0.32 | 0.25 | 0.13 | 0.09 | 0.06 |
| | Ratio | | | | |
| 1/2 X | 4.1 | 5.3 | 7.7 | 9.5 | 12.1 |
| X | 4.0 | 5.5 | 7.4 | 9.4 | 12.0 |
| 2 X | 4.1 | 5.6 | 7.4 | 9.9 | 12.4 |
| P-value | 0.7034 | 0.7346 | 0.5895 | 0.5204 | 0.5328 |
| lsd | 0.51 | 0.78 | 0.75 | 0.83 | 0.77 |

Table 4.6. Fruit quality of 'Nules Clementine' mandarin fruit harvested on a two-weekly basis at Greendale during the 2002-03 season. There were six replicates with ten fruit per replicate.

| | Sampling date | | | | |
|---|---|---|---|---|---|
| Treatment | 05/03/2003 | 20/03/2003 | 03/04/2003 | 16/04/2003 | 30/04/2003 |
| | Fruit diameter (mm) | | | | |
| 1/2 X | 42.8 a | 47.4 | 51.8 | 56.5 | 60.6 |
| X | 41.2 ab | 46.1 | 50.7 | 55.5 | 60.0 |
| 2 X | 40.8 b | 46.6 | 51.3 | 56.2 | 60.7 |
| P-value | 0.0966 | 0.4384 | 0.5261 | 0.5975 | 0.7061 |
| lsd | 1.99 | 2.00 | 1.95 | 1.90 | 1.76 |
| | Fruit weight (g) | | | | |
| 1/2 X | 43.9 | 55.1 | 71.2 | 88.6 | 104.1 |
| X | 40.1 | 52.5 | 66.6 | 84.8 | 102.7 |
| 2 X | 38.3 | 53.9 | 70.2 | 88.6 | 103.6 |
| P-value | 0.6276 | 0.9301 | 0.8441 | 0.8899 | 0.9834 |
| lsd | 12.89 | 14.02 | 17.26 | 17.00 | 16.36 |
| | Juice content (%) | | | | |
| 1/2 X | 44.3 | 49.3 | 50.1 | 52.3 | 52.7 |
| X | 43.5 | 46.1 | 49.1 | 50.8 | 52.7 |
| 2 X | 41.8 | 48.0 | 49.0 | 52.1 | 54.9 |
| P-value | 0.6573 | 0.5099 | 0.8182 | 0.7340 | 0.0901 |
| lsd | 6.18 | 5.77 | 3.88 | 4.15 | 2.32 |
| | SSC (°Brix) | | | | |
| 1/2 X | 10.3 ab | 10.2 | 10.6 | 10.7 | 11.7 |
| X | 10.7 a | 10.3 | 10.7 | 10.5 | 11.3 |
| 2 X | 9.7 b | 10.4 | 10.6 | 10.7 | 11.4 |
| P-value | 0.0548 | 0.7121 | 0.8260 | 0.5130 | 0.1919 |
| lsd | 0.82 | 0.67 | 0.42 | 0.39 | 0.45 |
| | Acidity (%) | | | | |
| 1/2 X | 2.97 | 2.33 | 1.58 | 1.07 | 0.93 |
| X | 3.29 | 2.42 | 1.67 | 1.16 | 0.93 |
| 2 X | 3.27 | 2.23 | 1.53 | 1.06 | 0.92 |
| P-value | 0.8246 | 0.8755 | 0.8253 | 0.4565 | 0.9712 |
| lsd | 1.25 | 0.77 | 0.49 | 0.18 | 2.13 |
| | Ratio | | | | |
| 1/2 X | 3.7 | 4.8 | 7.1 | 10.2 | 12.7 |
| X | 3.6 | 4.4 | 6.8 | 9.3 | 12.2 |
| 2 X | 3.2 | 5.0 | 7.2 | 10.3 | 12.4 |
| P-value | 0.6689 | 0.7766 | 0.9030 | 0.5006 | 0.8278 |
| lsd | 1.49 | 1.85 | 2.12 | 1.82 | 1.55 |

Table 4.7. Fruit quality of 'Nules Clementine' mandarin fruit harvested on a two-weekly basis at Backsberg during the 2003-04 season. There were six replicates with ten fruit per replicate.

| | Sampling date | | |
|---|---|---|---|
| Treatment | 02/04/2004 | 16/04/2004 | 30/04/2004 |
| | Fruit diameter (mm) | | |
| 1/2 X | 46.2 | 49.9 b | 53.1 |
| X | 46.9 | 52.2 a | 52.5 |
| 2 X | 47.6 | 53.0 a | 53.6 |
| P-value | 0.1451 | 0.0001 | 0.4618 |
| lsd | 1.41 | 1.44 | 1.68 |
| | Fruit weight (g) | | |
| 1/2 X | 46.8 b | 58.1 b | 67.9 |
| X | 49.5 ab | 65.5 ab | 66.0 |
| 2 X | 52.1 a | 69.3 a | 72.2 |
| P-value | 0.0720 | 0.0290 | 0.4570 |
| lsd | 4.47 | 8.01 | 10.36 |
| | Juice content (%) | | |
| 1/2 X | 45.5 | 54.1 | 54.7 |
| X | 48.4 | 54.1 | 56.2 |
| 2 X | 48.4 | 53.3 | 56.4 |
| P-value | 0.3784 | 0.8184 | 0.3860 |
| lsd | 5.15 | 2.77 | 2.91 |
| | SSC (°Brix) | | |
| 1/2 X | 11.1 | 10.8 | 11.2 |
| X | 11.2 | 10.8 | 11.0 |
| 2 X | 10.8 | 10.6 | 10.8 |
| P-value | 0.4025 | 0.3155 | 0.3507 |
| lsd | 0.59 | 0.29 | 0.55 |
| | Acidity (%) | | |
| 1/2 X | 2.20 | 1.47 | 1.27 a |
| X | 2.02 | 1.38 | 1.16 ab |
| 2 X | 1.90 | 1.34 | 1.05 b |
| P-value | 0.2976 | 0.5235 | 0.0863 |
| lsd | 0.40 | 0.25 | 0.20 |
| | Ratio | | |
| 1/2 X | 5.2 | 7.4 | 8.9 b |
| X | 5.6 | 7.9 | 9.5 ab |
| 2 X | 5.8 | 8.0 | 10.4 a |
| P-value | 0.4637 | 0.4837 | 0.1387 |
| lsd | 1.05 | 1.09 | 1.49 |

Table 4.8. Fruit quality of 'Nules Clementine' mandarin fruit harvested on a two-weekly basis at Greendale during the 2003-04 season. There were six replicates with ten fruit per replicate.

| | Sampling date | | |
|---|---|---|---|
| Treatment | 02/04/2004 | 16/04/2004 | 30/04/2004 |
| | Fruit diameter (mm) | | |
| 1/2 X | 46.9 | 51.3 | 53.7 |
| X | 46.5 | 49.6 | 52.0 |
| 2 X | 46.2 | 50.5 | 53.1 |
| P-value | 0.5909 | 0.1119 | 0.1291 |
| lsd | 1.50 | 1.58 | 1.68 |
| | Fruit weight (g) | | |
| 1/2 X | 52.3 | 65.7 | 75.4 |
| X | 50.0 | 59.3 | 67.9 |
| 2 X | 49.2 | 64.2 | 72.7 |
| P-value | 0.6007 | 0.3865 | 0.2713 |
| lsd | 7.26 | 9.87 | 9.51 |
| | Juice content (%) | | |
| 1/2 X | 48.7 | 56.3 | 58.0 |
| X | 51.2 | 56.7 | 58.6 |
| 2 X | 49.2 | 56.7 | 59.4 |
| P-value | 0.5114 | 0.9667 | 0.4705 |
| lsd | 4.65 | 3.61 | 2.53 |
| | SSC (°Brix) | | |
| 1/2 X | 10.4 | 10.5 | 10.9 |
| X | 10.6 | 10.6 | 11.0 |
| 2 X | 10.4 | 10.4 | 10.9 |
| P-value | 0.6337 | 0.9089 | 0.9228 |
| lsd | 0.62 | 0.54 | 0.71 |
| | Acidity (%) | | |
| 1/2 X | 1.81 | 1.28 | 1.00 |
| X | 1.91 | 1.40 | 1.10 |
| 2 X | 1.94 | 1.26 | 0.97 |
| P-value | 0.5575 | 0.3789 | 0.2330 |
| lsd | 0.28 | 0.22 | 0.15 |
| | Ratio | | |
| 1/2 X | 5.8 | 8.2 | 11.0 |
| X | 5.6 | 7.7 | 10.1 |
| 2 X | 5.3 | 8.3 | 11.2 |
| P-value | 0.4146 | 0.5522 | 0.4672 |
| lsd | 0.78 | 1.39 | 5.71 |

# CHAPTER 5

# OVERALL DISCUSSION AND CONCLUSION

In the first study, the importance of optimising and standardising the sampling procedure used for the quantification of stem water potential ($\psi_{stem}$) was illustrated. By minimising the variation in $\psi_{stem}$, the reliability of the procedure as a measure of plant water status can be enhanced. Bagging of leaves at sunrise with black polyethylene envelopes and measuring $\psi_{stem}$ 3 to 4 hours later allows the plant water status of the bagged leaves to reach equilibrium with the plant water status of the whole tree, and a realistic measurement of the current $\psi_{stem}$ can then be made. The use of aluminium foil to cover the bagged leaves reduces heat build-up by reflecting sunlight and dramatically reduces variation in $\psi_{stem}$. The time of day at which $\psi_{stem}$ measurements are made is important to ensure consistency in comparisons among treatments. The preferred time of day to measure $\psi_{stem}$ is prior to midday depression in photosynthesis (1100 HR). The east side of trees is more representative of transpiring leaves with open stomata and should be used for making $\psi_{stem}$ measurements. Under similar experimental conditions, only three leaves per replicate would be required to detect a difference in $\psi_{stem}$ between treatment means of 0.05 MPa.

In the second study, the effect of differential irrigation on sugar accumulation was determined. Withholding water by applying differential irrigation increased SSC by 0.3 to 0.6 °Brix under certain conditions. For example, SSC increased by 0.6 °Brix at Greendale in the 2002-03 season when irrigation volume was reduced resulting in a difference in $\psi_{stem}$ of 0.28 MPa between the 1/2X and 2X treatments within 3 weeks of applying differential irrigation, although this difference decreased to 0.16 MPa over a period of 4 weeks. SSC also increased at Backsberg by 0.3 °Brix in the 2003-04 season when a difference in $\psi_{stem}$ of 0.16 MPa was

achieved within 8 weeks of applying the differential irrigation treatments, but this differential only lasted for 2 weeks. However, where a $\psi_{stem}$ differential of 0.2 MPa could only be achieved after more than 2 months, e.g. Greendale in the 2003-04 season and Backsberg in the 2002-03 season, SSC was not increased.

Therefore, certain water-deficit stress conditions must be met to enhance sugar accumulation of 'Nules Clementine' mandarin. These conditions require that the difference in $\psi_{stem}$ should be of a sufficient intensity of between 0.16 and 0.3 MPa, and this difference should be maintained for a sufficient duration of between 4 and 6 weeks. Furthermore, deficit irrigation should be applied relatively early in fruit development (Barry et al., 2004b), namely during the sugar accumulation stage which starts within 4 weeks of the end of the fruit drop period and continues until harvest.

## LITERATURE CITED


Bain, J.M. 1958. Morphological, anatomical, and physiological changes in the developing fruit of the Valencia orange, Citrus sinensis (L.) Osbeck. Austr. J. Bot. 6:1-24.

Barry, G.H., W.S. Castle and F.S. Davies. 2004a. Soluble solids accumulation in 'Valencia' sweet orange as related to rootstock selection and fruit size. J. Amer. Soc. Hort. Sci. 129:594-598.

Barry, G.H., W.S. Castle and F.S. Davies. 2004b. Rootstocks and plant water relations affect sugar accumulation of citrus fruit via osmotic adjustment. J. Amer. Soc. Hort. Sci. 129:881-889.

Bar-Yosef, B., S. Schwartz, T. Markovich, B. Lucas and R. Assaf. 1988. Effect of root volume and nitrate solution concentration on growth, fruit yield, and temporal N and water uptake rates by apple trees. Plant and Soil 107:49-56.

Bar-Yosef, B. 1999. Advances in fertigation. Adv. Agron. 65:1-77.

Bean, R.C. 1960. Carbohydrate metabolism of citrus fruits: I. Mechanisms of sucrose syntheses in oranges and lemons. Plant Physiol. 35:429-434.

Begg, J.E. and N.C. Turner. 1970. Water potential gradients in field tobacco. Plant Physiol. 46:343-346.

Bentley, M. 1959. Commercial hydroponics: Facts and figures. Bendon Books. Johannesburg.

Boland, A.M., P.H. Jerie, P.D. Mitchell and I Goodwin. 2000. Long-term effects of restricted root volume and regulated deficit irrigation on peach: I. Growth and mineral nutrition. J. Amer. Soc. Hort. Sci.125(1): 135-142.

Boman, B.J. 1996. Fertigation versus conventional fertilization of flatwoods grapefruit. Fertilizer Research 44:123-128.

Boyer, J.S. 1967. Leaf water potentials measured with a pressure chamber. Plant Physiol. 42:133-137.

Boyer, J.S. and E.B. Knipling. 1965. Isopiestic technique for measuring leaf water potentials with a thermocouple psychrometer. Proc. Natl. Acad. Sci. 54:1044-1051.

Canny, M.J., B. Nairn and M. Harvey. 1968. The velocity of translocation in trees. Aust. J. Bot. 16:479-485.

Coetzee, E. 1998. More citrus from poor soil, with less water. Farmer's Weekly 5 June 1998 pp26-28.

Coombe, B.G. 1976. The development of fleshy fruits. Ann. Rev. Plant Physiol. 26:207-228.

Cooper, A. 1996. The ABC of NFT, Nutrient Film Technique. Casper Publications, Austalia.

Echeverria, E. and J.K. Burns. 1989. Vacuolar acid hydrolysis as a physiological mechanism for sucrose breakdown. Plant Physiol. 90:530-533.

Echeverria, E. 1992. Activities of sucrose metabolising enzymes during sucrose accumulation in developing acid limes. Plant Science. 85:125-129.

Elfving, D.C. and M.R. Kaufmann. 1972. Diurnal and seasonal effects of environment on plant water relations and fruit diameter of citrus. J. Amer. Soc. Hort. Sci. 107:139-142.

Elfving, D.C. 1982. Crop response to trickle irrigation. Hort. Rev. 4:1-48.

Falivene, S., I. Goodwin, D. Williams, A.M. Boland. 2005. Open Hydroponics: Risks and opportunities. Stage 1: General principles & literature review. NSW department of primary industries and Land & Water Austalia.

Fishler, M., E.E. Goldschmidt and S.P. Monselise. 1983. Leaf area and fruit size in girdled grapefruit branches. J. Amer. Soc. Hort. Sci. 108:218-221.

Fitter, A.H. and R.K.M. Hay. 1981. Environmental physiology of plants. Academic Press, New York.

Garcia-Luis, A., Didehvar, F., Guardiola, J.L. and D.A. Baker. 1991. The transport of sugars in developing fruits of Satsuma mandarin. Annals of Botany 68:349-357.

Garnier, E. and A. Berger. 1985. Testing water potential in peach trees as an indicator of water stress. J. Hort. Sci. 60(1):47-56.

Ginestar, C. and J.R. Castel. 1996. Responses of young Clementine citrus tress to water stress during different phonological periods. Journal of Horticultural Science 71(4):551-559.

Goldhamer, D.A. and M.L. Arpaia, 1998. Regulated deficit irrigation of citrus. Citrograph, Mar.

Goldhamer, D.A. and M. Salinas, 2000. Evaluation of regulated deficit irrigation on mature orange trees grown under high evaporative demand. Proc. Intl. soc. Citicult. IX Congr. 227-231.

Goldschmidt, E.E. and A. Golomb. 1982. The carbohydrate balance of alternate bearing citrus trees and the significance of reserves for flowering and fruiting. J. Amer. Soc. Hort. Sci. 107:206-208.

Goldschmidt, E.E. and K.E. Koch, 1996. In. Photoassimilate distribution in plants and crops: Source sink relationships. p 797-823. Ed. E. Zamski and A.A. Schaffer. Marcel Dekker Inc., New York.

Gonzalez-Altozano, P. and J.R. Castel, 1999. Regulated deficit irrigation in 'Clementina de Nules' citrus tree. I. Yield and fruit quality effects. Journal of Horticultural Science & Biotechnology 74(6):706-713.

Goode, J.E. and T.W. Hegarty. 1965. Measurement of water potential of leaves by methods involving immersion in sucrose solutions. Nature 206:109-110.

Groenewald, A. 2000. Hidroponiese stelsel sorg vir vet sitrus-bonus. Landbouweekblad 19 Mei 2000 pp 16-20.

Harding P.L and W.E. Lewis. 1941. The relation of size of fruit to solids, acid, and volume of juice in the principal varieties of Florida oranges. Proc. Fla. State Hort. Soc. 54:52-56.

Harris, D.A. 1971. Hydroponics: The gardening without soil. Purnell. Cape Town

Harris, D.A. 1987. Hydroponics: The South African guide to gardening without soil. Struik. Cape Town.

Ho, L.C. 1988. Metabolism and compartmentation of imported sugars in sink organs in relation to sink strength. Ann. Rev. Plant Physiol. Plant Mol. Biol. 39:355-378.

Hockema, B.R. and E. Echeverria. 2000. Factors involved in soluble solids accumulation in citrus fruit. Proc. Fla. State Hort. Soc. 113:126-130.

Hockema, B.R. and E. Etxeberria. 2001. Metabolic contributors to drought-enhanced accumulation of sugars and acids in oranges. J. Amer. Soc. Hort. Sci. 126:599-605.

Holtzhauzen, L.C.1969. Observations on the developing fruit of Citrus sinensis cv. Washington navel from anthesis to ripeness. Tech. Comm. 91. Department of Agricultural Technical Services, Univ. of Stellenbosch, South Africa.

Huang, T.B., R.L. Darnell and K.E. Koch. 1992. Water and carbon budgets of developing citrus fruit. J. Amer. Soc. Hort. Sci. 117:287-293.

Jackson, W.T. 1962. Use of carbowaxes (polyethylene glycols) as osmotic agents. Plant Physiol. 37:513-519.

Jensen, M.H. 1999. Hydroponics worldwide. Acta Horticulturae 481:719-729.

Jones, J.B. 1997. Hydroponics: A Practical guide for the soilless grower. St. Lucie Press. Florida.

Kato, T. and S. Kubota. 1978. Properties of invertases in sugar storage tissues of citrus fruit and changes in their activities during maturation. Physiol. Plant.42.67-72.

Kauffman, M.R. and P.J. Kramer, 1967. Phloem water relations and translocation. Plant Physiol. 42:191-194.

Kaufmann, M.R. 1970. Water potential components in growing citrus fruits. Plant Physiol. 46.:145-149.

Knipling, E.B. 1967. Measurement of leaf water potential by the dye method. Ecology 48:1038-1041.

Knipling E.B. and P.J. Kramer, 1967. Comparison of the dye method with the thermocouple psychrometer for measuring leaf water potentials. Plant Physiol. 20:67-72.

Koch, K.E. 1984. The path of photosynthate translocation into citrus fruit. Plant,Cell and Environment 7:647-653.

Koch, K.E. and W.T. Avigne. 1990. Postphloem, non-vascular transfer in citrus: Kinetics, metabolism and sugar gradients. Plant Physiol. 93:1405-1416.

Koch, N.C. 1995. Studies on the improvement of mandarin fruit size. MSc(Agric) Thesis, University of Stellenbosch, South Africa.

Kramer, P.J. 1934. Effects of soil temperature on the absorption of water by plants. Science 79:371-372.

Kramer, P.J. 1940. Root resistance as the cause of decreased water absorption by plants at low temperatures. Plant Physiol. 15:63-79.

Kramer, P.J. 1983. Water relations of plants. Academic Press, New York.

Kriedemann, P.E. 1969a. $^{14}C$ distribution in lemon plants. J. Hortic. Sci. 44:273-279.

Kriedemann, P.E. 1969b. $^{14}C$ distribution in orange plants. Aust. J. agric. Res. 20:291-300.

Kriedemann, P.E. 1970. The distribution of $^{14}C$-labelled assimilates in mature lemon trees. Aust. J. agric. Res. 21:623-632.

Krizek, D.T. 1985. Methods of inducing water stress in plants. HortScience 20(6):1028-1037.

Kruger, J.A., C.D. Tolmay and K. Britz. 2000a. Effects of fertigation frequencies and irrigation systems on performance of Valencia oranges in two subtropical areas of South Africa. Proc. Intl. Soc. Citricult. IX Congr. 232-235.

Kruger, J.A., K. Britz, C.D. Tolmay and S.F. du Plessis. 2000b. Evaluation of an open hydroponics systems (OHS) for citrus in South Africa: Preliminary results. Proc. Intl. Soc. Citricult. IX Congr. 239-242.

Kuperus, K.H., N. Combrink, K. Britz and J. Ngalo. 2002 Evaluation of an open hydroponic system (OHS) for the production of citrus in South Africa. Citrus Research International (Pty) Ltd. Annual Research Report.

Lagerwerff, J.V., G. Otaga and H.E. Eagle. 1961. Control of osmotic pressure of culture solutions with polyethylene glycol. Science. 133:1486-1487.

Lippert, F. 1993. Amounts of organic constituents in tomato cultivated in open and closed hydroponic systems. Acta Hort. 339:113-123.

Lombaard, S. 1994. Sproeibemesting van jong sitrusbome. Instituut vir tropiese en subtropiese gewasse. 262:10-11.

Lowell, C.A. 1986. Structure and sucrose metabolizing enzymes of the transport path: Implications for assimilate translocation in grapefruit. PhD dissertation. Univ. of Florida, Gainesville.

Lowell, C.A., P.T. Tomlinson and K.E. Koch, 1989. Sucrose-metabolizing enzymes in transport tissues and adjacent sink structures in developing citrus fruit. Plant Physiol. 90:1394-1402.

Mantell, A., E.E. Goldschmidt, S.P. Manselise. 1980. Turnover of tritiated water in calamondin plants. J. Amer. Soc. Hort. Sci. 105:741-744.

Martinez, R. and C. Fernandez. 2004. Preliminary results on citrus groves under the MOHT system. Xth Intl. Citrus Congr. pp103. (abstr.)

Mason, J. 1990. Commercial hydroponics: How to grow 86 different plants in hydroponics. Kangaroo Press.

McCutchan, H. and K.A. Shackel. 1992. Stem-water potential as a sensitive indicator of water stress in prune trees (*Prunus domestica* L. cv. French). J. Amer. Soc. Hort. Sci. 117(4):607-611.

Meyer, R.F. and J.S. Boyer. 1981. Osmoregulation, solute distribution, and growth in soybean seedlings having low water potentials. Planta 1151:482-489.

Miller, W.M. 1990. Internal quality attributes of Florida Valencia orange and their relationship to fruit density measurements. Appl. Eng. Agr. 6:461-464.

Monselise, S.P., R. Goren and I. Wallerstein. 1972. Girdling effect on orange fruit set and young fruit abscission. HortScience 7: 514-515.

Morgan, J.M. 1984. Osmoregulation and water status in higher plants. Ann. Rev. Plant. Physiol. 35:223-242.

Naor, A., I. Klein, H. Hpert, Y. Grinblat, M. Peres and A. Kaufman. 1999. Water stress and crop level interactions in relation to nectarine yield, fruit size distribution, and water potentials. J. Amer. Soc. Hort. Sci. 124(2):189-193.

Naor, A. and S. Cohen. 2003. Sensitivity and variability of maximum trunk shrinkage, midday stem water potential, and transpiration rate in response to withholding irrigation from field-grown apple trees. Hortscience 38(4):547-551.

Pijl, I. 2001. Drip fertigation: Effects on water movement, soil characteristics and root distribution. MSc(Agric) Thesis, University of Stellenbosch, South Africa.

Richards, L.A. and G. Otaga. 1958. Thermocouple for vapour pressure measurement in biological and soil systems at high humidity. Science. 128:1089-1090.

Richards, D. 1986. Tree growth and productivity – the role of roots. Acta Hort. 175:27-36.

Robinson, T.L. and W.C. Stiles. 1993. Fertigation of young apple trees to improve growth and cropping. Compact Fruit Tree 26:61-65.

Salisbury, F.B. and C.W. Ross, 1991. Plant Physiology. Fourth edition. Wadsworth, California.

Schaffer, A.A., E.E. Goldschmidt, R. Goren and E. Galili. 1985. Fruit set and carbohydrate status in alternate and non-alternate bearing *Citrus* cultivars. J. Amer. Soc. Hort. Sci. 110:574-578.

Schaffer, A.A., O. Sagee, E.E. Goldschmidt and R. Goren. 1987. Invertase and sucrose synthase activity, carbohydrate status and endogenous IAA levels during *Citrus* leaf development. Physiol. Plant. 69:151-155.

Schoeman, S.P. 2002. Physiological measurements of daily daylight fertigated citrus trees. MSc(Agric) Thesis, University of Stellenbosch, South Africa.

Scholander, P.F., H.T. Hammel, E.D Bradstreet, and E.A. Hemmington. 1964. Hydrostatic pressure and osmotic potential in leaves of mangroves and some other plants. Proc. Natl. Acad. Sci. 52:119-125.

Scholander, P.F., H.T. Hammel, E.D. Bradstreet and E.A. Hemmington. 1965. Sap pressure in vascular plants. Science 148:339-346.

Schwarz, M. 1995. Soilless culture management. Springer-Verlag.

Shackel, K.A., H. Ahmadi, W. Biasi, R. Bucher, D. Goldhamer, S. Gurusinghe, J. Hasey, D. Kester, B. Krueger, B. Lampinen, G. McGourty, W. Micke, E. Mitcham, B. Olson, K. Pelletrau, H. Philips, D. Ramos, L. Schwankl, S. Sibbett, R. Snyder, S Southwick, M. Stevenson, M. Thorpe, S. Weinbaum, and J. Yeager. 1997. Plant water status as an index of irrigation need in deciduous fruit trees. HortTechnology 7(1):23-29.

Slatyer, R.O. 1967. Plant-water relationships. Academic Press, New York.

Slatyer, R.O. and S.O. Taylor. 1960. Terminology in plant-soil-water relationships. Nature. 187:922-924.

Slavik, B. 1974. Methods of studying plant water relations. Academia Publishing House, Prague, Springer-Verlag, New York.

Song, K.J., E. Echeverria and H.S. Lee. 1998. Distribution of sugars and related enzymes in the stem and blossom halves of 'Valencia" oranges. J. Amer. Soc. Hort. Sci. 123:416-420.

Spanner, D.C. 1951. The Peltier effect and its use in the measurement of suction pressure. J. Exp. Bot. 2:145-168.

Spanner, D.C. 1964. Introduction to thermodynamics. Academic Press, New York.

Stanghellini, M.E. and S.L. Rasmussen, 1994. Hydroponics: A solution for zoosporic pathogens. Plant disease. December 1129-1137

Stassen, P.J.C., P.G. Mostert and B.L. Smith. 1999. Mango tree nutrition: A crop perspective. Neltropika, January 1999 pp41-51.

Steel, R.G.D. and J.H. Torrie. 1980. Principles and procedures of statistics. 2nd ed. McGraw-Hill, New York.

Sung, S.S., D. Xu and C.C. Black. 1989. Identification of actively filling sucrose sinks. Plant Physiol. 89:1117-1121.

Syvertsen, J.P. and L.G. Albrigo. 1980. Seasonal and diurnal citrus leaf and fruit water relations. Bot. Gaz. 141:440-446.

Syvertsen, J.P. and S.M. Sax. Fertigation frequency, wetting patterns and nitrate leaching from lysimeter-grown citrus trees. Proc. Fla. State Hort. Soc. 112:9-14.

Ting, S.V. 1969. Distribution of soluble components and quality factors in the edible portion of citrus fruit. J. Amer. Soc. Hort. Sci. 94:515-519.

Tolmay, C.D., K. Britz and B. Delamini. 2002 Effects of fertigation frequency and irrigation system on performance of Valencia oranges in a cool subtropical area of South Africa. Citrus Research International (Pty) Ltd. Annual Research Report.

Tomlinson, P.T., E.R. Duke, K.D. Nolte and K.E. Koch. 1991. Sucrose synthase and invertase in isolated vascular bundles. Plant Physiol. 97:1249-1252.

Turner, N.C. 1981. Techniques and experimental approaches for the measurement of plant water status. Plant and Soil. 58:339-366.

Tyree, M.T. and H.T. Hammel. 1972. The measurement of the turgor pressure and the water relations of plants by the pressure-bomb technique. J. Expt. Bot. 23:267-282.

Van Rooyen, C. 2000. Sitrus vaar goed in hidroponika stelsel. Lanbouweekblad 4 February 2000 pp36-37.

Venter, G. 1999. Oop en geslote hidroponiese stelsels. Landbouweekkblad. 15 January 1999 pp30-31.

Wallerstein, I., R. Goren and S.P. Monselise. 1978. Rapid and slow translocation of $^{14}$C-sucrose and $^{14}$C-assimilates in citrus and Phaseolus with special reference to ringing effect. J. Hortic. Sci. 53:203-208.

Wiebbe, H.H. 1966. Matric potential of several plant tissues and biocolloids. Plant Physoil. 41:1439-1442.

Woods, D. 1999. Fundamentals of open hydroponics in the citrus industry. S.A. Besproeiing April/Mei 1999 pp4-6.

Yakushiji, H., H. Nonami, T. Fukuyama, S. Ono, N. Takagi and Y. Hashimoto. 1996. Sugar accumulation enhanced by osmoregulation in Satsuma mandarin fruit. J. Amer. Soc. Hort. Sci. 121:466-472.

Yakushiji, H., K.Morinaga and H. Nonami. 1998. Sugar accumulation and partitioning in Satsuma mandarin tree tissues and fruit in response to drought stress. J. Amer. Soc. Hort. Sci. 123:719-726.

Yen, C.R. and K.E. Koch. 1990. Developmental changes in translocation and localization of $^{14}C$-labelled assimilates in grapefruit: Light and dark $CO_2$ fixation by leaves and fruit. J. Amer. Soc. Hort. Sci. 115:815-819.