

## Chapter 1

#### GENERAL INTRODUCTION

The genus, *Citrus* (family Rutaceae), which includes varieties of oranges, grapefruit, mandarin types, lemons, and limes is one of the most important fruit crops worldwide. *Citrus* species produce fruits known for their distinct colour, flavour, and nutritional content (Syvertson and Lloyd, 1994). The criteria for determining the internal quality of citrus fruits differ according to their ultimate use: fresh consumption or processing (Tzur *et al.*, 1992) and is an important factor in determining the value and marketability.

The demand for oranges for juicing in South Africa increased dramatically with the exports of frozen orange juice escalating from 2 500 t in 1997 to 6 500 t in 1998 (D. Scheepers, 1999, Department of Agricultural Economics, University of Pretoria). With the international demand for fresh citrus stagnant in the traditional markets due to an over-supply on the European wholesale market, it is becoming important to find new niche markets.

Under the 1992 USA standards, only juice from *Citrus sinensis* was allowed in orange juice products without limitations (Barros *et al.*, 1991). In 1992, 70% of the US citrus production originated from Florida of which 93% of the annual orange production is used in the processing industry (Behr and Brown, 1992). Citrus processing was forecast at 25 Mt in 1993/94 for selected Northern Hemisphere countries (USA, Spain, Morocco, Korea Republic, Japan and China), while for the same period citrus production was forecast at 60.6 Mt (Rosa and Kirby, 1994). Fifteen million ton was processed in the 1995/1996 season (Siedband and Goldammer, 1996). In 1997 the citrus production was forecast at 44.6 Mt of which oranges accounted for most of the increase, while 15.3 Mt was expected to be processed (Siedband *et al.*, 1997). Of the

15.3 Mt for processing, the USA was expected to account for 73%. Production was increased by 7% in the following season with the amount of oranges expected to be processed by Northern Hemisphere countries at 16.4 Mt (Siedband and Goldammer, 1998). Petry and Goldammer (1999) forecast a decrease in the production of the largest Northern Hemisphere countries at 42.5 Mt and processing at 14.2 Mt due to smaller orange harvests in the USA, Spain and Mexico. In New South Wales, Australia, 'Valencia Late' grown in the Murrumbidgee Irrigation Areas is the predominant cultivar of which 75% is used for juicing (Hutton and Landsberg, 2000).

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In South Africa research has mainly been aimed at the fresh fruit market and cultivar breeding and cultural practices were neglected for improving qualities important for the juicing industry, thus the scope for research in this regard is extensive.

Fruit quality is mainly determined by the organic constituents. Amount and kinds of organic materials are influenced by many factors (Jones, 1961). Fruit quality also varies among cultivars and stage of maturity (Chen, 1990). Processors receive whatever fruit is available as it matures throughout the season and process this into juice products. It is often necessary to blend early and late season juices to achieve optimum quality while utilising production from the entire season (Chen, 1990).

Producing a high quality product is a common goal of both fresh fruit citrus growers and processors (Chen, 1990). High quality processed citrus juices must come from high quality fruit. Traditionally, the production of high quality fruit was the responsibility of growers. Over the past years this has become the exception rather than the rule. A close relationship has evolved between the members and the board of the Magaliesburg Citrus Co-operative with both parties acknowledging that what benefits the one, will also benefit the other. More commonly, a citrus processing plant is secondary to a commercial fresh fruit packhouse. The Magaliesburg Citrus Co-operative is

the only organisation focused solely on the processing of citrus in South Africa. In the interest of increasing juice quality, a financed project was launched by the Magaliesburg Citrus Co-operative to better understand factors affecting the crop and ultimately the quality of the fruit.

In this study a broad approach of several horticultural orchard practices were evaluated in an attempt to identify factors responsible for the kilogram total soluble solids per metric ton (kg TSS/t) of 'Valencia Late' oranges grafted on Rough lemon. In an effort to alter soil and orchard microclimate, synthetic as well as organic treatments were included. The amendments were chosen on the grounds of commercial availability and costs. In pot trials the effect of rootstock was evaluated for soluble solids per fruit and per metric ton. An attempt was also undertaken to determine the effects of the prevailing climatic conditions on the quality of fruit taken in by the Magaliesburg Citrus Cooperative during the 1999 and 2000 seasons.

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## Chapter 2

## FRUIT CHEMICAL CHARACTERISTICS AS INFLUENCED BY VARIOUS CLIMATIC AND CULTURAL PARAMETERS:

#### A Literature Review

Orange fruits are made up of various combinations of organic and inorganic constituents which mainly determine fruit quality (Jones, 1961). Fruit quality varies with cultivars and stages of maturity as well as other factors throughout the season (Sinclair, 1961; Chen, 1990). Fruit quality is also affected by many environmental factors such as climate, soil conditions and cultural practices. These factors, from temperature and mulching to disease control, interact with each other making analysis of them on fruit quality complicated (Kuriyama et al., 1981).

## Fruit growth and maturity

It is customary to describe citrus fruit growth in three stages of development (cell division, cell enlargement and maturation) along a sigmoid growth curve (Fisher et al., 1983; Deidda et al., 1992; Zhang et al., 1992; Anon, 1997a). In Figure 2.1 a representation of 'Valencia Late' fruit growth and development is depicted (M.C. Venter, 2001, Magaliesberg Citrus Co-operative, Brits). Stage I lasts approximately nine weeks from blossoming and is characterised by maximum cell division and relatively slow fruit growth (Bain, 1958; Anon, 1997a). Increase in fruit size is primarily due to rind growth. At anthesis Juice sac primordia expand by cell division and enlargement to fill one-half to two-thirds of the pulp segment by the end of this stage. Cell division stops at the end of this stage except in the flavedo (Bain, 1958).

Stage II is characterised by the period of maximum fruit growth and lasts for about 30 weeks after stage I. A marked expansion of tissues due to cell enlargement is evident in especially the pulp. Juice sacs fill the locules

approximately 16 weeks after blossoming. Oil gland size continues to increase and more glands are formed (Bain, 1958; Anon, 1997a).

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Maturation, stage III, lasts approximately 11 weeks after stage II (38 weeks after anthesis) to the time of commercial maturity. It is characterised by decreased rates of morphological and physiological changes. The flavedo changes to orange while slight increases in fruit size and mass occur mainly due to pulp expansion (Bain, 1958; Anon, 1997a).

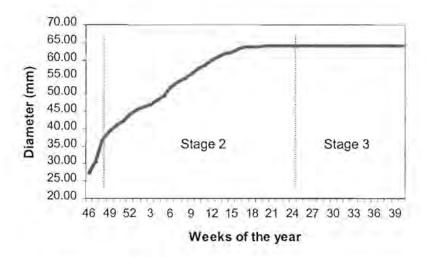


Figure 2.1 Fruit growth and development of 'Valencia Late' orange fruit during the 2001 season within the Brits production area (M.C. Venter, 2001, Magaliesberg Citris Co-operative, Brits). The dotted lines indicate the transition between stages of fruit growth and development as described by Anon (1997a).

Further, orange fruits undergo certain definite and progressive changes in chemical composition during growth and maturation (Sinclair, 1961). Increases in soluble solids, decrease in acid content and increase in solid to acid ratio are known to change during the season (Sinclair, 1961; Gardner, 1969; Syvertsen and Smith, 1983; Tadeo et al., 1987; Cohen, 1988; Howie and Lloyd, 1989; McAneney et al., 1995; Porras et al., 1992; 1995; Widodo et al., 1996; Yamanishi, 1995; Yamanishi and Hasegawa, 1995; Yakushiji et al., 1996). The accumulation of soluble solids during the ripening stage is not the outcome of starch breakdown since citrus fruit are non-climacteric (Tzur et al.,

1992). In general the soluble solids increase as fruit develop and ripen on the tree, but a decline in soluble solids occur in over ripe fruit left to hang on trees (Sinclair, 1961). The increase in total soluble solids of orange juice as fruit mature and the corresponding decrease in acid content, is expressed as the ratio of soluble solids to acid as a measure of maturity for commercial picking (Sinclair, 1961). As the solid to acid ratio is allowed to increase beyond the optimal, the fruit becomes overripe and tasteless (Anon, 1997a).

#### **Environmental factors**

#### Climate

More than most fruits, citrus fruit quality vary sharply with climate. All citrus fruits are of tropical origin (Grierson and Ting, 1978). Within the areas of the world in which oranges are grown, temperature appears to be the main climatic factor that influences fruit quality (Jones, 1961). Many authors have reported on the effect of air and soil temperature on the vegetative growth (Lenz, 1969; Khairi and Hall, 1976), root growth (Mohammad *et al.*, 1996), bud break (Richardson and Mooney, 1992; Al-Jaleel *et al.*, 1993), flowering (Hall *et al.*, 1977), and fruit quality (Ketchie, 1969; Richardson and Mooney, 1992; Yamanishi, 1995; Hutton and Landsberg, 2000) of various citrus cultivars.

Zhang et al. (1992) reported a positive correlation between the growth rate of citrus fruit and temperature, rainfall and the duration of sunshine, while being negatively correlated to evaporation. Richard and Mooney (1992) suggested that the increase in light, air temperature and heat accumulation within the canopy of trees mulched with reflective foil increased soluble solid levels within the fruit due to an increased photosynthetic activity of leaves.

Jones (1961) considered three aspects of temperature important. Minimum temperature, maximum temperature, and the total available heat. Jones et al. (1962) reported that fruit composition was rather more closely related to heat units than to other measured factors of climate such as maximum, minimum or mean temperature, total sunlight, etc. Mendel (1969) concluded that

accumulated temperatures (heat units) above the physiological threshold for citrus trees (12.5°C) are the decisive factors in the vegetative growth rate and that light (intensity, length of day, and quality) had a secondary influence under orchard conditions. Chen (1990) found linear models comparable to that of heat unit systems for early estimation in seasonal changes of °Brix and solid to acid ratios. Hutton and Landsberg (2000) found evidence that variation in fruit quality late in the harvest season could be accounted for by temperature conditions during the period that fruit were on trees and that gross changes in internal quality can be predicted by using effective heat units to calculate changes in °Brix and acid. The total available heat varies from year to year at a given location which accounts for differences in maturity dates or differences in maturity on a given date (Mendel, 1969).

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Although South Africa is not accustomed to freezes, 'Valencia' oranges, because they are less mature (lower soluble solid concentration) during the colder part of the year, freeze at a slightly higher temperatures than more mature fruit of early varieties (Jones, 1961). Bartholomew et al. (1950) found considerable physical recovery of cold injured fruit but little in percentage juice, total soluble solids, or in acid content of frozen 'Valencia' oranges. Freezing temperatures can thus induce a permanent reduction in fruit quality.

Extreme high temperatures may result in killing foliage, loss of fruit, and reduction in fruit quality (Jones, 1961). The effect is more marked if the preceding weather has been cool. Exposed fruit may become "sunburned", which greatly reduces quality (Jones, 1961). Temperatures at the centre of exposed 'Valencia' fruit did not increase appreciably until the albedo was damaged (Ketchie, 1969). Stage two of fruit growth for 'Valencia' oranges is described as the critical period (Anon, 1997a). Soil moisture, wind and heat are important factors determining growth during this period and interruptions in growth during this period are never fully recovered from (Anon, 1997a).

Ambient temperatures (irradiance) greatly affect soil temperatures. Decreasing root temperature limits vegetative growth and enhances flower production (Van Noort, 1969). Total bud break is increased by warmer soils

(Hall et al., 1977). Al-Jaleel and Williamson (1993) indicated soil temperature to be an important factor controlling budbreak and growth of citrus nursery trees. In New Zealand McAneney et al. (1995) postulated that delayed budbreak due to a cooler soil temperature, meant that flowering and the early stages of fruit development took place during a period of more favourable air temperatures and that this was an important determinant of subsequent sugar accumulation between orchards.

Materials introduced into the atmosphere by man may influence the composition and quality of oranges. Pollutants such as fluorides, herbicides, smog, sulphur dioxide and ethylene have been shown to reduce plant growth and production (Jones, 1961). Current levels of atmospheric oxidants, principally ozone, may be limiting yields of 'Valencia' oranges in the Riverside area of California by as much as 30% (Olszyk et al., 1990).

#### Light

It has long been recognised that light has an appreciable influence on the growth rate of citrus trees. Light intensity is a growth retarding factor, with the growth rate decreasing with increasing light intensity (Mendel, 1969) within certain borders. Yamanishi and Hasegawa (1995) found low light intensity to reduce the content of total soluble solids in the juice of citrus and deciduous fruits, with larger leaf areas required by fruit on internal and shaded branches (Fisher et al., 1983). Several papers have also been published on the effect of fruit position in the tree canopy with relation to its chemical composition and physical characteristics. Exposed, northern (southern hemisphere) fruit having higher soluble solids and soluble solid to acid ratios (Reitz and Sites, 1948; Syvertsen and Albrigo, 1980; Syvertsen and Smith, 1983; Cohen, 1988; Fallahi and Moon, 1989; Kender and Hartmond, 1999).

Besides the obvious effects of sun light on the macro-organ level of the tree, there is a specific path of photosynthate translocation into citrus fruit. The fruit quarter in direct vertical alignment below the source leaf receives the majority of translocated photosynthate (78%) while the quarter positioned opposite that aligned with the source leaf receives the lowest levels (Koch, 1984a; Koch

1984b). It is generally assumed that the size of photosynthetically active leaf area supplying the individual fruit is a main factor in determining its size (Fisher et al., 1983; Yamanishi and Hasegawa, 1995). The total leaf area per tree is, therefore, one of the factors determining fruit size. The photosynthetic efficiency of leaves is obviously important and the special relationship between leaves and fruit also has some effect; however, the photosynthetic activity of leaves is not constant (Fisher et al., 1983).

Reitz and Sites (1948) suggested that the warmer canopy positions yielded fruit higher in soluble solids. Cohen (1988) reported that citrus fruit from the warmer exterior or upper part of the tree were more mature and tastier than fruit from cooler, interior or lower parts of the tree. Fruit weight, juice content and peel thickness of internal fruit were found significantly higher when compared with external fruit (Fallahi and Moon, 1989). Green and Gerber (1967) showed that 90% of the direct irradiance on a clear day and 20 to 50% of diffuse radiation on a cloudy day are absorbed in the outer 1 m of canopy depth of a mature orange tree.

#### Water

In recent years a lot of literature has been reviewed on the influence of water on citrus fruit composition and quality by Shalhevet and Levy (1990). Trees grown under conditions of abundant soil moisture produce fruit high in juice, low in soluble solids, and low in acid content (Jones, 1961). It could be assumed that under most conditions the differences could be largely accounted for by dilution. On the other hand, Yakushiji et al. (1998) indicated that sugar accumulation in fruit was caused by and increase in translocation of photosynthates in fruit and especially into the juice sacs, under drought stress. This is in contradiction with the belief that sugar accumulation may be induced by dehydration after transpiration from stomata of fruit peel surfaces during conditions of drought stress. If this were the case, concentrations of sugars in fruit would be increased due to passive loss of water from cells in fruit rather than significant increased in amount of sugar content per fruit.

The effect of water stress is dependent on the duration and phenological stage in which the stress occurs. Fruit physical characteristics, e.g. peel thickness, fruit size and set, and juice yield are all affected by water stress (Levy et al., 1979). Stress during the third stage of development (refer to the section on Fruit growth and maturity) in citrus fruit growth increased peel thickness, soluble solid content and acid content while, during the first and second growth period, it decreased the above parameters (Cruse et al., 1982; Ginestar and Castle, 1996).

Fruit size is considered the major fruit characteristic influenced by irrigation. Drought increased the peel/pulp ratio (Shalhevet and Levy, 1990). Rain or irrigation during the latter part of the season or during harvest, lowers both the soluble solids and the titratable acid (Cruse *et al.*, 1982). It should not be forgotten that fruit size is reduced under conditions of restricted water which in turn also influences the soluble solids and acid contents as soluble solids increase with a decrease in the size of the fruit (Sinclair, 1961; Gardner, 1969).

## **Cultural practices**

#### Fertilisers and soils

Soils influence fruit composition and quality in that they vary in ability to supply the tree the various nutrient elements, including water. Soils of the arid regions are, in general higher in calcium, magnesium, sodium, phosphorus and potassium than the soils of humid regions (Jones, 1961). Soil of the humid regions have been leached for geological ages by heavy rainfall and as a consequence may be deficient in many of the nutrient elements (Jones, 1961).

Obreza and Rouse (1993) found that as fertiliser (nitrogen, phosphorus and potassium) rates increased, total soluble solid concentration in the juice of 'Hamlin' orange and the solid to acid ratio decreased, while weight per fruit and soluble solids per tree increased. Du Plessis and Koen (1988) observed the nitrogen/potassium ratio in the leaves of fruit bearing twigs was more

important in determining yield or fruit size than the absolute nitrogen or potassium values. In case of maximum yield the optimal nitrogen/potassium ratio was between 2.4 and 3.0 with nitrogen higher than 2.1% and potassium higher than 0.8%. For maximum fruit size the ratio was 1.6 and 2.2 with nitrogen higher than 1.8% and potassium higher than 0.9%.

Fruit number and size (the two main components of yield) are differently affected by nitrogen, phosphorus and potassium (Miller, 1990). In general fruit size, juice percentage, and solid to acid ratio are reduced by increasing nitrogen from a relatively low level to a relatively high level while fruit number, rind thickness and acid contents are increased, but has little effect on the total soluble solids (Jones, 1961). Phosphorus has a marked influence on fruit quality even when no effect on yield is noted. Increases in phosphorus leads to reduced acid and increased solid to acid ratio (Jones, 1961). Potassium exerts a stronger influence on fruit size in years in which all fruit are generally small. The most consistent effect of increasing potassium is to reduce the solid to acid ratio due to slight decreases in soluble solids and increases in acid contents (Jones, 1961).

## Mulching

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Citrus is well suited to benefit from mulches and the introduction of specific biocontrol agents has the potential for further increasing root health. The wild ancestors of citrus compete in forest ecosystems and have evolved with a distinct subtropical litter layer covering their root systems (Casale *et al.*, 1995). The benefits of applying organic mulches to crops are well documented and include improving soil structure and reducing soil temperature, resulting in irriproved root growth and more efficient use of water and nutrients, and increased biological activity (Schroth *et al.*, 1992; Casale *et al.*, 1995). Organic mulches also help to control weeds, nematodes, plant diseases and favours healthy roots (Casale *et al.*, 1995) while increasing yield (Agele *et al.*, 2000a; Agele *et al.*, 2000b). However, Walker and Morey (1996) found the application of *Pinus* or *Eucalyptus* sawdust increased nematode levels, while Al-Qasem and Abu-Gharbieh (1995) found that soils containing lower organic matter (0.77-1.43%) sustained higher numbers of citrus nematode in Jordan.



Wood compost consisting of *Eucalyptus* contains oils that can damage plant roots (Walker and Morey, 1996), while Casale et al. (1995) found that decay of mulch with high carbon to nitrogen ratio immobilises nitrogen and results in a temporary nitrogen shortage which was found less beneficial to citrus.

The addition of cattle manure significantly increased the organic carbon content of soil and increased yield of barley (Materechera and Mehuys, 1991). However, cow manure appeared to be unsuitable as it reduced citrus growth and root health and resulted in undetectable populations of applied biocontrol agents (Casale *et al.*, 1995), due to the release of large amounts of ammonia upon degradation.

Many studies have been conducted on the effect of the reflectivity of synthetic mulches on a wide variety of crops. The influence of mulching treatments is that they alter the orchard microclimate by the absorption and reflection of sunlight from the soil surface thereby influencing both canopy and soil temperatures (Richardson and Mooney, 1992). Synthetic mulches can increase soil temperatures throughout the season but their performance is dependent upon the reflectivity of the material, the thickness of the air layer between the mulch and the soil and the soil water content (Liakatas *et al.*, 1986). Black polyethylene and reflective foil have opposing effects on plant growth due to their different effects on the tree microclimate (Bacon, 1974; Richardson and Mooney, 1992). Special red plastic mulch which reflected red and far-red light, affected phytochrome-mediated allocation of photosynthates and directed more to developing fruit which lead to higher yields and fruit size (Kasperbauer, 2000).

#### Girdling

Girdling treatments have been applied in citrus production since the beginning of this century (Wallerstein et al., 1978). The effects of girdling on yield, fruit size, quality and ripening date in citrus have been widely studied (Peng and Rabe, 1996). However, citrus productivity and tree responses to girdling depend on many factors, such as girdling date, girdling procedures and techniques (e.g. the girdle width and position, i.e. trunks or branches),

different cultivars, and possibly even climatic differences between countries or regions (Yamanishi and Hasegawa, 1995; Peng and Rabe, 1996, Mataa et al., 1998). Girdling before flower bud differentiation induced flowering, while pre-bloom girdling promoted parthenocarpic fruit set in pummelo (*C. grandis*) trees (Yamanishi and Hasegawa, 1995). Girdling at anthesis increased fruit set (Mataa et al., 1998). Yamanishi (1995) observed that the combination of a higher temperature regime and girdling in the mid-autumn significantly increased the internal fruit quality and fruit size, and hastened fruit maturity of pummelo fruit. Girdling in early summer and autumn increased fruit size of 'Ponkan' mandarin while late season girdling had no effect (Mataa et al., 1998). Mataa et al. (1998) found no significant effect on soluble solids and titratable acid for various girdling dates. As far as the effect of girdling on citrus fruit quality and maturation are concerned, results are not conclusive (Peng and Rabe, 1996).

Peng and Rabe (1996) found that summer girdling at 2 to 4 and 2 to 5 weeks after physiological fruit drop (APFD) significantly increased soluble solids in the first season, although this effect diminished with consecutive annual girdling and lead to visibly reduced tree vigour and yield. Citric acid content in fruit has also been increased by summer girdling (Yamanishi and Hasegawa, 1995).

#### Rootstocks

That rootstock influence the quality of citrus fruits produced by the scion cultivar has long been recognised (Bitters, 1961; Monteverde et al., 1988; Wheaton et al., 1990; Sosa et al., 1992; Tuzcu et al., 1992; Econimides and Gregoriou, 1993; Alexander, 1996; Anderson and Beñatena, 1996; Castle and Baldwin, 1996; Muraro et al., 1996; Anon, 1997b; Georigiou and Gregoriou, 1999; Wright and Aubert, 1999), although the physiological basis of this influence has been a matter of conjecture (Gardner, 1969). This influence is believed more common and more profound in citrus than in other fruits, perhaps because of the large number and great diversity in character of citrus rootstocks in common use (Gardner, 1969). This could also be due to the fact that internal fruit quality, as measured by the percentage of total soluble solids

and acid of the fruit juice, is under almost constant surveillance as a criterion of consumer acceptance (Bitters, 1961; Gardner, 1969). This is important and frequently determines the choice of citrus rootstock. The rootstock and scion are in a sense two separate entities living in a symbiotic relation, and the interaction between the two is further complicated by the influence of different soil types, different climatic conditions, different cultural practises, and other factors (Bitters, 1961; Radogna *et al.*, 1992; Roose and Kupper, 1992). Rootstocks have been reported to affect fruit size and weight, rind thickness, juice content, total soluble solids concentration, and total acids (Bitters, 1961; Wheaton *et al.*, 1990; Econimides and Gregoriou, 1993). Besides the effect on fruit quality, rootstocks have also been found to cause variation in the nutrient concentrations in citrus leaves through the enhancement of absorption and translocation of some nutrients (El-Shazly *et al.*, 1992; Georigiou, 2000; Zerki, 2000).

Gardner (1969) observed that when the fruit (including a section of fruit stalk large enough to graft) of young 'Valencia' trees were reciprocally cross-grafted between 'Valencia' trees growing on Rough lemon (*Citrus limon* Burm. f.) and Sour orange (*C. aurantium* L.) rootstock, the fruits matured with total soluble solids in the juice characteristic of the rootstock on which they completed their growth and maturity. This indicated that the ultimate course of solids production is not fixed early in the ontogeny of the fruit nor by the variety of foliage, other than supplying carbohydrates to the growing fruit.

#### Pests and diseases

Soil borne pests, which feed and proliferate on the citrus root system can seriously affect root system function, directly affecting tree growth and yield. The citrus nematode *Tylenchulus semipenetrans* Cobb and the fungus *Phytophthora parasitica* Dastur are common parasites of citrus world-wide (Duncan *et al.*, 1993; McClure and Schmitt, 1996; Walker and Morey, 1996; Noling, 1997) and are the most devastating soilborne fungal pathogens involved in citrus slow decline (Le Roux *et al.*, 1991).

#### Nematodes

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Citrus tree growth and productivity is closely linked with root system health and both can be seriously affected by different soil borne pests and diseases (Noling, 1997). The citrus nematode *Tylenchulus semipenetrans* is one of the most debilitating pests of citrus world-wide (Duncan *et al.*, 1993; McClure and Schmitt, 1996) and responsible for an estimated 7-50% crop loss (Al-Qasem and Abu-Gharbieh, 1995; Reddy *et al.*, 1996; Walker and Morey, 1996, Noling, 1997). Because it does not devastate citrus trees, its presence may not be immediately detected and the damage it does may not be easily recognised. In time, however, its effects on trees will become noticeable and may have devastating effects (Heald and O'Bannon, 1994).

Fibrous roots emanating in bunches from woody lateral roots, are the primary sites where water and nutrient uptake from soils occur (Devlin, 1975; Noling, 1997). Root feeding by the citrus nematode results in the breakdown of root cells which are then frequently invaded by secondary organisms which eventually kill the root (Noling, 1997). If the root system is severely damaged and reduced in size, the normal utilisation of nutrients and water from the soil is inhibited (Heald and O'Bannon, 1994; Philis, 1995; Westerdahl, 1997). The nematode causes citrus slow decline characterised by poor growth (Philis, 1995) a reduction in fibrous root biomass and symptoms of root disfunction, such as small sparse leaves and reduced yield often resulting from smaller than normal fruit (Duncan et al., 1993; Niles et al., 1995; Philis, 1995, Walker and Morey, 1996). Damage caused by a citrus nematode infestation depends on the age and vigour of the tree, density of the nematode population, and susceptibility of the rootstock (Westerdahl, 1997). The citrus nematode is also known to influence channelling of carbohydrates produced in the leaves to storage organs, formation of storage reserves, and nutritional or chemical imbalances in infested trees (Noling, 1997). Wheaton et al. (1985) observed increased levels of potassium and calcium in the leaves of trees treated with These symptoms are typical of nematode infestation but not aldicarb. diagnostic. Nematode infestations may occur without inducing any aboveground symptoms (Westerdahl, 1997).

The nutritional quality of roots is determined by their age and temporal demands from other plant organs (Duncan and Eissenstat, 1993). Fibrous roots are the primary sites where water and nutrient uptake from soil occur (Noling, 1997) and where second-stage female nematode juveniles migrate through the rhizoshpere, feed and penetrate (Niles et al., 1995). In a study conducted by Duncan and Eissenstat (1993), *T. semipenetrans* responded to available carbohydrate in the roots, which meant that citrus fruit presented a major carbohydrate sink with which the nematode competes with for food. In other words the nematode population sizes respond positively to the excess carbohydrates found in roots only after plant demands have been satisfied. Le Roux (1995) indicated that the nutritional imbalance, which occur in citrus trees infected for many years with nematodes, may not be rectified immediately when the nematodes are eliminated. Cadusafos suppressed nematode populations for twelve months after application and significantly increased yields (McClure and Schmitt, 1996).

Population densities of nematodes generally increase in autumn and spring in response to flushes of new fibrous roots, which are the nutritional substrate for the nematode (Duncan and Eissenstat, 1993; Noling, 1997). As citrus roots grow, the mature sections become less susceptible to infection (Duncan and Eissenstat, 1993). Experiments with labelled CO2 indicated that reserve carbohydrates were utilised mainly to support reproductive development, while photosynthesis from mature leaves supplied the needs of vegetative growth (Goldschmit, 1999). Annual fruit production thus represents a major sink for plant carbohydrates as found by Duncan and Eissenstat (1993). Dry weight accumulation in 'Valencia' fruit is greatest two to four months after bloom, during which the competition for available carbohydrates likely occurs from October to January (Southern hemisphere). During spring and summer, available carbohydrates in older leaves and roots are relatively low (Goldschmit, 1999). Duncan and Eissenstat (1993) found higher starch levels in the roots of defruited trees to coincide with increased growth of nematode soil population densities. Thus decline in nematode population density can be linked to high carbohydrate demand by fruit and reduced carbohydrate concentrations in the remaining tissues of the plant (Duncan and Eissenstat,

1993). The density of the nematode population is then dependent on the available plant carbohydrate for fibrous root growth, which is in turn followed by an increase in nematode population density. The citrus nematode is then able to acquire excess carbohydrates after plant demands have been satisfied.

Population size of *T. semipenetrans* is also affected by soil texture (Heald and O'Bannon, 1994; Al-Qasem and Abu-Gharbieh, 1995), organic-matter content (Ritzinger *et al.*, 1998), soil moisture (Duncan *et al.*, 1993; Duncan and El-Morshedy, 1996), temperature (Duncan *et al.*, 1993; Noling, 1997), acidity, oxygen content, salinity, and host genotype (Niles *et al.*, 1995), developmental stage (Niles *et al.*, 1995; Noling, 1997) and chemical composition of citrus fibrous roots (Duncan *et al.*, 1993).

Fine textured, poorly drained soils with high organic matter content are known to favour citrus nematode reproduction and the development of other root rot problems which act in combination to reduce tree size, vigour and yield (Noling, 1997). Trees can become infected in almost any soil, but early infection in high numbers and subsequent population increase occurs more rapidly in fine-textured organic soils than in coarse-textured sands (Heald and O'Bannon, 1994). Walker and Morey (1996) stated that the application of organic mulches (*Pinus* or *Eucalyptus*, and composted green waste) increased nematode levels while Walker and Morey (1999) found organic amendments applied with nematophagous fungi did not further reduce nematode levels. Population densities of the citrus nematode decline rapidly under drought conditions differing in response depending whether all or part of the rhizosphere experiences drought (Duncan and El-Morshedy, 1996).

Most citrus rootstocks used commercially are attacked by the citrus nematode (Heald and O'Bannon, 1994). On susceptible rootstock, female second-stage juveniles migrate through the rhizosphere and feed ectoparasitically on the epidermal and hypodermal cells of fibrous roots. When they find a suitable site, they penetrate the root cortex, feed on parenchyma cells, induce the

formation of nurse cells around a permanent feeding site, and mature to adults (Niles et al., 1995; Noling, 1997).

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On resistant rootstocks, establishment of the permanent feeding site is impeded by plant defences such as hypersensitive reactions and formation of wound periderm (Niles *et al.*, 1995). The use of resistant rootstocks such as Troyer citrange is a good practice, although the tolerant rootstocks may loose their tolerance because the nematode will evolve a new biotype over time (Duncan *et al.*, 1994; Luck *et al.*, 1996). Tolerant rootstocks include Swingle citrumelo and Trifoliate orange (Duncan *et al.*, 1994; Noling, 1997) while susceptible rootstocks include Rough lemon, Sweet orange (Anon, 1997b; Walker and Morey, 1999), and Carrizo citrange (Niles *et al.*, 1995).

Walker and Morey (1999) found commercial products formulated with microbial antagonists, applied to soil, performed poorly against nematodes Tylenchulus semipenetrans on citrus, compared with the conventional aldicarb. There is a need to replace highly toxic and potentially polluting chemicals, used to control plant parasitic nematodes with less dangerous chemicals or, preferably with biological control agents (Walker and Morey, 1999). Paecilomyces lilacinus (strain 251) is a parasite of plant-parasitic nematodes, which has effectively been used on banana, tomato and tobacco under the trade name of PI Plus (Sandberg, 1999) and has not been registered on citrus thus far. Biostart® has been reported to enhance the function of growth stimulation and suppress soil borne pests and some fungal and bacterial diseases commonly encountered. The natural occurring microbial mix includes Bacillus laterosporus, B. chitinosporus and B. licheniformis. In the soil B. laterosporus stimulates root growth and suppresses bacterial diseases to a certain degree, B. chitinosporus neutralises the nematode egg and suppresses fungal disease (especially Fusarium), while B. licheniformis causes protein degradation assisting in nematode and fungal suppression.

Le Roux (1991) found the population of *T. semipenetrans* on roots and in soil was reduced by treatments containing aldicarb with corresponding increase in

yield. Cadusafos suppressed nematode populations for twelve months after application and significantly increased fruit size and yields in soils containing 85% sand, 7% silt, 8% clay and 0.3% organic matter (McClure and Schmitt, 1996). A 26% increase in exportable fruit was achieved after nematode control in Cyprus with various nematicides (Philis, 1995) and Bullock and Pelosi (1995) had significantly more fruit of preferred size at harvest, after annual treatments of aldicarb. Walker and Morey (1996) found multiple applications of cadusafos to the entire root zone was more effective than the standard industry practice of single, banded application of aldicarb.

Data available on yield increases from citrus nematode control in various citrus-growing countries suggest a world average range of 20 to 30% increase in citrus yield (Harris, 1983; Heald and O'Bannon, 1994). Stansly and Rouse (1994) observed significant improvement in yield, but not juice quality. An increase in fruit size for the first year and significant yield increases in the second year of aldicarb application was also observed by Wheaton *et al.* (1985). Yield increases are mainly caused by an increase in fruit size (Philis, 1995; McLure and Schmitt, 1996). Soil applications of K<sub>2</sub>SO<sub>4</sub> in combination with aldicarb significantly increased the level of potassium in leaves, and fruit size of 'Valencia' orange tree growing on soils previously planted to citrus in South Africa (Fouche *et al.*, 1977). Harris (1983) found residues of aldicarb in the fruit pulp, juice and rind of 'Valencia' orange trees eight months after two and three applications of 8 kg/ha making late applications of aldicarb very risky.

## Phytophthora

Phytophthora parasitica reduces fibrous root density of mature trees sufficiently to affect crop yield by destroying feeder roots (Duncan et al., 1993; Ohr and Menge, 1998). If the destruction of feeder roots occurs faster than their regeneration, the uptake of water and nutrients will be severely limited, and stored reserve levels will be depleted (Ohr and Menge, 1998). Leaves turn light green or yellow and may drop depending on the amount of infection (Ohr and Menge, 1998). The symptoms described are typical but not

diagnostic, as they could result from other causes as well and may even occur without inducing obvious aboveground symptoms.

Yield and total soluble solids per hectare where increased at sites treated with soil applications of metalaxyl due to reduction of infection and increase of feeder root densities (Timmer et al., 1989). A multiple-year treatment program with fosetyl-Al or metalaxyl resulted in significantly healthier tree canopies and higher root densities compared to non-treated trees; however, the population densities of *P. citrophthora* and *P. parasitica* did not differ significantly when non-treated trees were compared to those receiving fungicide treatments (Matheron et al., 1997).

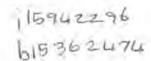
## Conclusion

The fact is that there are a variety of factors that influence fruit quality of which only a few have been discussed in the above sections. These factors can be divided into two groups namely those that can be controlled and those that we have no control over. Careful consideration should thus be taken when deciding on planting new orchards to combine factors conducive to high quality. For existing orchards, the focus to increase fruit quality can only be placed on factors that can be manipulated. All factors can be manipulated; the only question is the cost thereof.

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## Chapter 3

# INFLUENCE OF ON TREE FRUIT POSITION AND GIRDLING ON INTERNAL QUALITY OF 'VALENCIA' ORANGE FRUIT

## Abstract

During the 1999 season the internal quality of 'Valencia Late' orange was investigated with regard to fruit position and exposure in the canopy and the effects of winter girdling of branches. Fruit at shoulder height from northern and southern exposed positions were sampled during maturation. Fruit from eastern and western exposed positions were sampled on the crown while shaded fruit were sampled from bottom, inner canopy positions. Fruit from exposed canopy positions generally had higher soluble solids while no real differences were found for soluble solids per metric ton. Girdling of branches late in the season did not improve soluble solids. Quadratic trends were best fitted to the data in this study indicating an increase of soluble solids over the season peaking and then decreasing.

## Introduction

Pronounced differences in fruit quality have been shown to be related to the position of the fruit on the tree (Reitz and Sites, 1948; Syvertson and Albrigo, 1980; Syvertsen and Smith, 1983; Cohen, 1988; Fallahi and Moon, 1989). Reitz and Sites (1948) identified three factors affecting the soluble solid content of 'Valencia' oranges according to fruit position in the tree canopy. Soluble solid content increased with height regardless of the direction of exposure to light; outside, canopy and inside fruit decreased in soluble solid content respectively; and that soluble solid values are related to the direction of light exposure.

Girdling treatments have been applied in citrus production mainly to increase the quality of fruit under various environmental constraints (Fisher *et al.*, 1983; Yamanishi, 1995; Yamanishi and Hasegawa, 1995; Peng and Rabe, 1996, Mataa *et al.*, 1998). However, the productivity and tree responses to girdling depend on many factors such as girdling date, procedure and techniques, cultivar and variation in climate (Peng and Rabe, 1996).

The aims of this study was firstly to confirm that a considerable amount of variation among individual fruits can be related to the position of the fruit on the tree, and secondly to investigate the effect of branch girdling on internal fruit quality during the fruit maturation period.

#### Materials and Methods

#### Characteristics of the experimental plot

The study was conducted during 1999 in two separate orchards. The experimental sites consisted of 'Valencia' (*Citrus sinensis* (L.) Osb.) orange trees grafted on Rough lemon (*C. limon* (L.) Burm. f.) rootstock. The orchards were planted in 1987 and situated in the North West Province, near Brits (25°S 27°E, 1107 m.a.s.l.) in South Africa. The orchards were selected on the

basis of previous seasons production in soluble solids per metric ton. The first (300 trees / ha) having a history of high and the second (1 600 trees / ha) of low soluble solids per metric ton. Fruit from northern and southern canopy positions were sampled from trees spaced 5.8 m apart within rows and 5.8 m between rows (300 trees / ha). Fruit from heavily shaded interior as well as, top eastern and western positions of canopies were sampled from trees spaced 2 m apart within rows and 3 m between rows (1 600 trees / ha). Healthy vigorous trees, uniform in canopy size for each spacing, were included in the trial design. Commercial orchard practices were maintained throughout the duration of the trials.

#### Treatment details

Branches used in the girdling treatments were selected with enough fruit to sustain consecutive sampling over the maturation period. Girdling (7/6/1999) was carried out using a budding knife by making a single cut through the bark of ca. 1 mm effective width around selected branches according to the method of Peng and Rabe (1996) used for girdling of main trunks. No bark strip was removed and no wood layer was damaged. Fruit from ungirdled branches served as controls.

#### Fruit quality determinations

Fruit were picked from control and girdled branches, and consisted of three fruit per treatment replica. Fruit from northern and southern exposures were picked at shoulder height and for eastern and western exposures on the crown (top) position on the canopy. Shaded fruit were sampled from the inner core of the tree canopy. Fruit diameter and weight, juice weight, peel thickness and total soluble solids were measured throughout the season. Fruit were sectioned equatorally so that peel thickness could be measured with an electronic hand calliper and the juice extracted by hand with a Pineware CS2 citrus juicer. Total soluble solids were determined with a temperature-compensating digital refractometer (Palette PR-101) (Wardowski et al., 1979). Soluble solids per metric ton were calculated by multiplying the juice percentage and soluble solids (°Brix) and dividing the result by 10.

## Experimental design and statistical analysis

Data was statistically analysed as ten randomised single tree replications with two treatments per canopy position. Polynomials were compared per group by using a program developed by Groeneveld (1970), by means of solving a polynomial. Significance (5% level) of a contrast between any two polynomials was expressed in terms of the independent variables. The observation range was computed as days after 1 April 1999. Polynomials of the first degree are known as linear trends and that of the second degree as quadratic trends.

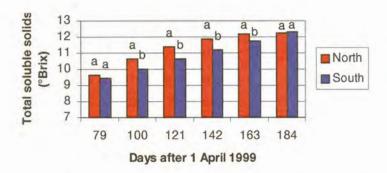
## Results

Soluble solids. As depicted in Figure 3.1, fruit on the external northern side of trees tended to have higher soluble solid concentrations over most of the season. Values were comparable at 184 days after 1 April (normal harvest period). For fruit from the northern exposure a quadratic trend was best fitted while for fruit from the southern exposure a linear trend was found. Thus, fruit from the southern exposures mature at a slower rate than fruit from northern exposures but that by the end of the maturation period, the soluble solid contents are comparable. Both were significant over the maturation period.

Fruit sampled from heavily shaded positions in the canopy core were significantly lower in soluble solid concentration than found for fruit in top external exposures (Figure 3.2). Quadratic trends displayed in Figure 3.2 indicate significant increase in soluble solids up to 142 days after 1 April 1999 where after the concentration of soluble solids in the juice decreased for all canopy positions.

Fruit from northern and southern ungirdled and girdled branches indicated a trend for higher values for control fruit over most of the maturation period (Figure 3.3a and b). Linear trends were best fitted to fruit from northern

girdled, southern and southern girdled treatments. Girdled branches at the top eastern and western sides of the canopy did not significantly increase the concentration of soluble solids above that of control fruit (Figure 3.3c and d). On both exposures fruit from ungirdled control branches indicated higher values. For top eastern and western ungirdled and girdled branches, the soluble solid concentration followed a significant quadratic trend.



**Figure 3.1** Seasonal changes in total soluble solids of 'Valencia Late' orange fruit as influenced by northern and southern canopy positions in the Brits production area (300 trees / ha). Means with different letters in each series differ significantly at 5% level.

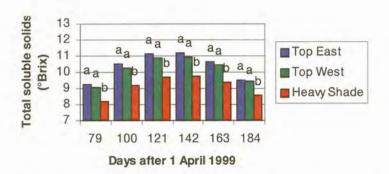
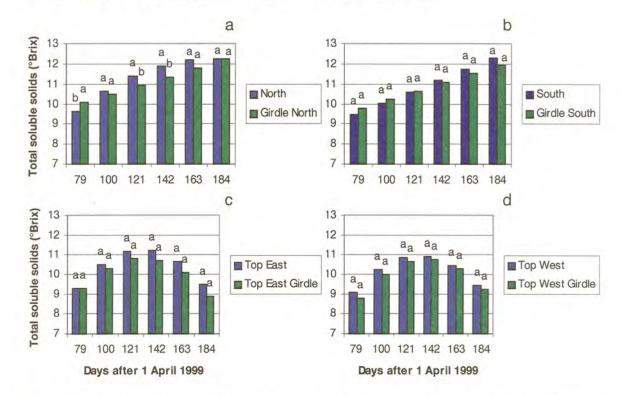


Figure 3.2 Seasonal changes in total soluble solids of 'Valencia Late' orange fruit as influenced by position on the canopy in the Brits production area (1 600 trees / ha). Means with different letters in each series differ significantly at 5% level.

Soluble solids per metric ton. In Figure 3.4 no significant trend was found with regard to differences between fruit from northern and southern

canopy exposures. For both northern and southern exposures significant quadratic trends over the maturation period were observed.



**Figure 3.3** Seasonal changes in soluble solids for 'Valencia Late' orange fruit from control and girdled branches on northern (a), southern (b), eastern (c) and western (d) positions on tree canopies in the Brits production area. Means with different letters in each series differ significantly at 5% level between exposures for single dates.

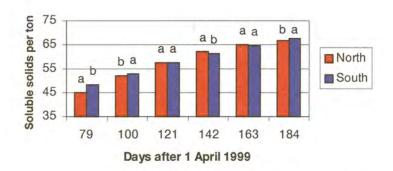
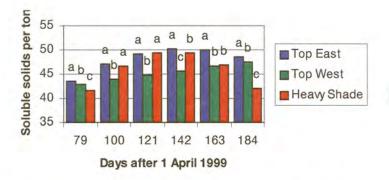


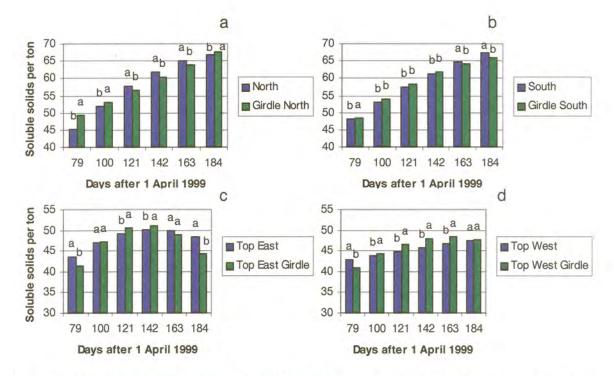
Figure 3.4 Seasonal changes in total soluble solids per metric ton of 'Valencia Late' orange fruit as influenced by northern and southern canopy positions in the Brits production area (300 trees / ha). Means with different letters in each series differ significantly at 5% level between exposures

Fruit from the top eastern position of the canopy had the highest soluble solid content per ton (Figure 3.5). A similar quadratic trend was followed over the

season as found in Figure 3.2. Fruit from top western canopy positions followed a linear trend continuing to increase after the soluble solids per ton in fruit from top eastern and shaded fruit had started to decrease.



**Figure 3.5** Seasonal changes in total soluble solids per metric ton of 'Valencia Late' orange fruit as influenced by position on the canopy in the Brits production area (1 600 trees / ha). Means with different letters in each series differ significantly at 5% level between exposures for single dates.



**Figure 3.6** Seasonal changes in soluble solid per metric ton for 'Valencia Late' orange fruit from control and girdled branches on northern (a), southern (b), eastern (c) and western (d) positions on tree canopies in the Brits production area. Means with different letters in each series differ significantly at 5% level between exposures for single dates.

No definite trends were displayed for girdling treatments on northern and southern canopy exposures (Figure 3.6a and b). Fruit from northern girdled branches indicated a linear trend, overtaking values for northern control fruit (quadratic trend) after 163 days. Girdled treatments significantly increased the soluble solids per ton for top exposed branches over most of the maturation period (Figure 3.6c and d).

# Discussion

The state of the s

Fruit sampled from various canopy positions and light exposures confirmed findings of Reitz and Sites (1948), Syvertsen and Smith (1983), Cohen (1988) and Fallahi and Moon (1989). The linear trend fitted to the soluble solid content in southern fruit (Figure 3.1) would seem to indicate that these fruit are still to reach a peak already reached by the more mature fruit on the northern side also found by Cohen (1988). Cohen's (1988) suggestion that the fruit from warmer exterior or upper parts of the tree are more mature than those from cooler, interior or lower parts of the tree, is probably related to microclimate conditions such as the amount of light and higher temperatures to which canopies are exposed. The practical implication of this information lies in the exposure of as much of the leaf area as possible by pruning windows in the canopy for increased light penetration.

Variation in soluble solid content of fruit from top eastern and western, and heavily shaded canopy positions (Figure 3.2) correspond with findings of Reitz and Sites (1948) who discovered a trend to higher soluble solids in the top of the tree as compared to the lower parts. Fallahi and Moon (1989) suggested that higher CO<sub>2</sub> assimilation rates, higher leaf to fruit ratio, and efficient transport of photosynthetic products to fruit in external canopy positions; and / or that fruit from internal canopy positions are closer to the main limbs (main xylem tissues) may receive more water, resulting in more dilution in soluble solids compared to external fruit, might account for the higher soluble solids of fruit from external canopy positions. Temperatures of sun-exposed leaves are

usually above air temperature while 90% of irradiance on a clear day is absorbed in the outer 1 m of canopy depth of a mature orange tree (Syvertsen and Lloyd, 1994). It can then further be understood that the core temperature of the canopy is cooler and that temperature driven metabolic processes proceed at slower rates (Sinclair, 1961) as compared to external canopy positions in accumulating soluble solids in fruit.

The influence of fruit position seemed to have little influence on the soluble solid content per metric ton contradictory to soluble solid content. As juice percentage has an appreciable influence on the soluble solid content per metric ton, it can be suggested that the juice percentage of fruit from southern fruit had higher values than those from northern positions (Figure 3.4). Further more, reasons for significant lower soluble solids per metric ton for fruit at top, exposed western positions (Figure 3.5) can be proposed by the same explanation that juice percentage was lower in comparison to other exposures. In the southern hemisphere, north-western exposures are generally regarded as being the hottest sides of trees leading to an increase in temperature and a decrease in humidity if all other variables kept constant. As the evaporative demand increases, an increase in vapour pressure deficit occurs between the inside and outside of citrus leaves (Syvertsen and Lloyd, 1994). This in turn causes an increase in transpiration even if stomatal conductance decreases leading to more negative leaf water potentials (Syvertsen and Lloyd, 1994). It is thus suggested that the warmer exterior part of tree canopies might have lower juice percentages in fruit due to lower availability of water in the tree and therefore that higher rates of photosynthate translocation are found in these fruit due to active osmoregulation to maintain cell tugor (Yakushiji et al., 1996; Yakushiji et al., 1998). However, Reitz and Sites (1948) observed that the position of the fruit on the tree had little if any effect on the percentage juice in the fruit.

The effects of girdling treatments have been widely studied (Fisher *et al.*, 1983; Yamanishi, 1995; Yamanishi and Hasegawa, 1995; Peng and Rabe, 1996, Mataa *et al.*, 1998). Results in this study indicated no practical increase in soluble solids also found by Mataa *et al.* (1998). This is possibly due to the

late application of girdling treatments not allowing enough time to get a significant response. Work based on girdled branched is also not identical to data obtained from entire trees. One major difference is the lack of strong alternative sink systems such as the stem and roots (Fisher *et al.*, 1983).

That fruit position has an influence on the concentration of soluble solids has been confirmed. However, in all the literature reviewed and even in this study, no parallel studies of temperature and light intensities within canopy positions and exposures have accompanied these findings. These include several articles reviewed by Syvertsen and Lloyd (1994) with regard to the environmental physiology of citrus. Studies in this regard would be highly insightful.

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# Chapter 4

# FRUIT CHEMICAL CHARACTERISTICS AS INFLUENCED BY SOIL AMENDMENTS IN TWO ORCHARDS

## Abstract

Problems with marginal internal quality exists in the South African processing industry. Ten treatments were evaluated in two non-adjunct orchards primarily according to their improvement in soluble solids per metric ton and yield. For the first orchard treatments included were the placement of a white reflective plastic mulch, application of bark chip mulch, nitric acid for pH adjustment, aldicarb, metalaxyl, PI Plus and a combination of cow manure and Treatments for the second orchard included treatments of molasses. synthetic commercial as well as biological nematicides used individually and in combination with organic amendments. White reflective plastic increased yield and soluble solid production per hectare above control trees. Aldicarb increased fruit weight and kilogram soluble solids per metric ton. None of the other treatments lead to significant improvement of soluble solids per ton. The addition of organic mulch increased fruit size, soluble solids per metric ton and per hectare and yield although nematode counts were highly comparable with the control at harvest. In both treatments of bark chips and manure and molasses in single application, nematode counts were not significantly suppressed in comparison to the addition of aldicarb and PI Plus® in combination treatments. Fruit chemical characteristics were severely influenced by prevailing weather conditions experienced during September 2000. Harvest should thus be avoided after severe weather or stress extremes as the results in this study indicate that a financially viable level of soluble solids per metric ton was only again recovered after two weeks following the stress condition.



## Introduction

Throughout the literature reference is made to the effects of soil amendments on internal fruit quality (Materechera and Mehuys, 1991; Schroth *et al.*, 1992; Casale *et al.*, 1995; Walker and Morey, 1996; Agele *et al.*, 2000a; Agele *et al.*, 2000b), pests and diseases on root health and yield (Timmer *et al.*, 1989; Duncan *et al.*, 1993; Le Roux, 1995; Niles *et al.*, 1995; McClure and Schmitt, 1996; Matheron *et al.*, 1997; Noling, 1997; Westerdahl, 1997; Ohr and Menge, 1998). Seldom if ever, mention is made of the effects thereof on internal chemical characteristics of fruit from treated trees. Although the improvement of internal quality is a direct result of tree health status, chemical characteristics of citrus fruit are an indirect effect of applied soil amendments whether organic, synthetic or chemical.

The aim of this study was to investigate a range of organic, synthetic and chemical soil applications for the improvement of soluble solids per metric ton for the juicing industry.

## Materials and methods

Characteristics of the experimental plot and surrounding area

The study was conducted during the 2000 and 2001 citrus seasons in two non-adjunct orchards of 2 ha each. The experimental sites consisted of 'Valencia' (*Citrus sinensis* (L.) Osb.) orange trees grafted on Rough lemon (*C. limon* (L.) Burm. f.) rootstock. Both orchards were planted in 1987 and situated in the North West Province, near Brits (25°S 27°E, 1107 m.a.s.l.) in South Africa. Trees were spaced 5.8 m apart within rows and 5.8 m between rows (300 trees / ha). Healthy vigorous trees, uniform in canopy size, were used in the trial designs. The second orchard was selected on the basis of proximity to the first, age of trees, and higher root nematode counts. In the first orchard drip irrigation was scheduled two times per week for five and four hours in the summer and winter respectively. Drippers delivered 8 L per hour

per tree. Nitrogen and phosphorus were delivered through the irrigation system at rates of 300 g urea and 250 g potassium chloride (20%) per tree per year. Soil applications of MAP consisted of 150 g per tree per year. Leaf applications of 1% oxifulvic acid (2%) (Enercom) were applied annually in September and December, and potassium nitrate (4000 g / 100 L) / urea combination as single application in November. In the second orchard micro irrigation was scheduled for once a week. Potassium nitrate was supplied through the irrigation system at 350 g per tree per year. Leaf applications of potassium nitrate (4000 g / 100 L) / urea combination were applied twice during November. For both orchards fertilisers were split over the recommended months by Central Agricultural Laboratories.

The general natural veld type and vegetation of the surrounding area consisted of tropical Bushveld of the Savannah biome (Anon, 2001). Soils in this area are generally regarded as being between 450 mm and 750 mm deep, with a clay percentage of 15 to 35%. Red, yellow and greyish, eutrophic soils with high base status are predominant, underlain by a plintic catena (Anon, 2001).

#### Treatment details

## Orchard 1

Silex plastic (2 μm) was applied in mid December 1999. The plastic consisted of black and white reversible sides. For purposes of this trial the white side faced upward (Figure 4.1b). The plastic was cut to the size of the drip line of each tree replica and holes punched for water infiltration. The rest of the treatments were applied early February 2000. Bark chips were applied as organic mulch at a rate of 0.5 m² per tree replica (Figure 4.1c). A litre of 60% nitric acid (HNO<sub>3</sub>) was diluted with 150 L water per treatment replica in an attempt to lower the natural soil pH (water) of 7.1. The fourth and fifth treatments consisted of single applications of 300 g of aldicarb (temik) and metalaxyl (ridomil) respectively, per tree. The sixth treatment was a biological nematicide. PI Plus® was applied at the recommended rate of 4 kg per ha.

PI Plus® applications were done twice during the season, the second application eight weeks after the first. The last treatment was a combination of cow manure and molasses. Three shovels of manure were applied together with 40 ml molasses diluted in water for even distribution for each tree repllica. The molasses was re-applied during the following spring.

### Orchard 2

Bark chips, aldicarb, Pl Plus®, and manure and molasses were applied as in Orchard 1. A combination treatment of aldicarb, manure, molasses and bark chips was also included into the trial. A second combination treatment consisted of Pl Plus®, manure, molasses and bark chips. Application amounts were as for single treatments. The last treatment was a microbial soil inoculant claiming to promote plant root growth and decrease nematode populations. Biostart® was applied at the recommended rate of 1 L per ha per month. No applications were made during the cooler winter months of May, June and July.

All treatments were applied within the drip line of the trees. Control and treatment trees were subjected to normal commercial cultural practices. All trees in each orchard respectively were subjected to the same irrigation and fertilisation regimes throughout the duration of the trial period.

#### Soil samples and temperatures

Soil samples were taken at the onset of the study period and analysed for the conformation of *Tylenchulus semipenetrans* (Orchard 1 and 2) and *Phytophthora* (Orchard 1) present in the orchard according to the method applied by Duncan *et al.* (1994). Nematode populations were determined according to the centrifugal-flotation method of Jenkins (1964). *Phytophthora* presence was confirmed by applying the method used by Jeffers and Martin (1986) on PARPH selective media. Sampling was repeated at the end of July and at harvest. A pH-curve for soil from Orchard 1 was determined for application rates of nitric acid.

Soil temperatures in Orchard 1 were measured according to the method of Richardson and Mooney (1992). Shielded thermistors were used to monitor daily soil temperatures at reference, 10, 30 and 60 cm (Figure 4.2). Data was stored using a CR21X datalogger (Campbell Scientific Inc, Utah) at hourly intervals, during late May and June.

#### Fruit quality determinations

Fruit were picked from the northern, external position of the trees, at shoulder height and consisted of six fruit per treatment replica. Fruit diameter and weight, juice weight, rind thickness, total soluble solids and titratable acidity were measured every three weeks beginning in June (19/6), through July (10/7, 31/7), August (21/8), September (11/9) and early October (2/10), 2000 and August (21/8) and October (2/10) 2001. The results represented focus mainly on the 2000 season unless specified otherwise. Fruit were sectioned equatorally so that peel thickness could be measured with an electronic hand calliper and the juice extracted by hand with a Pineware CS2 citrus juicer. Total soluble solids were determined with a temperature-compensating digital refractometer (Palette PR-101) and titratable acidity by titration of a 10 ml aliquot of juice using 0.1N NaOH to an endpoint with phenolphthalein as an indicator (Wardowski *et al.*, 1979).

#### Yield

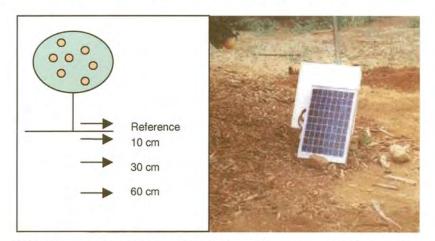
Individual tree yields were taken at the time of non-selective commercial picking at the beginning of October 2000. Yield was determined in kilogram per tree replica for each separate treatment.

## Experimental design and statistical analyses

Data for each study was statistically analysed as a complete randomised block design with eight treatments and four, six-tree replicates. The data was tested for normality by a Proc Universate and variance analysis was performed using the GLM procedure of SAS (Statistical Analysis System) computer program (Anon, 1989). Means were compared according to a protected Fisher test at a 1 and 5% level of significance.



**Figure 4.1** Applied treatments as discussed in Materials and methods. Bare soil as the control (a), white Silex plastic (b), and bark chips (c). Other treatments not represented in this figure were all applied within the drip line of tree canopies.



**Figure 4.2** Representation of soil temperatures monitored during late May and June 2000 in Orchard 1. Temperature at soil surface and depths of 10, 30 and 60 cm were measured according to the modified method of Richardson and Mooney (1992). A Campbell Scientific datalogger was used to store data at hourly intervals.

#### Results

#### Orchard 1

There were no definite trends over sample dates with regard to the different treatments. Diameter, rind thickness, fresh weight, juice weight and juice percentage, were non-significant between applied treatments for the first four sample dates (Table 4.1). For the fifth and sixth sample dates, there were significant differences found between treatments with regard to the physical

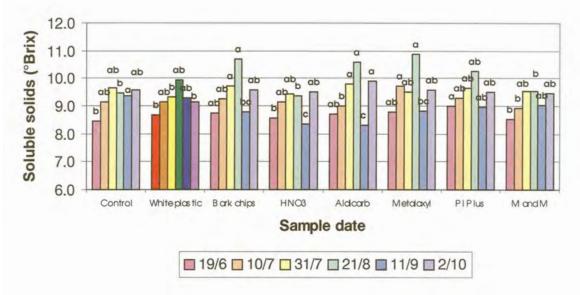
characteristics. Total soluble solids, titratable acid and the solid to acid ratio were most significant during sample date three through five. For the purposes of this study the results and discussion will be limited to the chemical characteristics and only where of significance will physical parameters be discussed.

Soluble solids between treatments were significant only for the first (19/6), fourth (21/8) and fifth (11/9) sample dates (Figure 4.3). Fruit from the first sample date differed significantly only between the control and PI Plus treatments with the latter indicating higher soluble solid values. During the fourth sampling, fruit from the bark mulch, aldicarb and metalaxyl treatments were significantly higher when compared to the fruit from control trees. At this time control fruit were second lowest only to fruit from the pH treatment. Fruit from the fifth sampling were highly significant between treatments. Soluble solids were lowest for trees treated with nitric acid and aldicarb. Control fruit indicated the highest soluble solids with significantly lower differences for the bark mulch and metalaxyl treatments respectively. By harvest no significant differences were found among treatments.

**Table 4.1** Significant differences between sample dates within treatments with regard to physical characteristics and chemical compositions analysed during the 2000 season. (P < 0.05 for significant and P < 0.01 for highly significant differences).

Sample No.	1	2	3	4	5	6
Sample Date	19/6	10/7	31/7	21/8	11/9	2/10
Diameter (mm)	ns	ns	ns	ns	*	*
Rind thickness (mm)	ns	ns	ns	ns	*	**
Fresh weight (g)	ns	ns	ns	ns	ns	*
Juice weight (g)	ns	ns	ns	ns	**	*
Percentage juice (%)	ns	ns	ns	ns	**	ns
TSS (°Brix)	*	ns	ns	*	**	ns
TA (%)	*	ns	**	*	ns	ns
Ratio TSS:TA	ns	ns	**	*	**	ns
Kg TSS/t	ns	ns	ns	ns	*	ns
Final yield (kg/tree)	.8:	4		9	2	*

The general trend of soluble solids over all treatments indicated a sharp decrease after the fourth sampling. However, when the treatments are displayed individually, the plastic mulch did not conform to this inclination (Figure 4.3). In the remaining treatments, the soluble solids increased from sample date five up to harvest with highest significant recovery from the aldicarb treatment, which was also found to have the largest decrease after the fourth sample date. In comparison, control fruit had the least decline in soluble solids after sample four and increase after sample five respectively.



**Figure 4.3** Seasonal changes in soluble solids as influenced by soil amendments during the 2000 season for 'Valencia Late' orange fruit. All treatments indicate a similar decrease in soluble solids during the fifth date of sampling in comparison to the fruit from trees to which plastic mulch was applied. Manure and molasses is referred to in the graph as 'M and M'. Means with different letters in each series differ significantly at 5% level between treatments for single sample dates.

The titratable acid decreased and solid to acid ratio increased significantly over the season for all treatments. Titratable acidity did not show a similar decline for the fifth sample as found in soluble solid, solid to acid ratio and soluble solids per metric ton. Similar to soluble solids, the titratable acid between treatments only differed significantly for three sample dates (Table 4.1). Titratable acid of fruit from the first sample date were significantly higher for the bark mulch treatment when compared to control, plastic mulch, HNO<sub>3</sub> and aldicarb treatments (results not shown). Six weeks later (31/7),

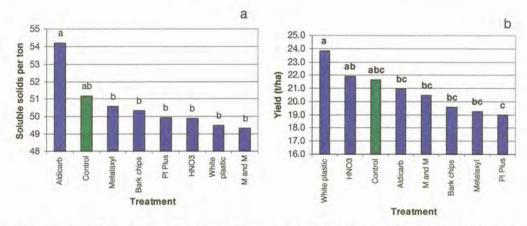
differences in titratable acid between treatments were highly significant. At this date, fruit from the bark mulch treatment were not significantly higher to control fruit, although fruit from the manure and molasses treatment was significantly lower than the bark mulch treatment. Fruit from the following sample indicated much the same trend. Bark mulch treated trees generally yielded fruit higher in titratable acid over the season although differences between treatments were not significant for all sample dates. Titratable acid in the fruit from the bark mulch treatment, when compared to the control and plastic mulch treatment had lost its significance by harvest. At harvest the titratable acid of the control and plastic mulch treatments were higher than of fruit from the bark chip treatment. This indicates that TA decreased at a faster rate in the bark mulch treatment in comparison to all the other treatments.

At the onset of sampling, no significant differences for the solid to acid ratios were found between treatments. By the third sample date (31/7), highly significant differences were found between treatments. Control fruit were significantly lower in the solid to acid ratio than fruit from the manure and molasses treatment. Fruit from the plastic mulch were significantly higher during the third sampling than the control and bark mulch treatments, which did not differ significantly from each other. Three weeks later (21/8), the difference between the later three treatments was much less pronounced. By the fifth sampling, control and plastic mulch treatments were significantly higher than that of bark mulched treatments. At this time all treatments, except fruit from the plastic mulch, were significantly lower than the control. And by harvest any significance between treatments had diminished.

Only during the fifth sampling were differences between treatments significant for soluble solids per metric ton. Control fruit were significantly higher when compared to all other treatments due to a higher juice percentage. The lowest soluble solids per metric ton were found for trees treated with HNO<sub>3</sub>. This effect diminished at harvest although values for this treatment were of the lowest (Figure 4.4a).

A significant difference was found for yield (t / ha) between treatments (Figure 4.4b). Yields were significantly lower for trees treated with Pl Plus® compared to plastic mulch and HNO<sub>3</sub> treatments. Walker and Morey (1999) found biological antagonists performed poorly in general and can even cause growth inhibition.

Soil temperatures for the period between 26/5 and 22/6 at soil level (reference temperature) and at depths of 10, 30 and 60 cm is shown in Table 4.2. The reference temperature for the white plastic was higher than the bare soil. Soil temperatures at 10, 30 and 60 cm were lower for the white plastic treatment, confirming the findings of Richardson and Mooney (1992) who found the same results for reflective foil.



**Figure 4.4** Effect of soil amendments on soluble solids per ton (a) and yield (b) of 'Valencia' orange fruit. Manure and molasses is referred to as 'M and M' in the graph. Means with different letters differ significantly at 5% level.

Three-weekly measurements of fruit diameter showed that fruit from the plastic mulched plots were slightly larger throughout the season compared to control fruit. However, although average fruit weight (g) and yield (kg / tree) at harvest was higher on plastic mulched trees, these differences were not statistically significant (Table 4.3), which confirmed results found by Richardson and Mooney (1992) on 'Satsuma' mandarins who found similar results.

**Table 4.2** Average soil temperatures at soil surface, 10, 30 and 60 cm soil depth during late May and June 2000 in Orchard 1.

Zone	Bare soil (°C)	White plastic
Reference temperature	12.59	12.91
Temperature at 10 cm	16.55	15.14
Temperature at 30 cm	16.26	15.78
Temperature at 60 cm	17.08	16.59

**Table 4.3** Yield, average fruit weight at harvest in 2000 for 'Valencia' fruit from control, plastic and organic mulched trees (as part of eight treatments).

Treatment	Yield (kg/tree)	Average fruit weight (g)	
Bare earth	72.2 abc	181.6 abc	
White polyethylene	79.4 a	194.0 a	
Bark chips	65.3 bc	173.6 bc	

Fruit samples collected during the 2001 season (21/8 and 2/10) did not indicate significant differences between mulched treatments for any of the measured parameters (fruit diameter and weight, juice percentage, rind thickness, total soluble solids and titratable acidity, solid to acid ratio and soluble solids per metric ton) although similar trends were found.

#### Orchard 2

During the first two sample dates no significant differences were found for the measured parameters among any of the treatments. Fruit diameter indicated no differences between treatments throughout the duration of the study and was only significant between dates of the 2000 season. Rind thickness differed between treatments for sample dates 31/7, 11/9 and at harvest, but not over time. Fresh weight of fruit differed only for the third sample date (31/7), and juice weight for the third (31/7) and fifth (11/9) sample dates. Fresh weight, juice weight and juice percentage increased significantly over

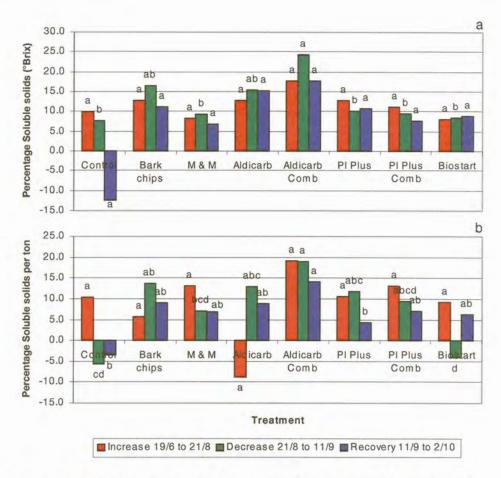
the duration of the trail period. For the two samples taken during the 2001 season, no significant differences were found fruit diameter and weight, juice percentage, rind thickness, total soluble solids and titratable acidity, solid to acid ratio and soluble solids per metric ton among control and mulched trees.

Neither titratable acidity nor the ratio of soluble solid content to acid were significantly different at harvest. For all treatments there were significant decreases in acid content and increases in the ratio over the sampling period. The chemical characteristics (soluble solids, soluble solid to acid ratio and soluble solids per metric ton) indicated differences for the fifth sampling date corresponding with the results in the previous section. As there were no significant differences noted among the treatments for any of the sample dates, excluding the fifth sample date, the percentage differences in increase over the first four samples, decreased after the fourth sample, and recovery after the fifth sample, were determined for total soluble solids and soluble solids per metric ton. No significant differences were found among treatments for percentages in increase and recovery.

Soluble solids in fruit from trees treated with PI Plus® and combination thereof indicated slightly higher increases over the control. Bark chips and aldicarb were similar in their percentage increase and decrease in soluble solids, while the Biotstart® and manure and molasses treatments were less than the control. The general trend among treatments indicated that a corresponding percentage decrease and recovery accompanied a high percentage increase in soluble solids.

However, percentages for total soluble solids (Figure 4.5a) and soluble solids per metric ton (Figure 4.5b) indicated an increase over the first four sample dates over the treatments. Fruit from the aldicarb combination treatment yielded the highest percentages increase, decrease and recovery for soluble solids per fruit and per metric ton. The soluble solids per metric ton indicated a decrease in fruit of trees treated with aldicarb alone (Figure 4.5b) for the first four sample dates due to the significantly lower juice percentage during the fourth sample. Recoveries in soluble solids per fruit were highest for fruit of

trees treated with aldicarb as single application and in combination treatment. In all treatments a positive recovery was noted after the fifth sampling except in the control. Fruit from control trees indicated a increase of solids followed a decrease, without the occurrence of a significant decrease in soluble solids for the fifth sample date.

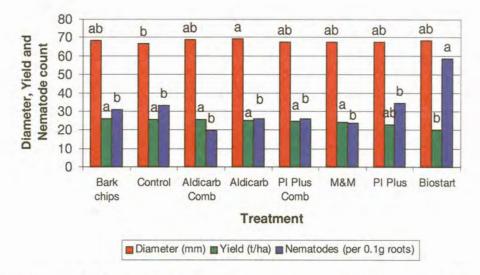


**Figure 4.5** Comparison of the percentage increase of soluble solids (a) and soluble solids per metric ton (b) over the first four samples, decrease after the fourth sample and recovery after the fifth sample. Means with different letters in each series differ significantly at 5% level among treatments.

Fruit from the aldicarb combination treatment indicated a 9% increase above that of control fruit for soluble solids per metric ton over the first four sample dates. In Figure 4.5a and 4.5b, percentages for the aldicarb combination treatment stand out above the control and either of the single application treatments included in the aldicarb combination treatment. As found for the soluble solid content in fruit from control trees, the soluble solid content per

metric ton did not follow the general trend found for fruit in the other treatments.

There was no significant difference between the yield for treatments in 2000, except of trees treated with Biostart®, which were significantly lower than control trees (Figure 4.6). Trees treated with bark chips had the highest yield with a nematode count slightly higher than in the control although not significant. Fruit were larger from trees treated with aldicarb for the 2000 season.



**Figure 4.6** Comparison between the size of fruit (mm), yield (t/ha) and nematode count (per 0.1 g roots) per treatment. Treatments are arranged in the graph according to decreasing yield. Means with different letters in each series differ significantly at 5% level.

Nematode counts on root samples taken at harvest were highest in the Biostart® treatment followed by the PI Plus® combination and bark chip treatment. For both samplings at the end of July and at harvest, nematode counts for the aldicarb and aldicarb combination treatments were highly comparable for both mean and standard deviation. High nematode counts did not seem to significantly reduce fruit size in this treatment but did appear to reduce yield.

#### Discussion

Sinclair (1961) found that as citrus fruits mature, soluble solids increase titratable acid decreases and the solid to acid ratio increases. Comparable trends were observed in the juice of fruit from all treatments in both orchards. The decrease in soluble solids for the fifth sampling coincided with prevailing weather conditions two days prior to and during sampling where windspeeds of 130 and 120 km/h were encountered, peaking at 207 km/h on the day of sampling, and relative humidities 50% lower for two days before and after.

The application of plastic mulch in Orchard 1 buffered the severity of the wind and high evaporative losses from the soil in Orchard 1 (Figure 4.3). Yakushiji et al. (1998) observed that moderate stress increased soluble solids in fruit as well as soluble solids per metric ton while severe stress lead to a decrease in soluble solids. Possibly suggesting in part an explanation for results found in this study for the sudden decrease in soluble solids during the fifth sampling.

Besides the plastic layer conserving soil moisture, a second benefit was that the reflectivity of the plastic led to an increased reference temperature (Table 4.3). As found by Richardson and Mooney (1992) the diurnal temperature range in the canopy of trees mulched with reflective foil increased by 4°C above that of un-mulched trees during spring and early summer. influence of temperature on source and sink regions primarily reflects the influence of temperature on the rate of translocation. That is, the effect of temperature on metabolic processes involved in the loading of sugar into the sieve tubes at the source and unloading out of the sieve tubes at the sink, primarily controls the rate of translocation (Devlin, 1975). Hence, warmer shoot temperatures compared to root temperatures, increases the translocation of photosynthates (sugars) to the aboveground sinks (Hartt, 1965). Also reflection of a high percentage of sunlight from reflective surfaces can significantly increase the canopy temperature during the day, thereby reducing the amount of energy stored in the soil which in turn delays the seasonal increase in soil temperature during spring (Richardson and Mooney, 1992). Considering the above discussion it is suggested that fruit from the

plastic mulched trees were buffered by the plastic layer and did not indicate as strong decline in soluble solids as observed in other treatments.

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However, besides the buffer effect of the reflective plastic, the results found in this study did not confirm findings by Richardson and Mooney (1992) who found significant increases in soluble solids in fruit from foil mulched trees. The reflective plastic did increase fruit size, yield (Table 4.3) and soluble solid production per hectare with soluble solids per metric ton lower than control trees, although not significantly. The higher conservation of soil moisture under the trees with plastic mulch compared to bare soil control trees may have lead to lower levels of water stress in trees compared to fruit of control trees reducing levels of soluble solids in the fruit during harvest as discussed by Yakushiji et al. (1998). Juice percentage was higher and soluble solids lower, explaining the lower kilogram soluble solids per metric ton compared to fruit from control trees. Significant reductions in soluble solids, citric acid, and suspended solids were found with increased frequency of irrigation by Cruse et al. (1982) and Yakushiji et al. (1998).

In Orchard 1 aldicarb increased soluble solids per metric ton and per hectare above control trees confirming result found by Bullock and Pelosi (1995). Although the presence of citrus nematode was confirmed in the treatment trees, aldicarb did not significantly improve measured parameters above the control in this study. According to Walker and Morey (1996) and Walker and Morey (1999), aldicarb stimulated citrus tree growth, while cadusafos plus metalaxyl was more effective than metalaxyl alone in stimulating citrus growth and preventing leaf chlorosis in soils infested with *Phytophthora nicotianae*. The effect is not a direct one. Improved root health due to a decrease in infection, leads to increased growth and productivity of citrus trees.

The health status of trees might not always be visible as specific symptoms or deficiencies, but can influence the manner in which trees react to external stimuli such as stress. Wheaton et al. (1985) found trees receiving aldicarb suffered less severe freeze damage than control resulting in more rapid recovery of production even though no increase in trunk growth, flowering or

vegetative growth characteristics were observed above the control. Although there were no significant trends among treatments in Orchard 2, the fifth sample date stands out. Fruit from the aldicarb combination treatment indicated the highest percentage recovery (18%) above the control fruit after conditions of high wind velocity and evapotranspiration. Nematode values were lower in the aldicarb combination treatment indicating that the healthier trees have the ability to recover faster than trees infested with higher population densities of citrus nematodes. This also indicates that applications of bark chips, manure and molasses, and aldicarb did not have the same potential as when used in combination with each other. Thus, a healthier tree poses the ability to accumulate higher percentages of soluble solids in their fruit over the same time period.

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In Orchard 2 juice quality was not significantly improved by the nematode treatments, as also found absent in the trials of Stansly and Rouse (1994). However, Wheaton *et al.* (1985) observed a significant increase in °Brix and solids per box in the second year of aldicarb application.

The application of nitric acid was found too diluted to create a significant effect (Orchard 1). Soil analysis did however indicate pH (water) for control and nitric acid treatments 7.03 and 6.98 during July and 6.69 and 5.68 at harvest, respectively. As the soil is a dynamic entity the decrease in soil pH (water) might be attributed to the dripper irrigation in the orchard (R. Abercrombie, 2001, ARC Institute for Tropical and Subtropical Crops, Nelspruit, South Africa).

The organic amendments in Orchard 1 did not improve measured parameters, and in some cases inhibited improvement in fruit quality in this study. Chicken manure, horse/cow manure mixture, and cow manure were found by Casale et al., (1995) to be poor substrates for growth of biocontrol agents and reduced growth and root health of avocado and citrus due to large amounts of released ammonia. Decay of bark chips with high carbon/nitrogen ratio immobilises nitrogen and results in temporary nitrogen shortages (Casale et al., 1995) possibly causing the reduction in fruit size and yield found in the

bark chip treatment found in Orchard 1. Pl Plus® applications were applied in the study as recommended by the manufacturers for conditions in banana plantations, which are very different from those, found in citrus orchards. The mulched plantations, which are shaded in most cases, provide a cool, moist environment for the micro-organisms in comparison to the citrus orchard, which receives drip irrigation in moderate amounts and where soil temperatures are much less buffered.

However, the addition of organic mulch in Orchard 2 increased fruit size, soluble solids per metric ton and per hectare and yield although nematode counts were highly comparable with the control at harvest. In both treatments of bark chips and manure and molasses in single application, nematode counts were not significantly suppressed in comparison to the addition of aldicarb and PI Plus® in combination treatments.

Previous reports indicate that aldicarb may or may not increase yield (Wheaton et al., 1985). Aldicarb may increase yield to a greater extent in trees producing well below their potential. Thus the extent of success is predetermined by the current health status of the trees involved. In Australia, aldicarb did not improve yield of 'Valencia' oranges (Harris, 1983), nor were benefits observed on 'Valencia's in an experiment in South Africa over a sixyear period where aldicarb was applied every third year (Fouche, 1977). The latter not rendering results may be due to the application amount and interval of timing. In Florida aldicarb applied for three consecutive years substantially increased third year yield at one location but had no effect at another (Wheaton et al., 1985). Because the dry weight in 'Valencia' fruit is the greatest two to four months after bloom (Phase 2 of fruit growth and development) and falls in the critical growth period, disruptions during this period are never fully recovered from (Anon, 1997). Gardner (1969) suggested the speculation surrounding the ultimate course that solid production might be determined early in the ontogeny of the fruit, to be without basis. Date of application is thus important in determining time required to observe a response. Applications in this trial may have been too late to obtain



a current season response in the first year. Earlier application dates should be evaluated in future studies over more growing seasons.

When considering yield in Orchard 2, microbial antagonists performed poorly even when used in a combination treatment. The lowest yield observed for trees treated with Biostart® was also the treatment with the highest nematode count confirming results found by Heald and O'Bannon (1994), Al-Qasem and Abu-Gharbieh (1995), Noling (1997) and Westerdahl (1997), due to a deteriorated root system. At harvest, even though fruit from the Biostart® treatment yielded of the highest solids per metric ton, it had the lowest solid production per hectare for all treatments.

Applications in this study may have been too late to obtain a current season response in the first year. The timing of applications is very important due to the dry weight accumulation in 'Valencia' fruit being the greatest two to four months after bloom (Phase 2 of fruit growth and development) and falls in the critical growth period (November to June). Disruptions during this period are never fully recovered from (Anon, 1997). Gardner (1969) who found that citrus fruit were characterised by the rootstock on which they completed their maturation opposed that the ultimate course of solids production might be determined early in the ontogeny of citrus fruit. Date of application remains important in determining time required to observe a response. Earlier application dates should be evaluated in future studies to coincide with main root growth flushes (August to November) and over longer periods. Also, the 2000 season was characterised by high rainfall, it would thus have been expected to observe greater differences between treatments during the 2001 season.

The highest soluble solids per metric ton in all treatments were obtained during the end of July and the beginning of October 2000. The late harvest did not significantly decrease the solids per ton but can have a negative effect on tree carbohydrate reserves and therefore inducing alternate bearing. In the longer term it would be a better option to harvest earlier in the season under conducive weather conditions.

The fact is that picking dates should be determined separately for fresh consumption and for processing, according to their particular needs as far as fruit quality is concerned (Tzur, 1992). Results obtained suggest that the highest soluble solids and juice percentages do not fall on the same dates. Results further indicated the strong influence of climatic conditions on the internal fruit quality and would suggest a more detailed study to confirm these findings. The lack of significant results in this study during the first year of application should not cast a shadow as to the possible effectiveness thereof. More attention should have been paid to chemical soil analyses as dominant soil factors can influence treatment effectiveness. According to Le Roux (1995), nutritional imbalances, which occur in citrus trees infested for many years with nematodes, may not be rectified immediately when the nematodes are eliminated. Thus the greater the damage to the trees, the longer it takes to recover.

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