SOME ASPECTS OF COLD STORAGE OF 'FUERTE' AVOCADOS (*Persea americana* Mill.) GROWN IN THE NATAL MIDLANDS

By

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ABSTRACT

The South African avocado industry is largely export orientated and export by sea to European markets necessitates cold storage for up to 4 weeks at temperatures around 5.5°C. Avocado fruit is subject to chilling injury which is manifested as mesocarp discolouration, and pitting and blackening of the rind. Of the South African cultivars exported, 'Fuerte' is the most susceptible to chilling injury, and accounts for >50% of avocado exports.

A number of temperature regimes where temperature was reduced in a step-wise fashion from 8.5 or 7.5°C to 4.5 or 5.5°C during 3 to 5 weeks of storage were tested weekly throughout the 1993 and 1994 'Fuerte' harvesting seasons in the Natal Midlands (a cool mesic subtropical area), in attempt to find cold storage temperature regimes which would minimise chilling injury. No definite trends with regard to certain temperature regimes resulting in fruit with less chilling injury were evident. Overall, stepped down temperature regimes produced fruit of quality no better than storage for 5.5°C for 4 weeks. There was no significant difference in concentration of total phenolics in 'Fuerte' fruit mesocarp throughout the 1994 harvesting season ($P < 0.05$). Levels of ethylene evolution during 4 weeks of storage at 7.5 and 5.5°C ranged from 0 to 5 $\mu l.kg^{-1}.h^{-1}$, and peaked at 109 and 75 $\mu l.kg^{-1}.h^{-1}$ in fruit stored at 7.5 and 5.5°C respectively at room temperature on removal from cold storage. Rapid moisture removal from 'Fuerte' fruit after harvest and before cold storage by placing the fruit in glass jars to which a suction of -75 kPa was applied, resulted in increased susceptibility to external chilling injury, the severity of which was proportional to the amount of moisture removed from the fruit.

Pre-storage heat treatments with a view to decreasing sensitivity of fruit to cold storage were carried out on 'Fuerte' fruit. Dry heat and warm water baths at temperatures of 36 to 40°C caused rind blackening of varying severity, depending on temperature and duration. Vapour heat treatments at temperatures of 36 to 48°C for 10 min to 48 h also caused rind blackening, with the exception of 10 min at 48°C and 1.5 and 3 h at 40°C which produced fruit of higher overall quality after 4 weeks of cold storage at 3.5°C than fruit not heat treated. These treatments however, could not be repeated in 1994 to confirm the results obtained as the harvesting season was over by the time the trial was completed.

DECLARATION

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

Signed 

(Derek John Donkin)

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INTRODUCTION

The avocado (*Persea americana*. Mill.) belongs to the family Lauraceae and has been classified into three botanical varieties or races viz. *Persea americana* var. *americana*, the lowland or "West Indian" types; *Persea americana* var. *drymifolia* - of Mexican origin; and *Persea americana* var. *guatemalensis* - of Guatemalan origin (Scora and Bergh, 1990). The following cultivars (in order of harvesting during the season) are grown for export in South Africa: 'Fuerte', 'Edranol', 'Hass', 'Pinkerton', 'Rinton' and 'Ryan'. 'Fuerte' is thought to be a natural hybrid of the Mexican and Guatemalan races and 'Hass' largely Guatemalan (Bergh, 1975).

Avocado production in South Africa is a rapidly expanding industry, which is largely export orientated. According to FAO estimates, South Africa was the 11th largest avocado producer in the world in 1993 with a crop of 47 000 t (Anon., 1994), of which 26 000 t were exported. In 1994, 8.8 million cartons ($\pm$ 35 000 t) with a value of just over R 100 million were exported (Suter, 1994[1]), and in the year 2000 a figure of 20 million cartons is expected (Toerien, 1994).

Transport by sea to European markets necessitates cold storage for 21 to 28 days which may result in the occurrence of chilling injury typified by symptoms of mesocarp discolouration, and external blackening known as cold damage (Swarts, 1984). 'Fuerte' is known to be the cultivar most susceptible to cold storage disorders (Vorster *et al.*, 1990), and made up 56% of South African exports in 1994 (Suter, 1994[1]). Controlled atmosphere storage with oxygen and carbon dioxide concentrations of 2% and 10% respectively have been found to reduce postharvest disorders and low temperature sensitivity in avocados (Eksteen and Truter, 1985). However, controlled atmosphere storage requires special gas-tight containers and carefully monitored gas levels and such containers are not available for avocado shipping at an economical tariff (Bower and Cutting, 1988).

Suter, B. 1994. Pers. Comm., S. Afr. Avocado Grow. Assoc., Tzaneen.

The standard temperature for export of South African avocados has traditionally been 5.5°C, but in recent years, South African researchers have experimented with variable storage temperatures based on seasonal differences and fruit maturity in an attempt to reduce the incidence of internal and external physiological disorders associated with cold storage of avocados, and have developed "stepped down" temperature regimes for avocado export, where cold storage is commenced at 8.5 or 7.5° C and is reduced during the storage period of 3 to 4 weeks in steps of 1 or 2 ° C at 5 to 7 day intervals to 5.5 or 4.5°C, depending on fruit maturity, as determined by fruit oil content. Lower initial and final temperatures are used when storing more mature fruit, as chilling sensitivity decreases with increasing maturity. Research in this area has been conducted mainly in the Northern Transvaal production area.

The development of an export based avocado industry in Natal is fairly recent. Export avocados grown in Natal reach maturity approximately two months later than the same cultivars grown in the Northern and Eastern Transvaal. This is due due to generally cooler growing conditions as a result of greater lattitude (± 30°S as opposed to 23 to 25°S). Temperature regimes developed for cold storage of Transvaal avocados may not be as effective for Natal fruit of similar maturity. For this reason a number of different temperature regimes for 'Fuerte' fruit were tested in the 1993 and 1994 harvesting seasons in co-operation with Everdon Estate, Howick, in an attempt to find temperature regimes suitable for cold storage of this cultivar at different stages of maturity.

The rationale behind reducing temperatures during cold storage in order to reduce the incidence of cold storage disorders was originally based on the fact that avocado fruit are most sensitive to low temperatures during the climacteric rise in respiration and least sensitive in the post-climacteric stage (Kosiyachinda and Young, 1976). Bezuidenhout (1983b) developed a model which supposedly determined that the majority of export avocado fruit had undergone their climacteric rise in respiration in cold storage during the sea voyage to Europe, and suggested that higher storage temperatures be used until the fruit had undergone its climacteric cycle and lower temperatures thereafter, in order to reduce the incidence of cold storage disorders. Although it seemed unlikely that the climacteric cycle occurs during cold storage, there is still a common industry perception that this does occur,

and consequently levels of ethylene evolution of 'Fuerte' fruit were measured during- and after cold storage to determine when the climacteric peak occurs and if a possible change in fruit sensitivity to low temperatures during cold storage was marked by a fluctuation in ethylene production.

Browning reactions causing mesocarp discolouration in avocados are a result of the oxidation of o-diphenols to o-quinones by the enzyme polyphenol oxidase (PPO), which in turn are oxidised to brown melanin pigments (Bower and Cutting, 1988) . Golan *et al.* (1977) found a positive correlation between browning tendency and total phenolic concentration in avocado fruit. Different rates of browning were correlated with the amount of PPO present in fruit tissue and/or the amount of substrate. Cutting *et al.* (1992) found that total phenolics in avocado increased with increasing maturity, as did mesocarp discolouration after cold storage. Levels of total fruit phenolics, therefore, were measured to determine whether phenolic concentration was related to the incidence of internal physiological disorders.

Pitting and blackening of the avocado rind is a symptom of chilling injury known as "cold damage" in the South African avocado industry and has been noted as one of the two most important criteria in determining fruit quality on the European market (the other being fruit firmness) (Bezuidenhout and Eksteen, 1994). Work was carried out to determine whether rapid fruit moisture loss after harvest and before cold storage had an effect on fruit sensitivity to this disorder, as the severity of rind pitting in grapefruit as a result of cold storage was found to be influenced by moisture loss during cold storage (Purvis, 1984).

With the movement away from chemical means of increasing postharvest life of fresh produce, there has been a move towards research involving physical treatments to increase postharvest life. Pre-storage heat shock treatments have been successful in reducing chilling injury in tomatoes (Lurie and Klein, 1991) and mangoes (McCollum, 1993). At the commencement of this study, very little literature was available on heat treatment of avocado fruit. Subsequent work reported that chilling injury symptoms were reduced in heat treated 'Hass' avocados but that heat treatment of 'Fuerte' caused heat damage (Florissen *et al.*, 1993). Reduction of chilling injury in 'Fuerte' fruit using heat shock treatment would be

advantageous to the industry as this cultivar is most susceptible to cold storage disorders (Vorster *et al.*, 1990). This thesis includes work attempting to find a time - temperature relationship for heat treatment of the above mentioned cultivar which may induce a certain degree of resistance to chilling temperatures.

In summary, the work carried out for the purpose of this thesis was aimed at improving the quality of cold stored 'Fuerte' avocados grown in the Natal Midlands by means of physical treatments prior to cold storage and step - wise temperature reduction during cold storage..

CHAPTER 1

LITERATURE REVIEW: POSTHARVEST PHYSIOLOGY AND SOME ASPECTS OF AVOCADO FRUIT STORAGE

Softening is almost always associated with fruit ripening, which leads to increased susceptibility to physical damage and pathological attack (Brady, 1987), which ultimately reduces the shelf life of the product. Cold storage is necessary to prevent ripening during shipping of South African Avocados to European markets but may result in physiological disorders associated with long periods of cold storage. An understanding of avocado ripening physiology is necessary in the search for possible causes of these disorders (Bower and Cutting, 1988), in order to develop pre- and postharvest practices effective in producing high quality fruit with extended shelf life. Fruit ripening in general has been recently reviewed by Brady (1987) and the biochemistry of avocado fruit ripening by Seymour and Tucker (1993).

1.1 RIPENING PHYSIOLOGY

Unlike most other fruit, the avocado does not ripen whilst still attached to the tree (Schroeder, 1953). Burg and Burg (1962) suggested that a ripening inhibitor, possibly an anion which moves from the foliage to the fruit may prevent on-tree ripening. The avocado is a climacteric fruit and ethylene consequently plays an important role in the ripening process. The inhibitor preventing on-tree ripening was postulated by Blumenfield *et al.* (1986) to inhibit ACC synthase which converts SAM (S-adenosyl-methionine) to ACC (1-aminocyclopropane-l-carboxylic acid), the precursor of ethylene in the ethylene biosynthetic pathway.

1.1.1 Structural changes

The understanding of the biochemical basis of avocado textural changes is still incomplete, but probably involves changes in cell wall structure (Seymour and Tucker, 1993). Platt-Aloia and Thomson (1981) reported that during ripening, loosening of the cell walls occurs,

followed by total cell wall degradation occurring after the climacteric peak. Swelling and vesiculation of the rough endoplasmic reticulum was observed. Although post-climacteric cell wall breakdown occurred, all the cell organelles remained intact. They concluded that ripening does not lead to a total loss of compartmentation; but that cell wall breakdown does occur with senescence.

1.1.2 Enzyme activity

Changes in cell wall structure during fruit ripening are probably brought about by changes in enzyme activities. Cellulase and polygalacturonase activities have been reported to increase during avocado ripening with a concurrent decrease in pectinesterase activity

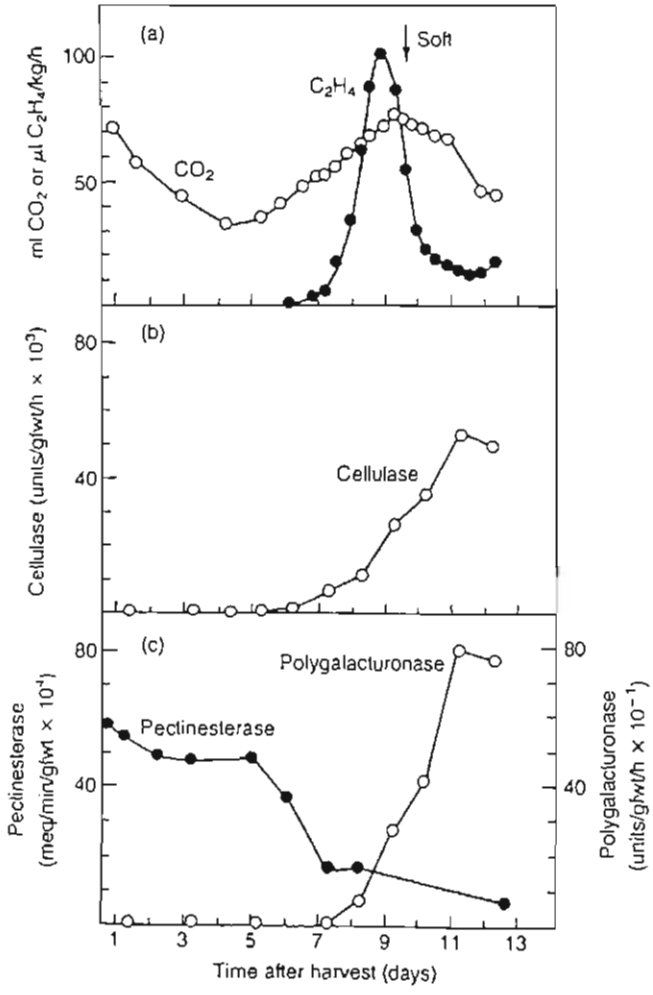

Fig. 1.1 Changes in cellulase, pectinesterase and polygalacturonase activity in relation to ethylene production and respiration in ripening 'Fuerte' avocado fruit (Awad and Young, 1979).

(Fig. 1.1) (Awad and Young, 1979). An increase in cellulase activity was observed with an increase in respiration rate and ethylene production at the respiratory climacteric, which is a plausible explanation for the total cell wall breakdown after the climacteric peak observed by Platt-Aloia and Thomson (1981). Pesis *et al.* (1972) measured an increase in cellulase activity in avocado fruit placed in an atmosphere containing ethylene gas and concluded that ethylene plays a role in controlling cellulase activity. Avocado cellulase was purified by Hatfield and Nevins (1986) and was found to be a (1-α_4)-ß-D-glucanase which hydrolysed only (1-4)-ß-glycosyl linkages, and did not appear to solubilise the cellulose polymers found in mature avocado cell walls. They concluded that the cell wall breakdown observed by Platt-Aloia and Thomson (1981) could not be explained by the activity of cellulase alone. Hatfield and Nevins (1986) proposed that xyloglucans or cellulose fibrils are hydrolysed, which would be consistent with microscopic observations of changes in cellulase fibres. Hydrogen bonding to other polysaccharides in the cell wall may be altered during ripening, disturbing the cell wall matrix and thus making the polygalacturans in the cell wall more accessible for enzymatic breakdown. This may explain why an increase in polygalacturonase activity occurs about 3 days after the first signs of an increase in cellulase activity (Awad and Young, 1979). Cellulase appears to be responsible for the early stages of fruit softening, controlled at least in part by ethylene and polygalacturonase responsible for final fruit softening (Bower and Cutting, 1988). Pectinesterase is believed to partially demethylate pectin, making it suitable for depolymerisation by polygalacturonase (Seymour and Tucker, 1993).

1.1.3 Ethylene

1.1.3.1 *Role in ripening*

Blumenfield *et al.* (1986) found that attached avocado fruits contain only trace amounts of ACC (which is the precursor of ethylene) and that an increase in ACC synthase activity after harvest leads to an accumulation of ACC. The first marked change after harvest leading to ethylene production was found to be an increase in ACC synthase activity which lead to an increase in ACC levels before the onset of the climacteric rise in ethylene production. Attached fruits had very low levels of ACC synthase which produced small amounts of ACC which was in turn converted to low levels of ethylene or MACC (1-

malonylaminocyclopropane-1-carboxylic acid). They hypothesised that ACC is the limiting factor to on-tree ripening, the production of which is limited by low levels of ACC synthase. The removal of some ripening inhibitor at harvest would increase ACC synthase activity and subsequent ACC and ethylene synthesis. The ethylene produced would begin an autocatalytic process by inducing more ethylene forming enzyme (EFE) and ACC synthase activity.

In work by Starret and Laties (1990), mature early season avocado fruit pulsed with ethylene for 24 hours immediately after harvest did not show a reduction in the time taken to the onset of the respiratory climacteric. However, ethylene pulsing 24 h after harvest shortened the time to the onset of the climacteric proportionally to the concentration of the pulse. They concluded that it was not known whether the inhibitor which prevents on-tree ripening is the cause of non-sensitivity to ethylene pulsing within the first 24h after harvest. The extent to which an ethylene pulse advanced ripening was dependent on fruit maturity.

1.1.3.2 *Effect on chilling sensitivity*

Ethylene production in avocado fruit occurs in a certain temperature range. Eaks (1978) found that ethylene production decreased between 25° and 35°C and at 35°C only trace amounts were produced. Virtually no ethylene was produced at 40°C. Storage of 'Hass' at 0° or 5°C for 4-6 weeks prevented normal climacteric ethylene production after transfer to 20°C and the fruit did not ripen normally. Storage at 10°C did not inhibit the ethylene climacteric and storage at 0 and 5°C for only 2 weeks only slightly reduced the magnitude of the ethylene peak on transfer to 20°C after storage (Eaks, 1983). Baile (1941) was unable to detect a climacteric rise in 'Fuerte' fruit stored at 4-5°C for 5 weeks. In 'Pinkerton' fruit, no ethylene evolution was detected during a cold storage period of 16 days (Fuchs *et al.*, 1986).

In 'Hass' and 'Fuerte' fruit, chilling sensitivity is dependent on the stage of the ethylene climacteric. Fruit at the climacteric rise are less sensitive than those at the climacteric peak. Post-climacteric fruit are least sensitive to chilling. It was proposed that the periods of sensitivity were correlated with periods of high metabolic activity. The rate of respiration is highest at the climacteric peak where pools of intermediates are likely to be at a maximum.

This points to chilling injury being caused by a change in the activity of regulatory enzymes at low temperatures allowing, intermediates to accumulate to levels which become toxic to the cells (Kosiyachinda and Young, 1976). Lee and Young (1984) found that 'Fuerte' fruit stored in an atmosphere containing 100 $\mu l.\ l^{-1}$ ethylene were more chilling sensitive, resulting in mesocarp discolouration at temperatures below 12°C. At 6°C and 9°C the ethylene did not induce a climacteric peak of respiration during 25 days of storage. In the light of this work, the increased sensitivity to chilling temperatures at the climacteric peak described by Kosiyachinda and Young (1976) may therefore be partly due to the presence of ethylene in the storage atmosphere.

1.2 PHYSIOLOGICAL DISORDERS

1.2.1 Chilling injury

"Chilling injury is the permanent or irreversible physiological damage to plant tissues, cells or organs, which results from the exposure of plants to temperatures below some critical threshold for that species or tissue. A chilling temperature is any temperature below the critical threshold temperature (but above freezing) that causes injury" (Lyons and Breidenbach, 1987).

The nature and severity of chilling injury symptoms are a function of species, cultivar, part of the plant and its maturity, severity and duration of exposure to chilling temperatures, ambient environment before and after chilling, and other stresses which may be experienced by the plant tissue (Saltveit and Morris, 1990). Chilling injury of plant tissue produces no unique and easily measurable physiological or visual symptoms (Morris, 1982) and often accentuates senescence or decay and the rate of water loss from affected fruit. Symptoms similar to those of chilling injury may occur which are not chilling-related (Saltveit and Morris, 1990). Chilling injury symptoms in avocado fruit include pitting and blackening of the exocarp, discolouration of the mesocarp and failure to ripen normally (Couey, 1982).

A number of physiological and biochemical responses have been observed in plant cells as a result of chilling temperatures. There is confusion and controversy however, as to whether

these are i) Primary responses to chilling temperatures, ii) part of the primary response, or iii) secondary responses to the initial event. These responses include changes in membrane structure and function, cessation of cytoplasmic streaming, alterations in respiration rates and patterns, changes in ethylene synthesis and many biochemical and compositional changes (Morris, 1982). A number of possible primary events have been put forward, e.g. a change in membrane lipid structure (Raison, 1974), or a conformational change in some structural proteins or regulating enzymes (Graham and Patterson, 1982) or cytoskeletal changes in the cell (Patterson *et al.*, 1979). The primary event is thought to occur instantaneously as the plant reaches its critical chilling temperature and is reversible if the chilling temperature is of short duration (Raison and Lyons, 1986).

Avocado fruit may not display chilling injury symptoms while in cold storage, but symptoms may develop once the fruit is allowed to ripen at higher temperatures (Couey, 1982).

1.2.2 Mesocarp discolouration

Mesocarp discolouration can be a limiting factor in the marketing of cold stored avocados, the severity of which may range from a light grey discolouration at the distal part of the fruit to a blackening of the entire mesocarp. Mesocarp discolouration is not necessarily accompanied by a browning of the vascular strands which may occur without mesocarp discolouration. Although mesocarp discolouration is generally indicative of chilling injury, it may occur in fruits which have not been cold stored (Vakis, 1982). Swarts (1984) classified the two commonly occurring forms of mesocarp discolouration as i) Grey pulp, being a general grey discolouration or browning of the mesocarp or ii) Pulp spot, being localised spherical grey spots in the mesocarp associated with the cut ends of vascular bundles. These different forms of the disorder are independent but they may both occur in the same fruit.

Browning reactions in fruit are a result of the oxidation of o-diphenols to o-quinones by the enzyme polyphenoloxidase (PPO). The o-quinones are irreversibly oxidised to melanin pigments which are brown in colour (Bower and Cutting, 1988).

1.2.2.1 *Phenolic compounds*

Plant phenolics may be divided into two groups: i) Phenolic acids and coumarins (C_6 - C_1 C_6 - C_5 structures) and ii) flavonoid compounds, including anthocyanins (C_6 - C_3 - C_6 structures). Phenolic acids have a benzene ring, a carboxylic acid and one or more phenyl hydroxyl groups that may become methylated to produce methoxy groups. Phenolic acids are precursors of many other plant compounds and are generally found in the cell vacuole or in special tissues. If the integrity of the cell membranes is upset or they are ruptured, the phenolic acids are oxidised atmospherically or enzymatically to quinones which are polymerised to produce a brown colour (Torres *et al.*, 1987).

Golan *et al.* (1977) found a positive correlation between browning tendency and total phenolic concentration in avocado fruit. Different rates of browning were correlated with the amount of PPO present in fruit tissue and/or the amount of substrate. Cutting *et al.* (1992) found that total phenolics in avocado increased with increasing maturity, as did mesocarp discolouration after cold storage.

1.2.2.2 *Polyphenol oxidase*

Polyphenol oxidase (PPO) is also known as catechol oxidase or reductase, or o-diphenol oxygen oxidaseductase (EC 1.14.18.1). In healthy green tissue, PPO exists in a latent form in the membranes of the chloroplasts as well as in rudimentary thylakoids and in leucoplasts, plastids and amyloplasts (Vaughn and Duke, 1984). Engelbrecht (1982) found the chloroplasts to be the major sites of PPO in avocado. Microbodies associated with the chloroplasts have also been reported to be important sites of PPO in the avocado (Sharon and Kahn, 1979).

PPO either exists in a bound or a latent form. The bound form is attached to membrane such as the thylakoids and the latent form is immediately available for reaction in the presence of a substrate and oxygen. For PPO to be activated, it appears that some form of cellular damage is necessary with subsequent removal of suppression and release from membranes (Kahn, 1977). Vaughn and Duke (1984) suggested that membrane structure and functioning

may be a means of activation. Bower and Cutting (1988) continued in a similar vein saying that for fruit browning to occur, PPO, oxygen and substrate must come into contact, which implies some sort of cellular disruption and that factors causing cellular disruption are important.

Kahn (1975) found that avocado cultivars which were more susceptible to mesocarp discolouration had higher PPO activity than those less susceptible. PPO activity has been used as a measure of avocado mesocarp browning potential in a number of experiments attempting to determine the cause of this disorder e.g. postharvest water stress and storage container ventilation (Bower and van Lelyveld, 1985) and moisture loss during cold storage (Bower and Cutting, 1987).

1.3 CALCIUM AND FRUIT QUALITY

Many of the physiological disorders occurring in fruit and vegetables are related to the calcium content of the tissues (Bangerth, 1974).

1.3.1 Uptake and transport

Most of the calcium taken up by the roots is transported by mass flow in the xylem. The concentration of intracellular calcium must be maintained at between 10^{-5} and 10^{-8}M to prevent interference with cellular functions. Very little calcium therefore moves in the living cells of the phloem because it is actively excluded. In contrast K^+ intracellular concentrations may range between 20 and 100 x 10^{-3}M (Clarkson, 1984). Calcium deficiency disorders are believed to be due to inefficient distribution of calcium rather than poor calcium uptake, and are restricted to organs and tissues which have low transpiration rates and a high demand for assimilates. Polar transport of indoleacetic acid (IAA) from an organ is also thought to affect calcium transport (Bangerth, 1979). Physiologically active organs such as developing shoots and fruits show greater IAA export and therefore increased calcium accumulation. Because of their greater physiological activity and transpiration rate, leaves and shoots are stronger sinks for calcium than fruit (Witney *et al.*, 1990).

Water stress or irregular water supply has been shown to increase the incidence of Ca deficiency disorders in tomatoes. Spray applications of TIBA (2,3,5 - tribenzoic acid), which inhibits auxin transport increased the incidence of calcium-related physiological disorders in apples (bitter pit) and blossom end rot in tomatoes, as well as decreasing the calcium content of the fruit (Bangerth, 1973).

1.3.2 Cell membranes

A cell membrane is a semi-permeable barrier formed by proteins and lipids. A central feature of the fluid mosaic model proposed by Singer and Nicholson (1972) is membrane fluidity. The degree of fluidity can be modulated by the activity of membrane-bound enzymes (Quinn and Williams, 1978) as well as by temperature and length and degree of saturation of the fatty acid chains (Raison, 1980). Other agents including calcium can also alter membrane fluidity. Divalent cations increase fluidity and monovalent cations decrease fluidity (Chapman, 1983). Borochov *et al.* (1982) found that membrane viscosity in rose petals increased with age due to a decrease in phospholipid content. Paliyath *et al.* (1984) found the same to be true in senescing apples and that calcium reduced the increase in membrane viscosity associated with senescence.

The cell membrane is made up of both neutral and polar phospholipids. Negatively charged phospholipids such as phosphatidyl serine are binding sites for calcium (Duzgunes and Papahajapoulis, 1983). Other cations, depending on their concentrations can displace calcium without a drastic change in membrane permeability (van Stevenick, 1965), however, in most plant tissues, only the concentrations of K, Mg and H are such that they are potentially antagonistic to calcium (Bangerth, 1974). On replacing calcium, these ions greatly increase permeability (Bangerth, 1979).

1.3.3 Cell walls

Calcium is a normal constituent of the middle lamella (Conway *et al.*, 1992). Calcium ions bind to the pectins in the middle lamella of the cell wall forming cross linkages which appear to maintain cell wall structure (Bangerth, 1979). Pectins are composed of chains of polygalacturonic and residues with rhamnose insertions which cause marked kinks in the

chain (Preston, 1979). This bunched configuration of the polygalacturonic acid chain allows spaces for the insertion of cations (Fig. 1.2). It is possible that all these spaces can be filled with cations as binding of an ion at one site facilitates binding of the next ion (Grant *et al.*, 1973). Cation bridges between pectic acids reduce the accessibility of enzymes which cause fruit softening (Conway *et al.*, 1992).

1.3.4 Avocado fruit physiological disorders

A number of workers have linked low fruit calcium levels to mesocarp discolouration in avocados. Resnisky and Sive (1993) found that in 'Ettinger' avocados, fruit with mesocarp discolouration had calcium levels three times lower than normal fruit. Chaplin and Scott (1980) found that the distal end of the avocado fruit, which is more susceptible to mesocarp discolouration than other regions of the fruit had, a lower concentration of calcium than the proximal end.

Pulp spot (browning of the procambial cells around the vascular bundles as opposed to the more general browning of mesocarp discolouration) has been associated with lower Ca and Zn levels than in normal fruit (Vorster and Bezuidenhout, 1988).

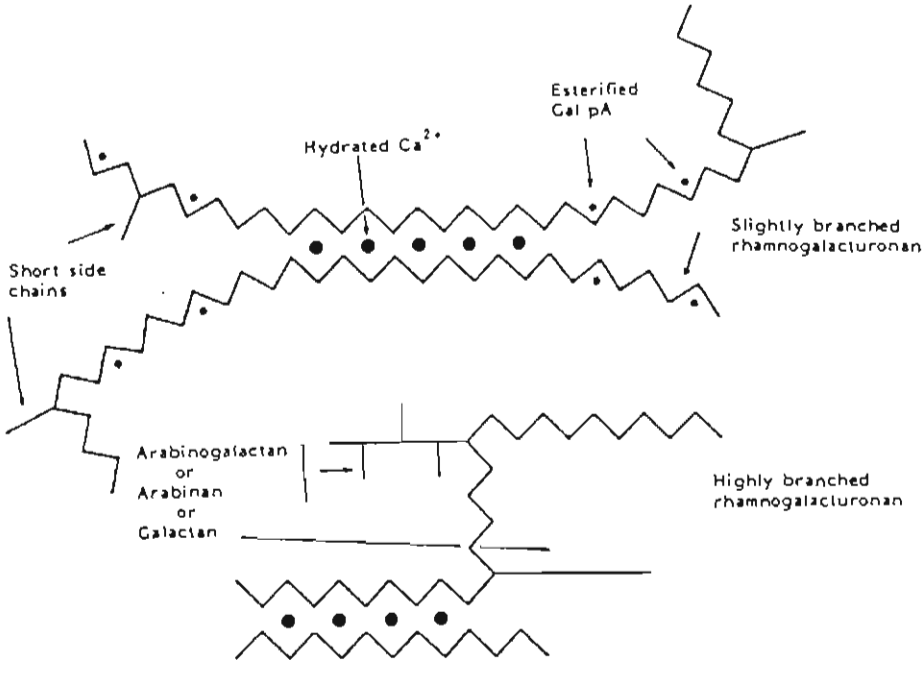

Fig. 1.2 Structural features of pectin (Selvendran, 1983).

Eaks (1985) managed to reduce the incidence of mesocarp discolouration in 'Fuerte' and 'Hass' fruit by vacuum infiltration of Ca, although the process caused exocarp browning. Ca infiltration also delayed the onset of - and decreased the respiratory rate at the climacteric. The extent to which this occurred depended on the concentration of the Ca solution. At 0,4 and 0,5M, no detectable climacteric occurred and the fruit failed to ripen.

Although the work mentioned so far suggests that mesocarp discolouration is a calcium deficiency disorder and could be controlled by increasing fruit calcium levels both before and after harvest, the following case shows that the cause of this disorder is more complex and involves resource partitioning according to the reproductive/vegetative balance of the tree. In a study of 'Fuerte' trees of two seasons, Kremmer-Köhne *et al.* (1993) noted the following: Late season fruit had a higher incidence (30-40%) of physiological disorders than early season fruit and the incidence of these disorders changed from year to year. Trees with lower yields had a higher proportion of fruits with physiological disorders. Fruit calcium content was constant over the two years (1990 and 1991), even though 1991 had a higher incidence of internal physiological disorders. Following N applications to the trees, fruit calcium levels decreased, indicating that increased vegetative vigour brought about by N application reduced fruit strength as a sink for calcium. Heavily N fertilised trees tended to be lower yielding and have a higher incidence of physiological disorders than unfertilised trees. But if K and Mg were applied with N, the incidence of physiological disorders was not increased, suggesting that mineral balance and interaction are important in determining avocado fruit quality.

Bower (1988) looked at the interacting factors affecting calcium uptake and distribution (Fig. 1.3). In avocado the early stage of fruit development, when the developing fruit is a strong sink for calcium coincides with a spring vegetative flush (Bower, 1988) which is an even stronger sink than the setting fruit (Witney *et al.*, 1990). Nitrogen fertilisation and other cultural practices should be carried out according to the phenological model of Whiley *et al.* (1988), so that the summer flush, rather than the spring flush is emphasised. Reduction in vegetative vigour was correlated with increased fruit calcium concentration by Witney, *et al.* (1990) when they showed that fruit from mildly *Phytophthora* infected trees which are less vigorous had a higher fruit calcium content.

The vegetative/reproductive balance in apples also has an effect on calcium allocation. Summer pruning reduces bitter pit (a calcium deficiency disorder) in apples and reduces vegetative growth. Heavy winter pruning encourages vegetative growth and increases the likelihood of bitter pit the following season. Increased vigour as a result of heavy winter pruning results in a high leaf to fruit ratio, making the leaves more competitive for water and those nutrients which are essential to prevent bitter pit occurring in the fruit (Preston and Perring, 1974).

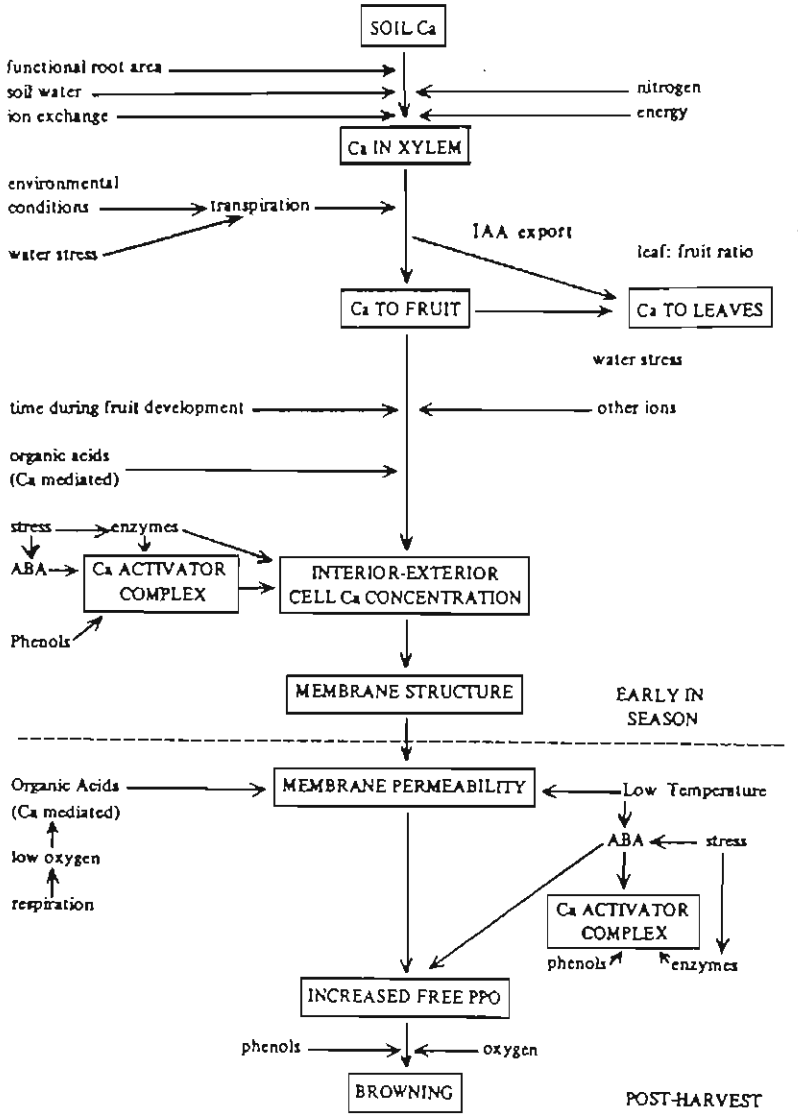

Fig. 1.3 Schematic representation of possible interactions between calcium and other plant and environment factors in the determination of fruit quality (Bower, 1988).

In avocado, chemical "pruning" of the spring vegetative flush using paclobutrazol foliar sprays increased initial fruit set, but enhanced summer fruit drop, thus nullifying the effect of increased fruit set on yield (Cutting and Bower, 1990; Wolstenholme *et al.*, 1990). However, Whiley *et al.* (1991) showed that accumulative yield over three seasons was significantly greater (ca. 63 %) in paclobutrazol treated trees. Fruit P, K, Ca and Mg levels were also enhanced (Cutting and Bower, 1990). This indicates that control of vegetative vigour is beneficial in enhancing post harvest quality in avocados, however problems such as increased fruit sunburn due to a lack of protection by new leaves (Cutting and Bower, 1990) have to be overcome.

1.4 MOISTURE LOSS AND POSTHARVEST QUALITY

A harvested fruit is a living commodity and as such, respiration and associated metabolic activity continues even when the fruit is detached from the tree. Water lost from the fruit after harvest is not replaced, unlike fruit still attached to the tree and consequently postharvest conditions should be such that moisture loss is minimised.

1.4.1 Cooling and cold storage

The aim of cold storage is to reduce the rate of metabolism of the stored commodity in order to prolong its postharvest life. The temperature and duration is ultimately determined by the interaction between natural deteriortion and decay, as well as the susceptibility of the product to chilling injury (Wills *et al.*, 1989).

Cooling and cold storage must be considered as separate operations. The refrigeration capacity required for cooling is far greater than that required for cold storage. For example it takes about 100 times more refrigeration capacity to cool pears for 24h than to store them for 24h. Increased cooling time decreases the required refrigeration capacity. Most fruit benefit from rapid cooling after harvest although some fruits cold store better if they are left at ambient temperature for a while e.g. South African freestone peaches are held at ambient temperature for $\pm$ 36h before cooling, which delays the onset of chilling injury (Mitchell, 1992).

During cooling, air speed over the product does not significantly increase water loss. However, during subsequent cold storage, high air speeds desiccate horticultural products (Mitchell, 1992). This is due to the constant removal of the thin layer of air around the produce which is near saturation point and consequently has a low evaporative demand. The moisture removed in this boundary layer is replaced with moisture from the fruit (Wills *et al.*, 1989). If the difference in temperature between the return and delivery air of a cooling system is $> 1\,^{\circ}C$ the evaporative demand of the air will be greatly increased, increasing the rate of water loss from the product. To reduce the difference in temperature between delivery and return air, air velocity should be increased to increase the volume of air available to take up heat from the fruit, which will result in a smaller increase in temperature (Woods, 1990).

Relative humidity (RH) is more important in cold storage than during cooling (Mitchell, 1992). At constant temperature, mass loss has a straight line correlation with temperature in the upper range (RH > 75%) (Grierson and Wardowski, 1978). Fruit is generally stored at a RH of 90% (Wills *et al.*, 1989).

1.4.2 Cooling methods

Room cooling, forced air cooling and hydrocooling are used for a number of commodities. Some commodities can be cooled by a number of methods, but most respond best to one or two of these methods. The time taken for initial cooling of fruit to storage temperature is important. The longer the cooling time, the more moisture is lost (in systems using cold air). Forced air cooling is therefore less desiccating in produce with a cuticle (Fockens and Meffert, 1972).

1.4.2.1 *Room cooling*

This method is widely used and is used in the South African avocado industry. Cold air is blown across the top of the produce in the room and then moves back past the fruit to the fan, with some moving through the containers at low velocity. The advantage of this type of cooling is that fruit can be cooled and stored in the same room. The disadvantages are that cooling is too slow for most commodities, which can result in excessive water loss, and more space is required in cooling rooms than for storage alone. Well ventilated containers

can greatly increase the rate of cooling. Because greater air velocity is required in cooling rooms than for storage, commodities stored in cooling rooms lose more water during storage than those kept in ideal storage environment (Mitchell, 1992).

1.4.2.2 *Forced air cooling*

This method of cooling is efficient because air is forced through the containers which facilitates faster heat removal from the produce. Air is forced through the containers by creating a pressure gradient between opposite faces of stacks of vented containers using an exhaust fan (Fig. 1.4). The rate of cooling is controlled by adjusting the volume of air passing over the product. An increase in the length of the stack or air volume increases the cost of cooling due to increased energy requirements. High relative humidity air is required when cooling commodities sensitive to desiccation (e.g. mushrooms) with forced air (Mitchell, 1992). Slabbert and Toerien (1984) found that forced air cooling extended the shelf life of 'Fuerte' avocados compared to room cooling but increased the severity of external chilling injury . The effect of air flow rate during forced air cooling on external chilling injury was not investigated in this work and was proposed as an area of future research.

1.4.2.3 *Hydrocooling*

This form of cooling makes use of cold water to bulk cool produce before packing. Advantages of this system are a rapid rate of cooling and the avoidance of water loss. The produce is either immersed in cold water or moved through a shower system. Effective cooling depends on adequate water flow over the commodity surface. For a shower system, a flow rate of 600-1000 $l.min^{-1}$ m^{-2} is necessary. Cooling times range from 10min-60 min depending on the size of product being cooled. A disadvantage of this system is that the produce undergoes a temperature increase during packing, which takes place after cooling (Mitchell, 1992).

1.4.3 Preharvest water stress

Bower *et al.* (1989) found that avocado fruit from trees under water stress showed more browning potential (as quantified by PPO activity) than fruit from trees which were not water stressed. They suggested that preharvest water stress increased postharvest disorders partly due to a lack of calcium in the fruit during the first 17 weeks after fruit set. (fig. 1.5).

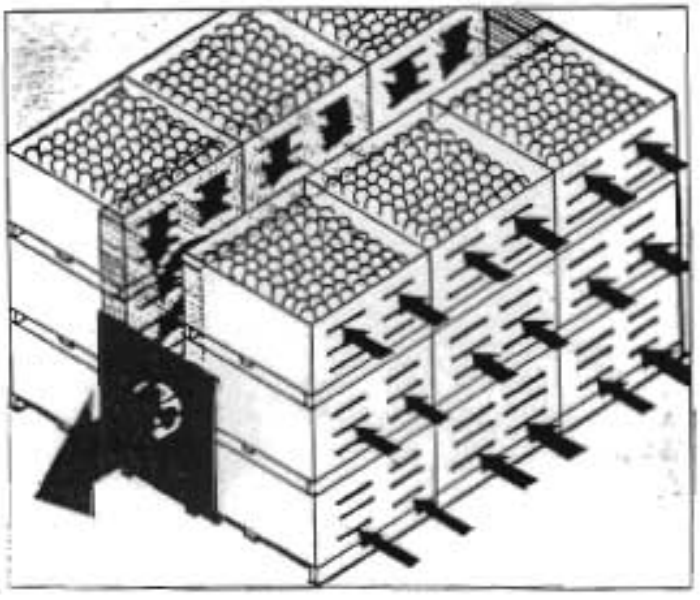

Fig. 1.4 Diagrammatic view of a forced air cooling tunnel. Containers are placed to form a tunnel from which air is exhausted. Cold air is drawn through the stack and comes into contact with the warm produce (Mitchell, 1992).

1.4.4 Postharvest water loss

Humidification of the storage atmosphere to prevent a decrease in RH during cooling and a consequent increase in moisture loss from the fruit, significantly reduced the incidence of pathological and internal physiological disorders in avocado fruit (Bower *et al.*, 1989).

The reduction of mesocarp discolouration in 'Hass' and 'Fuerte' avocados stored in sealed polyethylene bags observed by Scott and Chaplin (1978) was attributed to the modified atmosphere in the bags with CO_2 contents ranging from 3% to 7% and O_2 ranging from 2% to 6%. Storage atmospheres comprising 2% O_2 and 10% CO_2 were effective in reducing internal disorders in avocado (Spalding and Reeder, 1972). In the light of the results obtained by Bower *et al.* (1989) it would seem that a reduction in water loss during storage also played a role in mesocarp discolouration. Passive water infusion through the pedicel of avocado fruit during 28 days of cold storage at 5,5°C greatly reduced mesocarp discolouration (Cutting and Wolstenholme, 1992), further strengthening the evidence that water loss during cold storage increases susceptibility to mesocarp discolouration.

Moisture loss during cold storage also affects chilling injury in other fruits. Rind pitting is a symptom of chilling injury in grapefruit. Purvis (1985) found that wrapping Marsh grapefruit in polyethylene shrink film greatly reduced, but did not prevent rind pitting and the pattern of chilling injury response to temperature was not altered by shrink wrapping. It was concluded that moisture loss is a factor in chilling injury in grapefruit but not the primary factor. But shrink wrapping did not prevent all water loss and chilling injury severity increased with increased moisture loss. Rind pitting was found to occur in areas of the rind with the lowest diffusive resistance (Purvis, 1984). Pitting is also a symptom of chilling injury in eggplant and cucumber and is accelerated by low RH and reduced by shrink wrapping (Abe, 1990). During cold storage browning of the cells several layers below the epidermis occurs even at high RH without damage to the epidermal cells. This demonstrates that water loss is not the primary cause of pitting but aggravates the disorder (Abe *et al.*, 1974).

Moisture loss from avocado fruit after harvest and before storage is also important. Arpaia *et al.* (1992) found that the incidence and severity of mesocarp discolouration increased with increased holding time before cold storage. The higher the fruit temperature, the greater was the incidence of moderate to severe discolouration. Both holding time and fruit temperature would affect fruit moisture loss.

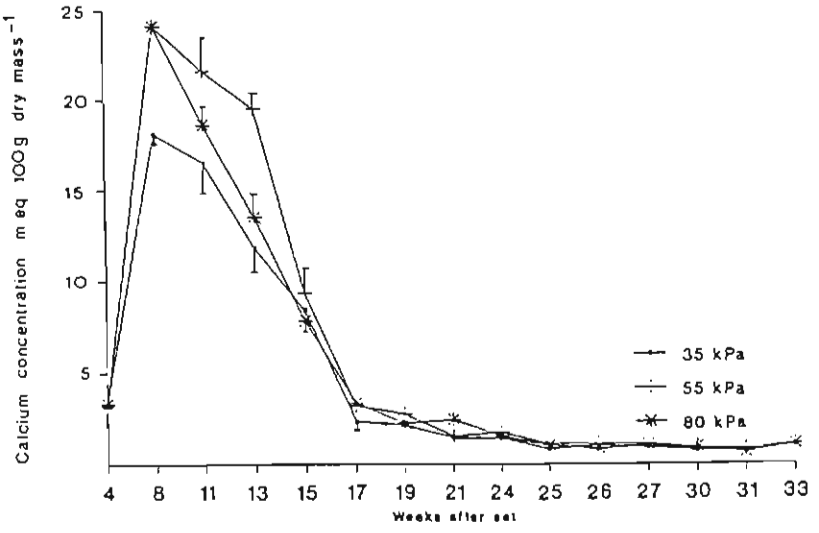

Fig. 1.5 Fruit calcium concentration changes in the distal portion of 'Fuerte' fruit from trees irrigated at different soil moisture tensions (Bower *et al.*, 1989).

1.5 COLD STORAGE OF SOUTH AFRICAN EXPORT AVOCADOS

Effective cold storage of South African export avocados is of major importance and has received considerable attention over the past decade. This has lead to the development of stepped down storage temperature regimes which are used in the industry today.

Bester (1982) described the optimum storage temperature for avocados as 5.5°C +/- 0.5°C with a maximum air delivery temperature of 4.5°C which was maintained throughout the entire shipping period of up to 28 days. Swarts (1982) suggested that the storage temperature could be reduced to less than 5.5°C as the season progressed because a good negative correlation (0.802) was found between the number of hours where orchard temperatures were less than 17°C in the two days prior to cold storage and the incidence of external chilling injury (cold damage). Cooler winter weather was thought to affect the tolerance of avocados to low storage temperatures. A poor correlation between fruit maturity based on flesh moisture content and cold damage was noted.

Sensitivity to cold storage in avocados was found to be cultivar dependent. Cold stored 'Hass' fruit showed a lower incidence of physiological disorders than 'Edranol' and 'Fuerte'

(Bezuidenhout, 1983a). 'Ryan' fruit was also found to be less sensitive to cold storage than 'Fuerte' (Vorster *et al.*,1987).

The idea of reducing temperatures during cold storage to reduce chilling injury came about when Bezuidenhout (1983b) formulated the 'climacteric model' which predicted the time for the avocado fruit to reach its climacteric peak as a function of storage temperature and fruit oil content. Using this model, it was supposedly determined that most of the fruits shipped in cold storage from South Africa had undergone their climacteric cycle by the time they reached Europe. Grey pulp was said to be related to the storage temperature after the climacteric peak with higher temperatures increasing the incidence of this disorder. It was suggested that storage temperatures should be higher initially and should be reduced after the fruit had undergone its climacteric cycle. The rationale behind this was deduced from work by Kosiyachinda and Young (1976) which showed that 'Fuerte' and 'Hass' avocados were most susceptible to chilling injury at the climacteric peak and least sensitive after the completion of the climacteric cycle. However, it seems unlikely that the climacteric peak did actually occur during storage at 5.5°C as Baile (1941), who was cited by Kosyachinda and Young (1976), was unable to detect a climacteric peak in 'Fuerte' fruit during 5 weeks of storage at 4 to 5°C.

Based on the climacteric model and the assumption that the climacteric peak occurs during cold storage (Bezuidenhout, 1983a), Toerien (1986) proposed a stepped down temperature regime to reduce cold damage by starting at temperatures of 7 to 8°C which were reduced in a step-wise fashion to around 3.5°C.

Vorster *et al.* (1987) observed a decrease in physiological cold storage disorders in avocados when using a higher temperature (7.5°C) during the early stages of storage and a lower temperature (3.5°C) during the later stages, compared to fruit cold stored at 5.5°C for the duration of the storage period. This was in agreement with the findings of Bezuidenhout (1983b) and Toerien (1986). A possible change in the degree of membrane lipid unsaturation which would affect membrane fluidity an hence permeability, was used by Vorster *et al.* (1987) to explain the acclimatization of the fruit to lower temperatures, which seems more plausible than the idea of a climacteric peak occurring during cold storage.

Table 1.1 Proposed storage temperatures South African 'Fuerte' avocados (Vorster *et al.*, 1990)

Fruit moisture %	Packhouse coldroom temp. (°C)	Cold truck temp (°C)	Holding store temp (°C)	Vessel temp (°C)
78.5	7.5	7.5	7.5	7.5 (last week 5.5)
77.5 - 78.5	7.5	7.5	7.5	7.5 (last week 5.5)
76.5 - 77.5	7.0	7.0	7.0	6.0 (last week 5.5)
75.5 - 76.5	6.5	6.5	6.5	6.0 (last week 5.5)
74.5 - 75.5	6.5	6.5	6.5	5.5
73.5 - 74.5	6.0	6.0	6.0	5.5
72.5 - 73.5	6.0	6.0	6.0	5.5
71.5 - 72.5	5.5	5.5	5.5	5.5
69.5 - 71.5	5.5	5.5	5.5	5.5 (last week 4.5)
< 67.5	5.5	5.5	5.5	5.5 (last week 3.5)

Cold storage temperature regimes currently used for South African 'Fuerte' fruit are based on those proposed by Vorster *et al.* (1990). Higher temperatures are used for early season fruit which is less mature and lower temperatures are used for more mature fruit. Fruit moisture content is used as a measure of maturity (Table 1.1). The climacteric model (Bezuidenhout, 1983b) was used to determine these regimes using the principal that the average time taken for avocados to arrive overseas from the Tzaneen area was 24 days, and so by using higher temperatures during the initial stages and lower temperatures during the later stages of storage, the climacteric peak would be reached after approximately 24 days, which was found to result in hard fruit after the storage period with little or no cold damage.

1.6 POSTHARVEST HEAT TREATMENT

The concern of the consumer that chemicals used to maintain postharvest quality are potentially harmful to humans has placed an emphasis on research to manage postharvest

disorders by non-chemical means (Klein and Lurie, 1992a). Prestorage heat treatment has received much attention lately with the following objectives in mind: i) To slow down ripening of climacteric fruits to enhance shelf life; ii) To reduce chilling sensitivity of tropical and subtropical crops, allowing longer storage periods at temperatures which would usually cause chilling injury; iii) To reduce rots by inactivating the pathogen or increasing host storage resistance; and iv) To control insect pests (Klein and Lurie, 1991).

Two approaches to heat treatments have been taken. Either long term - 12h to 48h at 38 to 46°C, or short term - up to 70 min at 45 to 55°C (Klein and Lurie, 1991). Methods of heat treatment include water dips (Couey, 1989), dry heat (Armstrong *et al.*, 1989) and vapour heat (air at 95-100% RH) (Mitcham and McDonald, 1993).

1.6.1 Duration and temperature

The duration and temperature of successful heat treatments differs from fruit to fruit. Tomatoes held at 38°C for 4 days (McCollum *et al.*, 1993) showed a reduction in chilling injury when placed in cold storage as opposed to control fruit which was not heat treated. Klein and Lurie (1992a) reported that avocados respond well to treatment at 36°C but develop heat injury at 38°C. Heat injury in avocados typified by surface browning and failure to ripen normally was reported by Kerbel *et al.* (1987) after heat treatment of 'Fuerte' avocados at 43°C for 3.5h to 12h. Similar results were obtained by Florissen *et al.* (1993) for 'Fuerte' fruit but heat treatment of 'Hass' for 6 and 12 h at 38°C reduced chilling injury. In general where successful long term heat treatment has been carried out, the relative humidity has been maintained at > 85% to prevent drastic water loss (e.g. McCollum *et al.*, 1993).

In 'Anna' and 'Granny Smith' apples, prestorage heat treatment using a higher temperature of shorter duration produced similar beneficial results to a lower temperature of longer duration. Storage at 46°C for 12h and 24h before being stored at 0°C produced firmer fruit with a higher soluble solids to acid ratio and a lower incidence of superficial scald than unheated fruit. Similar results were achieved in apples stored at 38°C for 72h or 96h before cold storage (Klein and Lurie, 1992b).

1.6.2 Effect on physiological processes

1.6.2.1 *Ethylene production and respiration rate*

Many climacteric fruit produce a surge of ethylene immediately after removal from cold storage. In mangoes, heat treatment at 38°C for 48h negated this surge in ethylene production and also greatly reduced the rate of ethylene production during the climacteric peak. This may indicate that heat treatment inhibits chilling-induced ethylene production. The respiration rate, measured as the rate of CO_2 evolution was reduced (McCollum *et al.*, 1993). In apples, heat treatment at 38°C for 4 days only inhibited ethylene production during the period of heat treatment (Lurie and Klein, 1990).

1.6.2.2 *Fruit softening and chlorophyll degradation*

In apples heat treated at 38°C for 3 to 4 days, the rate of fruit softening was reduced and chlorophyll degradation was enhanced, even after 6 months of storage of 0°C (Lurie and Klein, 1990). In avocados, heat treatment also reduced the rate of softening, probably due to a reduction in cellulase activity which is associated with avocado ripening (Klein and Lurie, 1991). Fruit softening and ethylene production both require protein synthesis (Brady, 1987), which is inhibited by heat treatment. A reduction in the rate of softening is probably as a result of inhibition of cell wall degradation systems, which have been shown to be synthesised at the onset of ripening (Tucker and Grierson, 1982).

Polygalacturonase (which is associated with fruit softening in tomatoes and stone fruit) is inhibited by high temperatures whereas pectinesterase has a temperature optimum of >40°C (Lee and Wiley, 1970). Lurie and Klein (1990) suggested that the de-esterification of pectins in the middle lamella by pectinesterase during heat treatment, and the subsequent binding of endogenous free calcium to the available sites, may explain the firmer texture of heat treated apples.

Increased rate of chlorophyll degradation as a result of heat treatment may occur because chlorophyllase, one of the enzymes involved in chlorophyll degradation has a temperature optimum of >40°C (Amir-Shapira *et al.*, 1986).

Although heat treatment prior to cold storage may reduce chilling injury in some commodities, the stage of fruit maturity and/or degree of ripening with regards to susceptibility to chilling injury should be considered. Heat treatment has been shown to reduce chilling injury in green tomatoes, but a 3 day ripening period prior to cold storage at 5°C for 20 days was shown to be more effective than a 3 days treatment at 38°C in reducing chilling injury in 'Rutgers' tomatoes (Whitaker, 1994).

1.6.2.3 *Protein synthesis*

Lurie and Klein (1990) hypothesised that whether a process is inhibited or accelerated during heat treatment depends on whether *de novo* protein synthesis is required for that process or not.

Storage of plant tissues at high temperatures for a few hours generally induces a heat shock response which is characterised by a suppression of normal protein synthesis and the accumulation of a special group of proteins called the heat shock proteins (HSP). Production of HSP confers greater thermotolerance and plant tissues can subsequently be exposed to high temperatures which are usually lethal without severe damage occurring (Lafuente *et al.*, 1991; Lurie and Klein, 1991). Subjection of plant tissues to one type of stress can confer resistance to another type of stress, due to a common response - a change in protein synthesis (Lafuente *et al.*, 1991; Lurie and Klein, 1991; Lurie and Klein, 1992).

The function of heat shock proteins has not been elucidated, but it seems that they allow plants to make structural adjustments which confer tolerance to stress conditions. Ion leakage, which is used as a quantitative measure of chilling injury was reduced in cucumber cotyledons which had been stressed prior to storage at a chilling temperature and there was a concomitant increase in heat shock proteins (Lafuente *et al*, 1991). Some heat shock proteins have been associated with the plasma membrane in maize roots (Cooper and Ho, 1987). This could explain the reduction of electrolyte leakage in chilled plant tissues in which heat shock proteins have accumulated.

1.7 PRACTICAL IMPLICATIONS

There is no shortage of literature on work done on preventing or reducing chilling injury in horticultural crops. In the South African avocado industry, temperature management has been the major tool used to reduce chilling injury in avocados. It has been found however, that the stepped down storage temperature regimes used for avocados grown in the Northern Transvaal are not as effective in reducing the incidence of chilling injury in 'Fuerte' avocados grown in the Natal Midlands (M.J. Slabbert, 1993[2]). There is a need therefore, to determine storage temperature regimes specific to 'Fuerte' fruit grown in the Natal Midlands, according to fruit maturity.

Moisture loss during cold storage has been implicated as a factor influencing chilling injury in avocados (Bower and Cutting, 1987). Fruit moisture loss after harvest and prior to cold storage may also influence chilling injury, and work in this regard was carried out in this thesis.

Levels of total phenolics and polyphenoloxidase activity have been found to be higher in avocado fruit with mesocarp discolouration (van Lelyveld *et al.*, 1984), and an increase in mesocarp discolouration has been associated with increasing maturity and concentration of total phenols in the fruit mesocarp (Cutting *et al.*, 1992). If total phenolic concentration in avocado mesocarp can be correlated to the incidence of mesocarp discolouration, total phenolic concentration could be used as an indicator of fruit susceptibility to the disorder.

As noted in this review, postharvest heat treatments have been successfully used to reduce chilling injury in a number of crops. Very little literature is available on heat treatment of avocados. Reduction of chilling injury in 'Fuerte' avocados by heat treatments would be of great advantage to the South African avocado industry, as this cultivar is one of the export cultivars which is more susceptible to chilling injury.

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[2]Slabbert, M.J. Pers. comm. Everdon Estate, Howick.

CHAPTER 2

POSTHARVEST HEAT TREATMENTS OF 'FUERTE' FRUIT

2.1 INTRODUCTION

One of the objectives of prestorage heat treatments is to reduce chilling sensitivity of tropical and subtropical crops, allowing longer storage periods at temperatures which would usually cause chilling injury (Lurie and Klein, 1991). Sensitivity of tomatoes (Lurie and Klein, 1991) and mangoes (McCollum *et al.*, 1993) to low temperatures has been reduced by vapour heat treatments. In the literature, two different approaches to heat treatment have been taken, viz. long term: 12 to 48 h at 38 to 46°C or short term: up to 70 min at 45 to 55°C (Lurie and Klein, 1991). Short term heat treatments have been used for insect disinfestation and disease control but have also been noted to reduce the rate of fruit ripening (Paull, 1990). Long term heat treatments have been carried out with the aim of reducing chilling injury (e.g. McCollum *et al.*, 1993). In a review on heat treatment, Lurie and Klein (1991) mention that in work done by themselves, heat treatment of avocados at 36°C reduced chilling injury but that treatment at 38°C caused heat injury. This work however, has not been published to date. Heat treatment of 'Sharwil' avocados at 37 to 38°C for 17 to 18 h reduced external chilling injury after storage at 1.1°C for 14 days for insect disinfestation purposes. However, the average external appearance of the heat treated fruit was only rated as "marginally acceptable" which was below a "marketable" rating (Sanxter *et al.*, 1994). Florissen *et al.* (1993) reported a reduction in chilling in 'Hass' fruit treated for 6 and 12 h at 38°C.

'Fuerte' avocados are susceptible to cold storage disorders. Reduction of susceptibility to these disorders using heat treatments would be advantageous to the export industry as improved quality fruit will fetch higher prices. Heat treatments were carried out in an attempt to determine a time-temperature relationship for heat treatment which could possibly reduce the susceptibility of 'Fuerte' fruit to cold storage disorders.

2.2 MATERIALS AND METHODS

As very little literature was available on heat treatment of avocados at the commencement of this study, a number of exploratory trials were carried out throughout the 'Fuerte' harvesting seasons of 1993 and 1994. Trails were conducted using different types of heat treatment in an attempt to find a type of heat treatment which did not damage the fruit and reduced fruit sensitivity to cold storage. The following methods of heating were employed: i) Dry heat: the fruit was placed in an incubator, ii) Water bath: the fruit was heat treated in a hot water bath, and iii) Vapour heat: where the fruit was heat treated in a high relative humidity atmosphere (> 90%). This high humidity atmosphere was achieved using a Paxton Electrotherm® 18 kW heater and humidifier which was attached to a standard shipping container. The heating and humidification equipment was supplied by Agrelek, the agriculture branch of Eskom. Temperature control on the unit was accurate within 1°C on either side of the set temperature. Fruit mass loss as a result of heat treatment was determined by massing the fruit before and after heat treatment. The majority of mass lost was assumed to be due to moisture loss, with losses due to respiration assumed to be negligible.

2.2.1 1993 Trials

'Fuerte' fruit harvested on 1993/05/19 and 1993/06/01 was heat treated in dry heat at 38°C for 48 h, and fruit harvested on 1993/06/12 and 1993/06/29 was heat treated at 36°C for 48 h. After heat treatment , the fruit was stored at either 3.5°C, 5.5°C or 6.5°C for 28 days.

Forty two count 14 fruits (mass 206 -305 g) were stored at each temperature and a further 42 fruits which had not been heat treated were stored at each temperature and served as controls. On removal from cold storage, firmometer readings (Swarts, 1981) were taken and the fruit was allowed to ripen at room temperature in a laboratory (ca. 18 to 22°C). A firmometer measures fruit firmness non-destructively. The softer the fruit, the higher the firmometer reading. The stage at which an avocado is ready to eat is indicated by a firmometer reading of 100. On ripening, as indicated by a firmometer reading of 100, each fruit was rated on a scale of 1 to 3 (1= mild; 2= moderate; 3= severe) externally for exocarp (rind) pitting and discolouration, and internally for anthracnose, stem end rot, vascular browning, pulp spot and grey pulp (Swarts, 1984).

Table 2.1 Heat treatment trials carried out in the 1994 'Fuerte' harvesting season.

Trial	Date	Treatment	Type of heat
1	1994/05/17	0, 12, 24, 48h @ 36°C	Dry, fruit in plastic bags
2	1994/05/24	0, 4, 6, 8h @ 40°C	Dry
3	1994/06/02	0, 4, 6h @ 40°C	Water bath
4	1994/06/08	36h @ 36°C & 12h @ 32°C + 24h @ 36°C	Water bath
5	1994/06/24	0, 6, 12, 24, 30, 36, 42, 48h @ 36°C	Vapour
6	1994/07/13	0, 0.5, 1.5, 3.0, 4.0h @ 40°C	Vapour
7	1994/07/13	0, 10, 20, 30, 45, 60 min @ 48°C	Vapour
8	1994/07/13	0, 2, 4, 6h @ 36°C	Vapour
9	1994/07/20	0, 3, 5, 12h @ 36°C	Vapour

2.2.2 1994 Trials

Nine heat treatment trials using different forms of heat, temperatures and durations of treatment were carried out during the 1994 'Fuerte' harvesting season in the Natal midlands (Table 2.1). Fruit for these trials was supplied by Everdon Estate. More trials were carried out than in 1993 as fruit was also obtained from the Wartburg area, where 'Fuerte' fruit is harvested a few weeks later than at Everdon Estate in spite of a warmer climate. Trial numbers 6 to 9 (Table 2.1) were carried out using fruit from the Wartburg area. Heat treated fruit was stored at 3.5°C, as this is known to cause cold storage disorders in 'Fuerte' fruit and the aim of the heat treatments was to reduce fruit sensitivity to low temperatures. Twenty three fruit were used per treatment in trial 1 and 32 per treatment in trails 2, 3, and 4. Trial 5 had 48 fruit per treatment. In trials 6 to 8 only 8 fruit were used per treatment with the intention of testing a number of different time and temperature combinations as exploratory trials, so that any treatment which appeared to reduce sensitivity to chilling injury could be carried out at a later stage with sufficient replication for statistical analysis. Thirty fruits per treatment were used in trial 9.

2.3 RESULTS AND DISCUSSION

2.3.1 1993 Trials

External appearance is of paramount importance in the marketing of 'Fuerte' avocados and any rind blemishes will reduce the value of the fruit. The effect of heat treatments on external appearance is very important. Fig. 2.1 shows the average external rating for all treatments of the dry heat trials carried out in 1993. The higher the rating, the greater the degree of rind blackening or browning. In all four trials, there was very little external blackening in the control fruit stored at all temperatures. Heat treated fruit always displayed

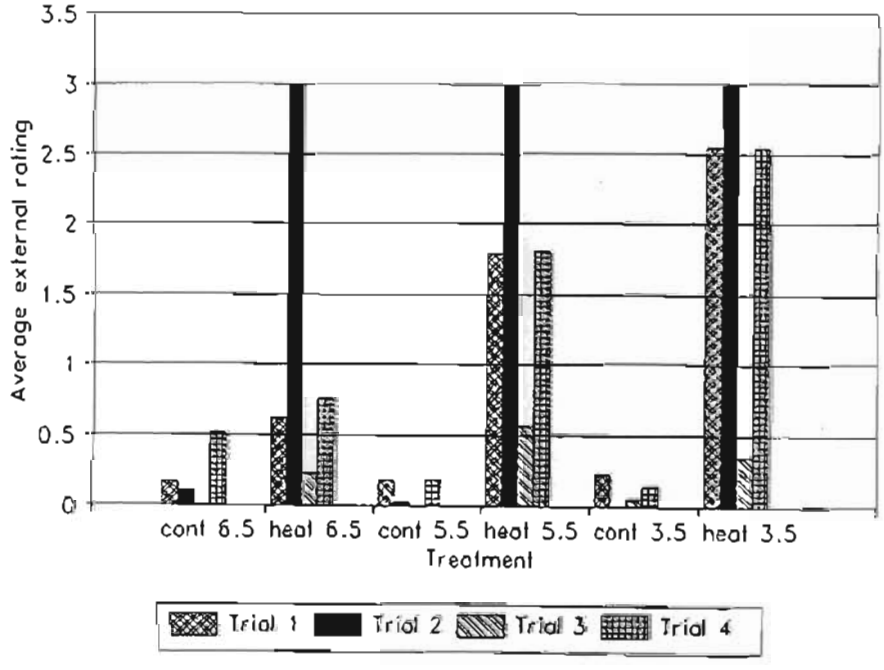

Fig. 2.1 Average external rating of control and heat treated 'Fuerte' fruit (1993) after storage at 3.5°C, 5.5°C and 6.5°C for 28 days.

a greater severity of this disorder than the controls at all temperatures for all four trials. A reduction in storage temperature of heat treated fruit resulted in increased severity of external blackening in trials 1 and 4 although the control fruit of the same two trials did not show the

same trend, and storage temperature of the controls seemed to have little effect on external blackening known as cold damage (Swarts, 1984). It appears therefore that heat treatment increased fruit susceptibility to cold damage. In trial 2, all heat treated fruit showed severe rind blackening as indicated by the average external ratings of 3. Varying levels of external blackening between fruit of the four different trials for the same storage temperature shows that pre- and postharvest factors must play a role in determining fruit susceptibility to this disorder as a result of heat treatment. Especially noticeable is the difference between heat treated fruit in trials 2 and 3 where the degree of external injury was less in trial 3.

Added shelf life is advantageous in the marketing of avocados. Fig 2.2 shows average ripening times of heat treated and control fruit in the four trials conducted in 1993. In all four trials, heat treated fruit took longer to ripen than the control fruit stored at the corresponding temperatures. There was a trend of increasing time to ripen in control fruit as the storage temperature was reduced. Increased ripening time as a result of heat treatment could be as a result of reduced cellulase activity (Lurie and Klein, 1990) and the failure of some fruits to ripen normally after heat treatment could be due to the reduction of cellulase activity to a greater extent. If the problem of external blackening as a result of heat treatment can be overcome, heat treatments may be useful in extending the ripening time of 'Fuerte' avocados. As with external blackening, there was variation in ripening time between trials, for example, trial 2 fruit took longer to ripen over all treatments than fruit in the other trials.

Fig 2.3 shows the average firmometer readings of heat treated and control fruit, which give an indication of fruit firmness on removal from cold storage. There was no trend with regard to fruit firmness and heat treatment over the four trials conducted. Heat treated fruit may have been softer (e.g. trial 3 fruit stored at 6.5°C), or harder (e.g. trial 4 fruit stored at 6.5°C) after storage than their respective controls. As with external blackening and ripening times, differences in firmness between trials were noticeable over all treatments, for example, fruit in trial 2 was firmer in all treatments than trial 4 fruit.

The heat treatments carried out all caused surface browning (Fig. 2.1) and were therefore not effective. Similar results were for 'Fuerte' avocados were reported by Kerbel *et al.*, (1987) and Florissen *et al.* (1993). Heat treatment increased the ripening time of the fruit

after cold storage (Fig. 2.2) which is in agreement with the findings of Lurie and Klein (1991) .

In some cases, heat treated fruit was softer than control fruit on removal from cold storage, although it took longer to ripen, e.g. in trial 1, fruit stored at 6.5°C (Fig. 2.3). This was probably due to moisture loss from the heat treated fruit and not due to a more advanced

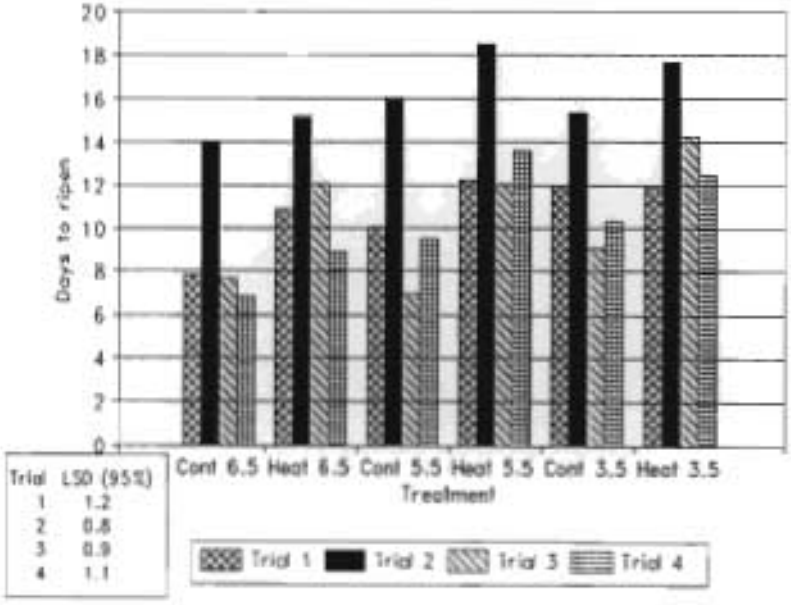

Fig. 2.2 Average ripening times of control and heat treated 'Fuerte' fruit (1993) after storage at 3.5°C, 5.5°C and 6.5°C for 28 daysstage of ripening.

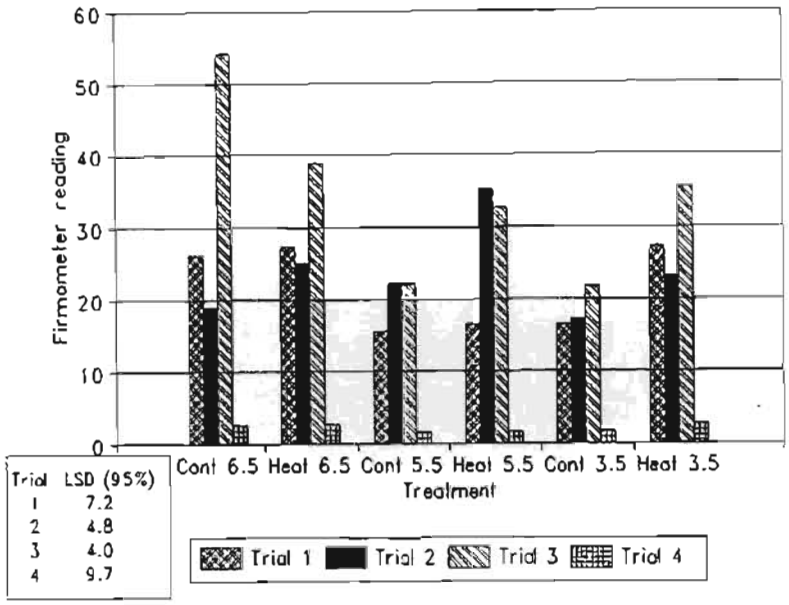

Fig. 2.3 Average firmometer readings of control and heat treated 'Fuerte' fruit (1993) after storage at 3.5°C, 5.5°C and 6.5°C for 28 days.

2.3.2 1994 Trials

2.3.2.1 *Vapour heat*

Table 2.2 Shows the results of pre-storage vapour heat treated fruit obtained from the Wartburg area. Each of the following three paragraphs contains a discussion of the three temperatures tested viz. 40, 48 and 36°C. The aim of these trials was to determine time - temperature relatinships for heat treatment which may be effective in reducing avocado fruit susceptibility to cold storage disorders, so that larger trials with sufficient replication for statistical analysis could be carried out on such treatments.

At 40°C, treatment for 4 h resulted in fruit with the most severe external blackening, with an average rating of 0.875. The average number of days to ripening was similar for all treatments at 40°C and no treatment extended the ripening time appreciably compared to the

control (0 h). Firmometer readings were very similar with the exception of fruit treated for 4 h , which was softer than the other treatments.

A temperature of 48°C was used to determine whether a higher temperature for a shorter period of time would elicit the desired reduction in disorders associated with low storage temperatures, and this was the highest air temperature which the heating equipment could deliver. 10, 20 , 30 and 60 min at 48°C all decreased the average ripening time, which is not desirable. All the abovementioned durations of treatment reduced fruit firmness after cold storage, as indicated by higher firmometer readings than the control fruit. The incidence of grey pulp did not show a trend with increasing or decreasing duration of treatment. A trial with greater replication would be necessary to determine whether of not heat treatment at 48°C has an influence on this disorder a some fruit seem to be pre-disposed to it as mesocarp discolouration may occur in fruits which have not been cold stored (Vakis, 1982). Further trials could not be carried out in 1994 as the 'Fuerte' harvesting season lasts approximately 7 weeks in the Natal Midlands and was over once this trial had run to completion.

Two separate trials (a week apart) (Table 2.1), were carried out heat treating fruit at 36°C. Differences in external blackening, days to ripen and incidence of grey pulp between the controls of these two trials shows that differences in maturity, and other pre-harvest factors have an effect on the quality of cold stored avocado fruit. Treatment for 4 h at 36°C in the first trial resulted in less external blackening than 3 h at 36°C in the second trial, which highlights the variable response to heat treatment from week to week. With the exception of

Table 2.2 Quality parameters of 'Fuerte' fruit from Wartburg heat treated at different temperatures for different lengths of time prior to cold storage at 3.5 °C for 28 days.

Temp (°C)	Duration (h)	External rating when ripe	Days to ripen	Grey pulp (proportion of fruit affected)	Firmo-meter reading
Control	0	0.625	8.3	0.25	15,0
40	0.5	0.625	7.8	0.50	15,3
40	1.5	0.125	8.4	0.00	15,4
40	3.0	0.250	8.4	0.13	15,4
40	4.0	0.875	7.1	0.38	16,7
Control	0	0.625	8.3	0.25	15,0
48	10 min	0.125	7.1	0.00	16,1
48	20 min	0.500	6.4	0.38	19,5
48	30 min	0.375	6.4	0.38	18,9
48	45 min	0.875	7.0	0.25	19,3
48	60 min	1.625	7.1	0.13	16,7
Control	0	0.625	8.3	0.25	15,0
36	2	0.438	8.3	0.44	16,4
36	4	0.625	8.1	0.32	16,5
36	6	1.000	8.8	0.19	16,6
Control	0	0.467	7.3	0.47	16,0
36	3	0.800	9.9	0.33	14,8
36	5	0.800	8.7	0.60	15,4
36	12	1.237	10.3	0.33	16,2

Fig. 2.4 'Fuerte' fruit vapour heat treated for 24 h at 36°C and stored at 3.5°C for 11 days (right) and fruit not heat treated (left).

2 h at 36°C, external blackening was more severe than in the control fruit and increased in severity with increased duration of treatment. Treatment at 36°C for 3, 5 and 12 h increased the ripening time, but external damage caused by these treatments overrides this desirable response.

Table 2.3 contains the results of vapour heat treatments for lengths of time ranging from 6 to 48 h on 'Fuerte' fruit from Everdon Estate. External blackening after cold storage was worse in the heat treated fruits and increased in severity with increased duration of heat treatment, as was the case in the second 36°C trial on fruit from the Wartburg area (Table 2.2). Ripening time was significantly greater than the control ($P < 0.05$) for treatment times greater than 6 h. Flesh tissue breakdown as characterised by brownig and flesh sponginess, became more prominent with increased duration of heat treatment in this trial. Fruit firmness on removal from cold storage did not show a trend with duration of heat treatment even though there were significant differences between treatments. There was no definite trend in grey pulp incidence with increasing duration of heat treatment. Although grey pulp was incident in 10% of the fruit heat treated for 6, 12, 18 h as opposed to 29% in the control fruit, the incidence of 23% in the 24 h treatment and 13% in the 30 and 36 h treatments tends to place doubt on whether or not heat treatment was responsible for reducing the incidence of grey pulp.

Table 2.3 Quality parameters of 'Fuerte' fruit heat treated at 36°C for different lengths of time prior to cold storage at 3.5°C for 28 days.

Temp (°C)	Duration (h)	External rating when ripe	Days to ripen*	Grey pulp (% fruit affected)	Firmo-meter reading
36	0	0.08	10.7 a	29	15,3 b
36	6	1.15	11.2 ab	10	13,5 a
36	12	1.03	11.9 bc	10	15,9 bc
36	18	1.18	12.9 d	10	15,8 b
36	24	2.00	13.7 e	23	17,3 c
36	30	2.00	13.9 ef	13	15,7 b
36	36	2.28	14.6 f	13	19,1 d
36	42	2.70	14.4 ef	28	15,2 b
36	48	3.00	12.3 cd	25	16,3 bc

* Values followed by the same letter do not differ significantly at P = 0.05.

Table 2.4 Quality parameters of 'Fuerte' fruit heat treated in plastic bags at 36°C for 12h, 24 h and 48 h prior to 28 days cold storage at 3.5°C.

Dura-tion (h)	Ave. exter-nal rating	Mean % mass loss during heat treat.	Grey pulp (% fruit affected)	Tissue Break-down (% fruit affec-ted)	Days to ripen*	Firmo-meter	Fungal infec-tion (% fruit affected)
0	0.43	-	26.1	0.0	12.0 ab	18,0 a	0.0
12	1.1	0.79 a	0.0	4.3	11.7 a	16,2 a	0.0
24	1.7	1.05 b	0.0	13.0	13.0 bc	17,7 a	13.0
48	2.3	1.50 c	8.6	95.7	13.9 c	20,6 b	13.0

* Values followed by the same letter do not differ significantly at P = 0.05.

2.3.2.2 *Dry heat*

Table 2.4 shows the results of 'Fuerte' fruit dry heat treated at 36°C, sealed in plastic bags. Even though individual fruits were placed in plastic bags, there were significant differences in moisture loss (as measured by change in mass) between treatments of 12, 24 and 48 h duration. External blackening was more severe in heat treated fruit and increased in severity with increased duration of heat. The incidence of grey pulp was highest in the control fruit, indicating as in the vapour heat trials, that heat treatment may reduce the incidence of this disorder. The incidence of flesh tissue breakdown increased with increased duration of treatment. Only fruit heat treated for 48 h took significantly longer to ripen than the control fruit but was significantly softer on removal from cold storage. This was probably not as a result of a more advanced stage of ripening, but due to reduced turgidity as a result of greater moisture loss during heat treatment. Anthracnose fungal infection was observed in the 24 and 48 h treatments and was probably as a result of moisture stress and tissue breakdown making the fruit more susceptible to pathogenic attack.

Table 2.5 shows the results of 'Fuerte' fruit dry heat treated for 4, 6 and 8 h at 40°C. There was a significant increase in moisture loss with each increase in duration of heat treatment. Once again, the incidence of grey pulp appears to have been reduced by heat treatment of 6 and 8 h. Ripening time was significantly greater in fruit heat treated at 40°C for 8 h. The control fruit was significantly softer than the heat treated fruit on removal from cold storage. The severity of external blackening increased with increased duration of heat treatment and was always greater than that of the control fruit.

Table 2.5 Quality parameters of 'Fuerte' fruit after dry heat treatment at 40°C for 4 h, 6 h and 8 h prior to 28 days cold storage at 3.5°C.

Duration (h)	Ave. external rating	Mean % mass loss during heat treat.	Grey pulp (% fruit affected)	Tissue Breakdown (% fruit affected)	Days to ripen*	Firmometer	Fungal infection (% fruit affected)
0	0.3	-	28.1	0	10.8 a	17,1 c	0
4	0.6	0.19 a	31.2	0	11.5 ab	16,5 ab	0
6	1.6	0.49 b	18.8	0	11.9 ab	15,2 a	0
8	2.1	0.81 c	15.6	0	12.4 b	15,1 a	13.0

* Values followed by the same letter do not differ significantly at P = 0.05.

Table 2.6 Quality parameters of 'Fuerte' fruit heat treated in a water bath at 40°C for 4 h, 6 h and 8 h prior to cold storage at 3.5°C for 28 days.

Duration (h)	Tissue breakdown (% fruit affected)	% fruit failing to ripen	Ave. external rating	Firmometer reading
0 h	0	0	0.4	16,8 b
4 h	21.9	3.1	1.8	13,9 a
6 h	100	100	3.0	17,7 b
8 h	100	100	3.0	18,1 b

* Values followed by the same letter do not differ significantly at P = 0.05.

2.3.2.3 *Water bath*

Table 2.7 shows the results of 'Fuerte' fruit heat treated in a water at 40°C for 4, 6 and 8h. This form of heat treatment caused severe external blackening even before cold storage, (Fig. 2.5) and was probably due to the rate of heat transfer to the fruit being more rapid than fruit

heat treated in air. Ripening was adversely affected and treatment for 6 and 8 h totally inhibited ripening. Treatment for 6 and 8 h caused flesh tissue breakdown in all fruit.

Table 2.7 Quality parameters of 'Fuerte' fruit heat treated in a water bath at 36°C for 36 h or 32°C for 12 h followed by 24 h at 36°C prior to cold storage at 3.5°C for 28 days.

Treatment	Tissue breakdown (% fruit affected)	% fruit failing to ripen	Ave. external rating	Firmo-meter reading
0 h (control)	0	0	0.1	16,3 a
36 h @ 36°C	100	97	3.0	30,3 c
12 h @ 32°C & 24 h @ 36°C	100	100	3.0	26,7 b

* Values followed by the same letter do not differ significantly at P = 0.05.

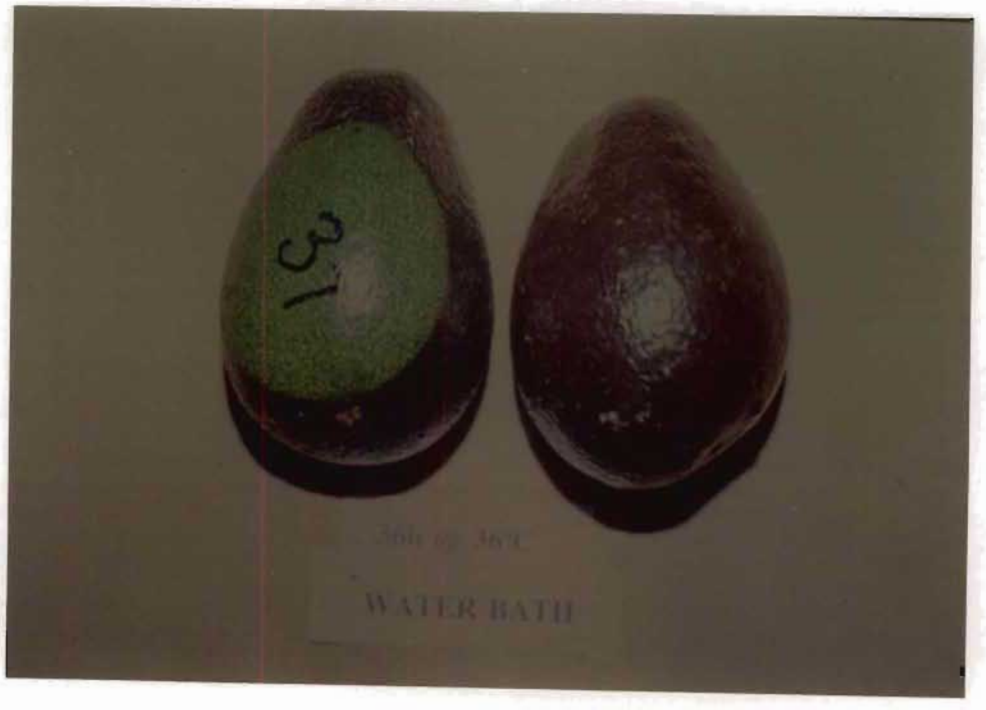

Fig. 2.5 'Fuerte' fruit heat treated in a water bath at 36°C for 36 h. The fruit on the left was not fully submerged, hence the green area which did not come into contact with the water

Dry heat treatment at 40°C increased exocarp browning with increased heat duration and all treatments produced fruit of inferior quality to the controls after cold storage. No tissue breakdown was observed and moisture loss (measured as a change in mass) and ripening time increased with increased heat duration (Table 2.5).

Table 2.7 shows the results of stepping up the temperature from 32 to 36°C during heat treatment of 'Fuerte' fruit in a water bath in an attempt to reduce the external and internal damage caused by water bath treatment at 36°C (Table 2.6). Stepping up the temperature was no less damaging to the fruit than holding it at 36°C for 36 h, with no reduction in tissue breakdown or external blackening.

Heat treatments in a water bath were tested as it was thought that moisture loss was causing exocarp browning and pitting. However, this form of heat treatment caused a greater degree of exocarp browning than dry heat (cf Tables 2.5 and 2.6). Skin browning is therefore not as a result of moisture loss during heat treatment but is rather a symptom of heat scald. The water bath treatment probably caused greater damage because of a greater rate of heat transfer to the fruit than air. Fig. 2.5 clearly shows exocarp browning where the fruit was in contact with the water. The more rapid transfer of heat to the fruit probably had a greater inhibiting effect on the enzymes involved in ripening as fruit heat treated at 40°C for 6 h and 8 h failed to ripen. A "stepped up" heat treatment where the fruit was first treated at 32°C then the temperature was increased to 36°C, was not effective in reducing heat scald and inhibition of ripening (Table 2.7).

Vapour heat treatments were generally less damaging to the exocarp and the ripening process than dry heat and warm water treatments (Tables 2.2 and 2.3). Three treatments produced fruit with less exocarp damage than their controls, viz. 1.5 h and 3 h at 40°C and 10 min at 48°C. These fruits also had a lower incidence of grey pulp than their controls. Heat treatment at 48°C reduced the ripening time which is not desirable. Only 8 fruits per treatment were used in this trial. Another trial will have to be carried out with greater replication to confirm these results. It was not possible to do this as the 'Fuerte' harvesting season was over by the time these trials had run to completion. If these treatments are found to be successful in reducing storage disorders, they will have to be tested throughout a season as changes in fruit maturity may elicit different responses to heat treatment.

2.4 CONCLUSIONS

Heat treatments caused heat damage in 'Fuerte' avocados, which is characterised by exocarp browning and pitting. Vapour heat was less damaging to the fruit than dry heat or warm water baths. In all the trails run in 1993 and 1994, only two treatments produced fruit with less external blackening and pulp spot than fruit not heat treated, without reducing ripening time, viz. 1.5 h and 3 h at 40°C. Further trials using these treatments would have to be fun for an entire harvesting season to confirm these results, as fruit of different maturity may respond differently to heat treatment. If in subsequent trials, a time - temperature relationship is found which improves the quality of cold stored avocados, the cost of heat treatment relative to higher prices for better quality fruit will determine whether heat treatments are used commercially or not.

2.5 SUMMARY

One of the objectives of pre-storage heat treatments is to reduce the chilling sensitivity of tropical and subtropical crops allowing longer storage periods at temperatures which usually cause chilling injury (Lurie and Klein, 1991). 'Fuerte' avocados are sensitive to cold storage, resulting in physiological disorders such as grey pulp and cold damage (Swarts, 1984). Pre-storage heat treatments of 'Fuerte' fruit has been reported to cause blackening of the rind (Sanxter *et al.*, 1994; Florissen *et al.*, 1993). A number of exploratory heat treatment trials were carried out on 'Fuerte' avocado fruit using dry heat, vapour heat and warm water baths, in an attempt to find a time - temperature relationship which would confer greater chilling resistance to 'Fuerte' fruit. Dry heat treatments were carried out in 1993 using temperatures of 36 and 38°C for 48 h. These treatments all caused rind blackening but extended ripening time after 28 days of cold storage at 6.5, 5.5 or 3.5°C.

In 1994, a wider range of temperatures and treatment durations were tested and ranged from 48 h at 36°C to 10 min at 48°C. Fruit in all of the trials was stored at 3.5°C for 28 days after heat treatment. Warm water treatments caused severe rind blackening, even before cold storage and inhibited ripening after cold storage. Dry heat treatments did not produce fruit of quality superior to that of untreated fruit after cold storage. Vapour heat treatment for 1.5 and 3 h at 40°C produced fruit with less external blackening and pulp spot than untreated

fruit, without reducing ripening time after storage. These two treatments show promise, but need to be tested throughout an entire harvesting season to confirm these results.

CHAPTER 3

COLD STORAGE

3.1 INTRODUCTION

The South African avocado industry is largely export orientated and transport by sea to European markets necessitates cold storage for three to four weeks. The avocado is a subtropical fruit, making it susceptible to chilling injury which limits its storage life (Eaks, 1976). Susceptibility to chilling injury is dependent on a number of factors e.g. cultivar (Vorster *et al.*, 1990); stress history and container ventilation (Bower and van Lelyveld, 1985); postharvest fruit temperature (Arpaia *et al.*, 1992); season (Bezuidenhout and Eksteen, 1994) and geographic location (Rowell, 1988). External cold damage and fruit firmness are the two most important quality determining criteria on the European market (Bezuidenhout and Eksteen, 1994) making effective cold storage techniques a necessity. The standard for transport and storage of South African avocados has traditionally been 5.5°C, with emphasis on postharvest maintenance of the cold chain in the packhouse, during road transport to Cape Town or Durban, in transit at the docks, on the 14 day sea voyage to Europe, and subsequent to offloading (Bezuidenhout, 1993; Ginsburg, 1985). In spite of the best efforts of exporters, invariably a proportion of the crop arrives overseas "breaking" (firmometer reading 36 to 45), soft (46 to 75) or even very soft (76-99). In an attempt to reduce this problem, South African researchers have experimented with variable storage temperatures, dependent on seasonal differences, based on fruit maturity. Research in this area has been conducted mainly in the Northern Transvaal production area centred on Westfalia Estate near Duivelskloof and Tzaneen. Toerien (1986) proposed a system of reducing storage temperatures throughout the season as the fruit became more mature and less sensitive to low storage temperatures. Vorster *et al.* (1990) found that higher temperatures during the earlier stages and lower temperatures during the later stages of cold storage tended to decrease the incidence of internal physiological disorders. Temperature management of avocados grown in the Eastern and Northern Transvaal is currently carried out according to these principles. The development of an export based avocado industry in the Natal Midlands is fairly recent. The 1991 avocado census showed 162 405 trees and a production potential of 6% of the South African crop (Finnemore, 1991). The main feature of this industry is the relatively

later maturity of each cultivar due to the greater latitude (+/- 30°S as opposed to 23 to 25°S) and the generally cooler growing conditions. It has been found that temperature regimes which are effective in storing Transvaal avocados of similar maturity are not effective for Natal fruit (M.J. Slabbert, 1993[3]). Nine different temperature regimes were tested, in co-operation with Everdon Estate, Howick, throughout the 1993 and 1994 'Fuerte' harvesting seasons in an attempt to determine which regimes will produce firm fruit with minimal internal and external disorders after a 4 week storage period.

Mesocarp discolouration in the form of grey pulp and/or pulp spot is as a result of the oxidation of o-diphenols by the enzyme polyphenol oxidase to o-quinones which are in turn oxidised to brown melanin pigments (Bower and Cutting, 1988). Levels of total phenolics and polyphenol oxidase activity were found to be higher in avocado fruit with mesocarp discolouration than healthy fruit (van Lelyveld *et al.*, 1984). Cutting *et al.* (1992) found that total phenols in 'Fuerte' avocado fruit increased with increasing maturity, as did mesocarp discolouration.

The concentration of total phenolics in 'Fuerte' mesocarp was determined throughout the harvesting season at Everdon Estate to determine whether an increase in physiological disorders with increasing maturity could be explained by an increase in total phenolic concentration.

During a cold storage period of 24 to 28 days, higher temperatures during the early stages and lower temperatures during the later stages have been found to decrease the incidence of pulp spot in 'Fuerte' fruit as opposed to those kept at 5.5°C throughout the storage period (Vorster *et al.*, 1987). It was hypothesised that at some point during cold storage, there was a decrease in sensitivity to cold storage, and that this may be marked by a change in ethylene evolution associated with the climacteric rise in respiration. Consequently, ethylene evolution was measured during cold storage to see if a change in sensitivity to chilling temperatures was marked by a fluctuation in ethylene production.

Slabbert, M.J. 1993. Pers. comm. Everdon Estate, Howick.

3.2 MATERIALS AND METHODS

3.2.1 Source of fruit

All the fruit used in these trials was grown at Everdon Estate near Howick in the Natal Midlands. The farm falls into bioclimatic group 3 (mist belt) (Phillips, 1973) and has a mean annual rainfall of 1052 mm for the past 77 years (Anon., 1994b). Mean maximum and minimum temperatures are 25.9 and 15.2°C for January, and 19.5 and 7.0°C for July, and the climate can be characterised as cool subtropical. The mean elevation of the farm is 1082 m above sea level. 'Fuerte' fruit was harvested from trees ranging in age from 7 to 11 years on 'Duke 7' clonal rootstocks. Tensiometers at 30 cm and 60 cm depths were used for irrigation scheduling. The trees were irrigated with a microjet system when the soil tension reached -40 kPa. Fertilisation was carried out according to tree phenological events and leaf and soil analyses.

3.2.2 Temperature regime trials

Standard 4 kg export cartons of count 14 and 16 fruit (236 g to 305 g) were taken from the pack line at Everdon Estate every week during the 1993 and 1994 'Fuerte' harvesting seasons (Table 3.2). Ten Cartons were used for each treatment (Table 3.1). Fruit moisture content was determined for each trial as an index of maturity by the laboratory staff at Everdon Estate by oven drying a known mass of thinly sliced mesocarp tissue (Swarts, 1976). The moisture contents for the last five trials of 1994 were misplaced at Everdon Estate so moisture contents for these trials were obtained from Clive Kaiser[4] who had taken samples from the same batches of fruit. The 1993 and 1994 'Fuerte' harvesting seasons lasted from 19 May to 14 July and 17 May to 12 July respectively. After the designated period (three to five weeks) in cold storage (Table 3.1), fruit firmness was determined using a firmometer (Swarts, 1981) whereafter the fruit was placed in a room at +/- 20°C and allowed to ripen. When eating ripe, as determined by a firmometer (Swarts, 1981) the fruit was rated internally and externally on a "presence or absence" basis for the following disorders: Cold damage (also known as black cold), which is characterised by pitting and blackening of the

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exocarp; pulp spot characterised by spherical grey spots in the mesocarp associated with the cut ends of the vascular bundles; grey pulp which is a general grey discolouration of the mesocarp; and vascular browning (Swarts, 1984); a general browning of the distal end of the fruit known in the industry as "bolverkleuring" or "dusky cold"; anthracnose rot caused by the fungus *Glomerella cingulata* (Pegg and Coates, 1993) and stem end rot caused by *Colletotrichum, Dothiorella, Phomopsis, Thyronectria, Rhizopus* and *Botryodiplodia spp.* (Swarts, 1984). The occurrence of lenticel damage was also recorded.

The data collected were analysed using a logistic regression model for a binomial distribution (Gujarati, 1988). The incidence of each disorder was analysed separately for each week that a trial was run.

Table 3.1 Temperature regimes used for 'Fuerte' fruit

Regime	Temperature (°C)				
	Week 1	Week 2	Week 3	Week 4	Week 5
1	8,5	8,5	7,5	6,5	-
2	8,5	7,5	6,5	5,5	-
3	8,5	7,5	5,5	4,5	-
4	7,5	7,5	6,5	5,5	-
5	7,5	7,5	5,5	4,5	-
6	5,5	5,5	5,5	5,5	-
7	4,5	5,5	7,5	7,5	-
8	7,5	7,5	6,5	-	-
9	7,5	7,5	6,5	5,5	5,5

Table 3.2 Dates on which temperature regime trials were commenced

Trial	1993	1994
A	19 May	17 May
B	26 May	24 May
C	2 June	1 June
D	9 June	6 June
E	16 June	15 June
F	23 June	21 June
G	30 June	29 June
H	8 July	6 July
I	15 July	12 July
J	-	19 July

3.2.3 Quantification of total phenolics

Eight 'Fuerte' avocado fruit of mass 236 g to 265 g (count 16) were taken from the pack line at Everdon Estate once a week for the duration of the 1994 'Fuerte' harvesting season, which lasted from the first week in June to third week in July. A 1.5 cm ring of mesocarp tissue from the widest point of each fruit was freeze-dried and stored at -20°C until analysis.

A modification of the method used by Osborne (1992) to quantify total phenols in *Pinus patula* cuttings, which is a modification of the method of Torres et. al (1987) to determine total phenols in avocado mesocarp, was used.

Freeze dried avocado mesocarp tissue was ground to a powder in liquid nitrogen using a mortar and pestle. A sample of 0.2 g of the powder was placed into a polypropylene centrifuge tube to which 10 ml of 100% chloroform and 10 ml of 100% hexane was added. The tube was capped and shaken on a laboratory shaker for 2 h after which it was centrifuged at 5000 rpm (2510g) for 10 min. The extract was filtered through Whatman® no. 1 filter paper and the supernatant discarded. Any material remaining on the filter paper was

scraped off back into the tube. 20 ml of 60% methanol in water solution was added to the tube which was capped and shaken on a laboratory shaker for 2 h. The extract was filtered through Whatman® no. 1 filter paper. A standard curve was prepared using a dilution series of gallic acid starting at 160 $\mu g.ml^{-1}$ and ending at 2.5 $\mu g.ml^{-1}$. A blank against which the spectrophotometer was calibrated, was prepared using 0.1 ml of water. Aliquots of 0.1 ml were taken from each sample and standard and placed into 20ml test tubes with two replications. 6ml of water and 0.5 ml Folin-Ciocalteu reagent was added to each tube which was vortexed thoroughly and allowed to stand between 1 and 8 min. 1.5 ml of 20% sodium carbonate solution was added, followed by 1.9 ml of water to bring the volume to 10ml. The solution was mixed thoroughly and incubated in a water bath at 50°C for 2 h. Absorbance was read on a Beckman® DU-65 spectrophotometer at 765 nm.

There is no completely satisfactory method for the quantitative extraction of plant phenolic compounds, but relative amounts can be compared using the same extraction technique (Ribereau-Gayon, 1972). An attempt to optimise phenolic extraction was made by testing different solvents and solvent concentrations. Acetone (60%) in water, 60% methanol in water, 100% acetone, 100% methanol and water were tested. The 60% methanol in water mixture gave the best results and was used instead of 100% methanol as used by Osborne (1992). To test whether phenolics were being lost in the extraction of tannins, carotenoids, chlorophyll and lipids using chloroform and hexane in the first extraction described in the method, thin layer chromatography was carried out on the supernatant. The plate was viewed under ultra-violet light to detect phenolic compounds (which fluoresce under ultra-violet light). No phenolics were detected and it was assumed that no phenols were lost during this extraction.

3.2.4 Ethylene determination

Mature 'Fuerte' fruit of mass 236 g to 265 g were harvested at Everdon Estate on 15 July 1993. Twenty fruits were stored in open glass jars either at 5.5°C or 7.5°C (ten at each temperature) for 28 days and then ripened at room temperature. Ethylene evolution was determined daily by sealing the jars for 2 h and drawing off a 1 ml sample of head gas in a gas tight syringe. The samples were injected into a Varian® 3400 gas chromatograph equipped with a flame ionisation detector and a 30 m (diameter 0.53 mm) 1,5 micron film bonded megabore column with OV-5 packaging. The injector, detector and column

temperatures were set at 120°C, 125°C and 60°C respectively. Gas flow rates used were as follows: N_2: 20 $ml.min^{-1}$; H_2: 20 $ml.min^{-1}$ and air: 300 $ml.min^{-1}$. A standard curve was constructed using 101 $\mu l.l^{-1}$ ethylene gas in nitrogen (accuracy of analysis: +/- 5%) at concentrations ranging from 0 to 100 $\mu l.l^{-1}$. A Delta software programme was used to calculate the ethylene concentration.

3.3 RESULTS AND DISCUSSION

3.3.1 Temperature regime trials

Tables 3.3 to 3.23 show the incidence of the various physiological and pathological disorders for which the ripe fruit was rated in the trials run in 1993 and 1994. Tables 3.23 and 3.24 indicate whether there were significant differences between all treatments in each trial (table 3.1) for each disorder. In most cases in 1993 and 1994, there were significant differences in the incidence of disorders between treatments in any one trial. This was often due to the incidence of disorders in treatments 8 and 9 which were of 3 an 5 weeks duration respectively, instead of the standard 4 week duration of the other treatments. Increased duration of storage is known to increase the incidence of physiological storage disorders. Because it was not possible to see any trends in the incidence of disorders in relation to storage temperature from these analyses, further analyses of the incidence of disorders in treatments one to six (Table 3.1) were carried out. Treatment 6 (5.5°C throughout the storage period) was used as a control against which the other treatments were compared. Significant differences between treatment 6 and the other five treatments are shown in Table 3.26.

Table 3.3 1993 Temperature regime trial A. Average firmometer readings and percentage fruit with various physiological and pathological disorders.

Treat	Black cold	Dist. end brown	Pulp spot	Grey pulp	Vasc. brown	Anth.	Stem end rot	Firmo*
1	16.4	0	2.5	0.8	0	0.8	0	25.9
2	8.2	0	0	0	0	0	0	24.9
3	19.7	0	0	16.4	0.8	0	0	27.3
4	7.4	0	0	0	0	0.8	0	30.1
5	5.8	0	0	0	0	3.3	0	29.7
6	2.5	0	0	0	0.8	3.3	0	24.2
7	20.0	0	0	0	0	0	0	29.5
8	50.0	0	0	0	0	0	6.0	22.3
9	53.6	0	0.9	0.9	0	0.9	0	27.0

*LSD (5%) = 2.9

1993 Trial A (Table 3.3)

Comparing all nine treatments, black cold was the only disorder which showed statistically significant differences ($P < 0.01$) in incidence between treatments (table 3.24). The incidence of black cold was highest in treatment 9 which was of 5 weeks duration (Table 3.1) instead of 4 weeks as in all the other treatments except treatment 8 which was of 3 weeks duration. One would tend to expect a higher incidence of black cold with increased duration of storage, however treatment 4 had a lower incidence of this disorder than treatment 8 which was identical to the first 3 weeks of treatment 4 (Table 3.1). Pre-harvest factors such as environmental conditions seem therefore to play as role in fruit susceptibility to black cold. Treatment 8 produced the hardest fruit after storage which was to be expected as this treatment was of the shortest duration.

Comparing treatment 6 (5.5°C for 4 weeks) to treatments 1 to 5, treatments 1 and 3 showed a significantly higher incidence of black cold than treatment 6 (Table 3.26). Treatment 6 produced hard fruit which was not of inferior quality to fruit in treatments 2 and 5 which were stepped down temperature regimes (Table 3.1).

Table 3.4 1993 Temperature regime trial B. Average firmometer readings and percentage fruit with various physiological and pathological disorders.

Treat	Black cold	Dist. end brown	Pulp spot	Grey pulp	Vasc. brown	Anth.	Stem end rot	Firmo*
1	0.8	0	1.6	0	0	0	1.6	24.7
2	1.6	0	0	0	0	0	0	30.2
3	0	0	0.8	0	0	0	0	23.4
4	2.3	0	2.3	2.3	0	0	0	23.2
5	2.3	0	0.8	0.8	0	3.1	0	23.2
6	39.7	0	2.4	7.9	0	0	0	29.6
7	56.1	0	12.9	7.6	0.8	6.8	3.0	72.0

*LSD (5%) = 3.0

1993 Trial 3 (Table 3.4)

The fruit in treatments 8 and 9 was mislaid during the course of this trial. There were significant differences between treatments in the incidence of all disorders noted, with the exception of vascular browning (Table 3.24). Treatment 7 (starting at 4.5°C and ending at 7.5°C) (Table 3.1) had the highest incidence of black cold, grey pulp, pulp spot, anthracnose and stem end rot and the fruit was soft on removal from storage.

Comparing treatment 6 to treatments 1 to 5, treatments 1 to 5 all had a significantly lower incidence of black cold than treatment 6, indicating that stepped down regimes may reduce the incidence of this disorder.

Table 3.5 1993 Temperature regime trial C. Average firmometer readings and percentage fruit with various physiological and pathological disorders.

Treat	Black cold	Dist. end brown	Pulp spot	Grey pulp	Vasc. brown	Anth.	Stem end rot	Firmo*
1	0	0	2.9	1.4	0.7	4.3	7.9	27.0
2	2.9	0	6.4	2.1	2.1	6.4	8.6	25.5
3	0	0	2.1	0	2.1	1.4	4.3	26.0
4	0	0	0.7	0.7	0	1.4	1.4	27.0
5	0.7	0	2.1	0	1.4	1.4	5.0	25.1
6	3.6	0	4.3	3.6	0	5.0	7.1	25.7
7	7.9	0	9.3	11.4	0.7	16.4	18.6	25.6
8	1.4	0	2.1	2.1	0	2.9	3.6	29.0
9	1.4	0	12.9	11.4	3.6	3.6	12.1	26.5

*LSD (5%) = 2.2

1993 Trail C (Table 3.5)

There were significant differences between treatments in the incidence of all disorders noted (Table 3.24). Treatment 7 produced fruit with the highest incidence of black cold as was the case in trial B (Table 3.4) This may indicate that low storage temperatures initially may increase fruit susceptibility to this disorder. Treatment 9 had the highest incidence of internal disorders which was expected as grey pulp and pulp spot incidence is known to increase with increased duration of cold storage. All treatments produced hard fruit (firmometer reading < 30).

Comparing treatment 6 to treatments 1 to 5, treatment 4 had a significantly lower incidence of stem end rot than treatment 6.

Table 3.6 1993 Temperature regime trial D. Average firmometer readings and percentage fruit with various physiological and pathological disorders.

Treat	Black cold	Dist. end brown	Pulp spot	Grey pulp	Vasc. brown	Anth.	Stem end rot	Firmo*
1	0	0	8.6	5.0	2.9	4.3	6.4	24.8
2	10.0	0	7.1	0	5.7	0	2.9	26.7
3	5.2	0	28.6	27.7	0.9	5.4	8.9	26.3
4	4.3	0	13.6	7.9	5.0	1.4	1.4	25.0
5	12.9	0	11.4	1.4	4.3	2.9	2.9	24.0
6	1.4	0	0	0	0	0	0	24.5
7	6.4	0	4.3	0	4.3	2.1	2.1	23.8
8	1.4	0	0	0	0	0	0	26.0
9	5.7	0	16.4	10.7	7.1	2.9	8.6	27.9

*LSD (5%) = 2.2

1993 Trial D (Table 3.6)

There were significant differences between treatments for all disorders noted (Table 3.24). Treatment 3 produced fruit with the highest incidence of grey pulp and pulp spot. Treatment 8 showed no internal disorders, probably due to the shorter storage period 3 weeks instead of 4 or 5 weeks (Table 3.1).

Comparing treatment 6 to treatments 1 to 5 (Table 3.26), Treatments 2, 3 and 5 all had a significantly higher incidence of black cold which is contrary to the results in trial B (Table 3.4) where treatments 1 to 5 all had significantly lower incidences of black cold than treatment 6 and so it cannot concluded that stepped down storage temperature regimes may reduce fruit sensitivity to this disorder.

Table 3.7 1993 Temperature regime trial E. Average firmometer readings and percentage fruit with various physiological and pathological disorders.

Treat	Black cold	Dist. end brown	Pulp spot	Grey pulp	Vasc. brown	Anth.	Stem end rot	Firmo*
1	0	0	1.4	0	0	1.4	6.4	28.6
2	1.4	0	5.7	0.7	0.7	1.4	4.3	26.5
3	0	0	2.9	0	0.7	0.7	2.9	27.8
4	0.7	0	1.4	1.4	0	0.7	1.4	27.6
5	0.7	0	2.1	0	0	0	2.1	27.6
6	0.7	0	1.4	0	0	1.4	3.5	27.3
7	0.7	0	0	0.7	0.7	0	5.0	28.1
8	2.1	0	4.3	2.9	2.9	2.1	2.9	23.9
9	0	0	5.0	7.9	2.1	0.7	2.1	27.5

*LSD (5%) = 1.9

1993 Trail E (Table 3.7)

There were significant differences between treatments in the incidence of grey pulp, pulp spot and black cold (Table 3.24). Once again, treatment 9 had the highest incidence of grey pulp and pulp spot. The incidence of stem end rot and anthracnose was fairly uniform over all treatments, suggesting that storage temperature regimes do not have an effect on the incidence of these pathological disorders.

There were no significant differences in any of the noted disorders when comparing treatment 6 to treatments 1 to 5 (Table 3.26).

Table 3.8 1993 Temperature regime trial F. Average firmometer readings and percentage fruit with various physiological and pathological disorders.

Treat	Black cold	Dist. end brown	Pulp spot	Grey pulp	Vasc. brown	Anth.	Stem end rot	Firmo*
1	0	0	0	0	0	0	0.7	24.5
2	0	0	6.4	2.9	4.3	0	1.4	27.6
3	0	0	5.0	2.1	3.6	1.4	1.4	26.2
4	2.9	0	10.0	5.0	7.9	0.7	8.6	25.3
5	0	0	7.9	2.9	3.6	0	0.7	27.6
6	1.4	0	3.5	2.1	2.1	0	2.9	25.9
7	0	0	1.4	0.7	2.9	0	2.9	27.3
8	2.9	0	5.7	4.3	3.6	0	4.3	28.9
9	0	0	5.0	2.1	7.9	0	7.1	25.7

*LSD (5%) = 2.1

1993 Trial F (Table 3.8)

There were significant differences between treatments for all disorders with the exception of grey pulp (Table 3.24). Treatment 8 had fruit significantly softer fruit than treatments 9 and 4 which was not as expected as treatment 8 was the same as the first 3 weeks of treatments 4 and 9 and only of 3 weeks duration (Table 3.1).

Comparing treatment 6 to treatments 1 to 5, treatment 4 had a significantly higher incidence of pulp spot than treatment 6 . Treatment 1 yielded the highest quality fruit with only a 0.7% incidence of stem end rot (only 1 fruit affected) and was significantly firmer than fruit in all other treatments except treatment 3.

Table 3.9 1993 Temperature regime trial G. Average firmometer readings and percentage fruit with various physiological and pathological disorders.

Treat	Black cold	Dist. end brown	Pulp spot	Grey pulp	Vasc. brown	Anth.	Stem end rot	Firmo*
1	1.4	0	15.0	6.4	17.9	0.7	2.1	26.4
2	5.7	0	16.4	5.7	14.3	0	2.1	27.8
3	2.9	0	11.4	0	10.7	0	3.6	26.2
4	3.6	0	20.0	2.9	15.7	0	0	27.4
5	2.3	0	7.1	4.3	7.1	0	2.1	27.3
6	4.3	0	7.9	4.3	7.9	0.7	0.7	27.1
7	5.0	0	10.0	0.7	9.3	0	2.1	27.8
8	0	0	0.7	0	5.0	0	1.4	25.1
9	0.7	0	10.0	6.4	14.1	0	0.7	29.5

*LSD (5%) = 2.0

1993 Trial G (Table 3.9)

There were significant differences between treatments for all disorders with the exception of stem end rot and anthracnose (Table 3.24). Treatment 9 produced fruit significantly softer than the other treatments, which was expected due to a longer storage period. 1993 Trail F generally had lower incidences of pulp spot and grey pulp (Table 3.8) than trial G which suggests that fruit sensitivity to these disorders changes with maturity and other pre- and postharvest factors.

Comparing treatment 6 to treatments 1 to 5, treatments 2 and 4 had a significantly higher incidence of pulp spot and treatments 1 and 4 had a significantly lower incidence of vascular browning (Table 3.26).

Table 3.10 1993 Temperature regime trial H. Average firmometer readings and percentage fruit with various physiological and pathological disorders.

Treat	Black cold	Dist. end brown	Pulp spot	Grey pulp	Vasc. brown	Anth.	Stem end rot	Firmo*
1	0	0	0.7	0	2.1	0	2.1	29.6
2	0	0	0.7	0.7	1.4	0	1.4	26.2
3	0	0	0.7	0	0.7	0	0.7	26.3
4	0	0	0	0	0.7	0	5.0	29.9
5	1	0	3.6	1.4	5.0	0	6.4	25.7
6	0	0	0.7	0	1.4	0	2.1	27.3
7	0	0	0	0	0	0	1.4	27.3
8	0	0	2.9	0	0.7	0.7	1.4	28.6
9	0	0	2.9	3.6	2.1	3.6	3.6	27.5

*LSD (5%) = 4.8

1993 Trial H (Table 3.10)

There were significant differences between treatments in the incidence of grey pulp, vascular browning and anthracnose (Table 3.24). Treatment 9 showed the highest incidence of grey pulp which was expected with a longer storage period. The high LSD figure for firmometer readings (4,8) shows that there was greater variation between treatments in this trial than in most of the other trials run which had LSD figures in the region of 2.0. There was no significant differences in the incidence of disorders when comparing treatment 6 to treatments 1 to 5 (Table 3.26).

Table 3.11 1993 Temperature regime trial I. Average firmometer readings and percentage fruit with various physiological and pathological disorders.

Treat	Black cold	Dist. end brown	Pulp spot	Grey pulp	Vasc. brown	Anth.	Stem end rot	Firmo*
1	0	0	7.1	0	6.4	0	0.7	24.6
2	0.7	0	11.4	0.7	5.0	0.7	2.1	25.2
3	0	0	16.4	1.4	10.0	0	0	24.1
4	0	0	5.0	0	4.3	0	0	26.0
5	2.1	0	12.1	7.1	2.9	2.9	5.0	25.5
6	0.7	0	8.6	2.1	3.6	0.7	2.1	24.3
7	0.7	0	5.7	1.4	4.3	0	0.7	26.5
8	0	0	7.1	1.4	10.0	0	1.4	26.9
9	0	0	42.9	37.9	38.6	7.1	10.7	27.7

*LSD (5%) = 2.0

1993 Trial I (Table 3.11)

There were significant differences between treatments in the incidence of all disorders noted with the exception of black cold (Table 3.24). The incidence of vascular browning did not seem to be affected by storage temperature regime as treatment 4 had a lower incidence of this disorder than treatment 8 even though treatment 8 was identical to the first 4 weeks of treatment 4 (Table 3.1). Treatment 9 produced fruit with the highest incidence of pulp spot, grey pulp, and vascular browning. These disorders have showed a trend of increasing incidence with increased duration of storage throughout the temperature regime trials conducted in 1993. Comparing treatment 6 to treatments 1 to 5, a significantly higher incidence of vascular browning was observed in treatment 3 (Table 3.26).

Table 3.12 1994 Temperature regime trial A. Average firmometer readings and percentage fruit with various physiological and pathological disorders.

Treat	Black cold	Dist. end brown	Pulp spot	Grey pulp	Vasc. brown	Anth.	Stem end rot	Firmo*
1	0	0	0	0.7	1.4	0	0.7	25.5
2	0	0	0	0	0.7	0	12.9	30.0
3	3.6	0	0	0.7	0	0	5.7	25.3
4	0.7	0.7	0.7	0.7	1.4	0	0.7	26.5
5	2.1	0.7	2.9	2.1	2.1	0	1.4	25.6
6	0	0	0.7	1.4	2.1	0	4.3	29.4
7	7.1	0	0	2.9	2.1	0	2.1	25.1
8	2.1	0.7	2.1	3.6	5.7	0	2.9	26.1
9	0.7	0	2.9	3.6	5.7	0.7	5.7	24.1

*LSD (5%) = 2.0

1994 Trail A (Table 3.13)

There were significant differences between treatments in the incidence of all disorders noted with the exception of grey pulp and stem end rot (Table 3.25). The incidence of all disorders was generally low, as was the case at the beginning of the 1993 season (Table 3.3). Comparing treatment 6 with treatments 1 to 5, only treatment 2 had a significantly higher incidence of stem end rot than treatment 6 (Table 3.27).

Table 3.13 1994 Temperature regime trial B. Average firmometer readings and percentage fruit with various physiological and pathological disorders.

Treat	Black cold	Dist. end brown	Pulp spot	Grey pulp	Vasc. brown	Anth.	Stem end rot	Firmo*
1	2.9	4.3	4.3	4.3	2.9	0	5.0	25.6
2	1.4	0	2.9	2.2	1.4	0	2.9	24.7
3	1.4	0.7	0.7	1.4	0.7	0	1.4	26.1
4	2.9	0	0	0.7	2.1	0	2.1	26.1
5	1.4	1.4	3.6	2.2	4.3	0	0.7	25.4
6	3.7	1.5	0.7	0.7	3.7	0	6.7	23.9
7	1.4	0	0.7	0.7	1.4	0	0.7	26.0
8	2.9	0	0	0	0	0	0	25.0
9	10.7	3.3	5.7	8.2	6.6	0	17.2	30.3

*LSD (5%) = 2.4

1994 Trial B (Table 3.13)

There were significant differences between treatments in the incidence of all disorders noted with the exception of anthracnose (Table 3.25). Treatment 9 fruit was significantly softer than fruit in all the other treatments and had the highest incidence of black cold, pulp spot, grey pulp, and vascular browning as was expected with a longer period of cold storage than the other treatments. Comparing treatment 6 to treatments 1 to 5, treatments 3, 4 and 5 all had a significantly lower incidence of stem end rot than treatment 6 (Table 3.27).

Table 3.14 1994 Temperature regime trial C. Average firmometer readings and percentage fruit with various physiological and pathological disorders.

Treat	Black cold	Dist. end brown	Pulp spot	Grey pulp	Vasc. brown	Anth.	Stem end rot	Firmo*
1	0	0	0	0	0	0	0	27.2
2	0.7	0	3.7	1.5	5.9	0	5.1	26.5
3	0.7	0	0	0	15.4	0	3.7	25.1
4	0.7	2.2	1.5	0.7	4.4	0	10.2	27.7
5	0.7	0	0	0	10.9	0	5.8	26.6
6	0	0	0	0.7	16.8	0	0	27.1
7	0	0	0.7	0.7	1.5	0	0.7	28.1
8	0	0	0.7	0	0	0	6.4	26.2
9	2.1	7.1	8.6	7.9	7.9	0	2.9	26.0

*LSD (5%) = 3.6

1994 Trial C (Table 3.14)

There were significant differences between treatments in the incidence of all disorders except for anthracnose which was absent in this trial (Table 3.25). There was greater variation in fruit firmness within treatments than in most of the other temperature regime trials as indicated by the LSD for firmometer readings of 3.6 whereas this figure is around 2.0 in most of the other trials.

Comparing treatment 6 to treatments 1 to 5, treatment 1 had a significantly lower incidence of vascular browning than treatment 6 Table (3.27), and produced the highest quality fruit with which was hard and had no physiological or pathological disorders.

Table 3.15 1994 Temperature regime trial D. Average firmometer readings and percentage fruit with various physiological and pathological disorders.

Treat	Black cold	Dist. end brown	Pulp spot	Grey pulp	Vasc. brown	Anth.	Stem end rot	Firmo*
1	0	5.4	0.9	0	9.8	0.9	2.7	29.3
2	0.9	11.8	7.3	7.3	9.1	6.4	5.5	25.3
3	4.5	1.8	1.8	2.7	7.1	0.9	5.4	28.7
4	3.1	0	1.0	1.0	2.0	0	1.0	26.6
5	1.8	0	1.8	1.8	4.5	0.9	1.8	26.4
6	0.9	0.9	2.7	2.7	11.8	0	0.9	27.1
7	0	0	1.8	1.8	0.9	0	0	26.1
8	0	1.4	1.4	2.9	5.7	0.7	1.4	27.5
9	0	3.8	7.5	2.8	13.2	1.9	4.7	25.8

*LSD (5%) = 3.0

1994 Trial D (Table 3.15)

There were significant differences between treatments in the incidence of all disorders noted with the exception of grey pulp (Table 3.25). Treatment 2 had the highest incidence of distal end browning which was associated with a relatively high incidence of grey pulp, pulp spot and vascular browning.

Comparing treatment 6 to treatments 1 to 5, treatment 2 had a significantly higher incidence of distal end browning, and treatment 4 had a significantly lower incidence of vascular browning (Table 3.27).

Table 3.16 1994 Temperature regime trial E. Average firmometer readings and percentage fruit with various physiological and pathological disorders.

Treat	Black cold	Dist. end brown	Pulp spot	Grey pulp	Vasc. brown	Anth.	Stem end rot	Firmo*
1	0	2.1	5.7	2.1	10.7	2.9	7.1	27.7
2	0.7	0	3.6	2.9	11.4	4.3	5.0	26.0
3	0.7	2.9	5.7	2.9	12.9	2.9	8.6	26.1
4	2.1	3.6	5.7	7.1	19.3	0.7	3.6	27.4
5	1.4	2.9	3.6	4.3	14.0	4.3	12.1	27.0
6	2.1	2.1	2.1	2.9	9.3	0	5.0	26.5
7	0	5.6	5.6	4.8	10.3	1.6	2.4	25.7
8	5.0	5.0	1.4	0.7	10.7	0	0.7	27.0
9	1.4	4.3	8.6	10.7	12.1	3.6	10.7	27.7

*LSD (5%) = 1.7

1994 Trial E (Table 3.16)

There were significant differences between treatments in the incidence of black cold , anthracnose and stem end rot (Table 3.25). Treatment 8 had the highest incidence of black cold (5%) which was not expected as treatment 8 was of the shortest duration (Table 3.1), and treatment 4 only had an incidence of black cold of 2.1 % even though the first 3 weeks of treatment 4 were identical to treatment 8 (Table 3.1), which implicates pre-harvest factors as determining sensitivity to this disorder.

Comparing treatment 6 to treatments 1 to 5, treatment 4 had a significantly higher incidence of vascular browning (Table 3.27), which also did not seem to be related to storage temperatures as treatment 9 which was identical to treatment 4 for the first 4 weeks (Table 3.1) had a lower incidence of this disorder (12% as opposed to 19.3%).

Table 3.17 1994 Temperature regime trial F.Average firmometer readings and percentage fruit with various physiological and pathological disorders.

Treat	Black cold	Dist. end brown	Pulp spot	Grey pulp	Vasc. brown	Anth.	Stem end rot	Firmo*
1	1.4	8.6	7.1	11.4	17.1	10.0	18.6	23.5
2	0	9.3	5.0	9.3	16.4	0.7	6.4	23.3
3	0	7.1	7.1	3.6	8.6	1.4	2.9	25.6
4	2.9	3.6	6.4	11.4	17.1	2.1	5.7	23.7
5	0	0.7	8.6	11.4	17.1	2.9	9.3	27.0
6	6.4	12.1	9.3	10.0	18.6	4.3	11.7	23.7
7	1.4	5.0	2.9	2.9	16.4	0	6.4	25.6
8	0.7	3.6	5.7	5.0	14.3	4.3	6.4	28.7
9	0.7	2.1	4.3	5.7	10.0	2.9	6.4	26.3

*LSD (5%) = 2.3

1994 Trial F (Table 3.17)

There were significant differences between treatments in the incidence of black cold, grey pulp, anthracnose and stem end rot. The incidence of grey pulp over all treatments was generally higher than in 1994 trial E (Table 3.17), which suggests that pre-harvest factors play a role in determining fruit sensitivity to this disorder. An increase in fruit maturity was not responsible as trails E and F had similar fruit moisture contents of 71.5% and 72.4% respectively (Table 3.28).

Comparing treatment 6 to treatments 1 to 5, treatments 1 to 5 all had a significantly lower incidence of black cold , treatments 4 and 5 had a significantly lower incidence of distal end browning, and treatment 3 had a significantly lower incidence of grey pulp, and vascular browning (Table 3.27). The incidence of distal end browning was highest in treatment 6 and a high incidence of grey pulp, pulp spot and vascular browning was associated with this disorder, as was the case with treatment 2 in 1994 trial D (Table 3.15).

Table 3.18 1994 Temperature regime trial G. Average firmometer readings and percentage fruit with various physiological and pathological disorders.

Treat	Black cold	Dist. end brown	Pulp spot	Grey pulp	Vasc. brown	Anth.	Stem end rot	Firmo*
1	0	5.7	6.4	7.1	13.6	2.9	5.7	24.5
2	2.1	3.6	3.6	4.3	11.4	5.7	4.3	24.6
3	1.4	2.1	5.5	4.1	11.0	1.4	4.8	24.0
4	0.8	2.4	7.1	7.1	12.7	0	6.3	25.3
5	1.6	3.2	5.6	4.8	12.7	2.4	7.1	24.3
6	0.7	2.1	8.6	11.4	11.4	2.1	10.7	24.6
7	0	0.7	4.8	6.8	15.1	6.8	11.6	23.1
8	2.1	3.6	6.4	6.4	14.3	3.6	9.3	25.2
9	4.1	4.8	6.8	11.0	17.8	6.8	13.7	26.7

*LSD (5%) = 1.3

1994 Trial G (Table 3.18)

There were significant differences between treatments in the incidence of anthracnose, stem end rot and black cold (Table 3.25). There were no significant differences between treatments in the incidence of distal end browning which probably explains why there were also no significant differences in the incidence of pulp spot, grey pulp or vascular browning (Table 3.25) as they are associated with distal end browning.

Comparing treatment 6 to treatments 1 to 5, treatments 2 and 3 had a significantly lower incidence of grey pulp and treatment 2 had a significantly lower incidence of stem end rot (Table 3.27).

Table 3.19 1994 Temperature regime trial H. Average firmometer readings and percentage fruit with various physiological and pathological disorders.

Treat	Black cold	Dist. end brown	Pulp spot	Grey pulp	Vasc. brown	Anth.	Stem end rot	Firmo*
1	0.7	2.9	10.7	8.6	12.9	0	2.9	26.3
2	3.6	3.6	5.0	7.1	10.7	5.0	9.3	24.6
3	0.7	0	1.4	2.1	14.3	5.7	10.7	25.6
4	7.1	2.1	9.3	3.6	16.4	3.6	6.4	27.3
5	4.3	12.1	16.4	16.4	9.3	2.1	2.1	25.2
6	5.0	0	14.3	5.0	20.0	7.9	14.3	24.6
7	4.3	2.1	2.1	5.0	20.0	1.4	7.1	25.2
8	2.1	2.1	5.0	3.6	7.1	1.4	6.4	25.1
9	0	0.7	8.6	13.6	22.1	3.6	10.7	25.5

*LSD (5%) = 2.6

1994 Trial H (Table 3.19)

There were significant differences between treatments in the incidence of all disorders noted (Table 3.25). Treatment 5 had the highest incidence of distal end browning (12.1%), as well as pulp spot (16.4%) and grey pulp (16.4%) which were associated with distal end browning.

Comparing treatment 6 to treatments 1 to 5, treatment 5 had a significantly higher incidence of distal end browning and grey pulp but a significantly lower incidence of vascular browning, anthracnose and stem end rot. Treatment 2 had a significantly lower incidence of pulp spot, vascular browning, anthracnose and stem end rot and treatment 3 had a significantly lower incidence of pulp spot (Table 3.27).

Table 3.20 1994 Temperature regime trial I. Average firmometer readings and percentage fruit with various physiological and pathological disorders.

Treat	Black cold	Dist. end brown	Pulp spot	Grey pulp	Vasc. brown	Anth.	Stem end rot	Firmo*
1	2.0	0	11.3	6.7	18.7	10.0	13.3	24.7
2	3.4	1.4	16.4	15.1	29.5	4.8	6.2	25.0
3	2.1	1.4	7.5	5.5	22.6	4.8	13.0	25.3
4	4.9	2.1	4.9	6.9	16.0	13.9	6.9	25.9
5	4.9	0	0.7	4.2	28.9	4.9	9.9	25.4
6	0.7	2.1	8.2	6.8	16.4	6.8	4.8	24.8
7	13.9	0	4.2	5.6	25.7	3.5	9.7	25.3
8	2.7	0	6.8	7.4	9.5	2.7	9.5	26.3
9	2.7	0	1.3	0.7	4.0	0.7	1.3	27.5

*LSD (5%) = 1.6

1994 Trial I (Table 3.20)

There were significant differences between treatments in the incidence of all disorders noted with the exception of black cold (Table 3.27). Treatment 2 had the highest incidence of pulp spot, grey pulp and vascular browning.

Comparing treatment 6 to treatments 1 to 5, treatment 2 had a significantly higher incidence of pulp spot, grey pulp and anthracnose contrary to the results of trial H run the previous week (Table 3.19), where the incidence of pulp spot and vascular browning was significantly lower than in treatment 6. Treatment 5 had a significantly lower incidence of pulp spot than treatment 6 but a higher incidence of vascular browning. Treatment 4 had a higher incidence of anthracnose than treatment 6 and treatments 1 and 3 had a higher incidence of stem end rot than treatment 6 (Table 3.27).

Table 3.21 1994 Temperature regime trial J. Average firmometer readings and percentage fruit with various physiological and pathological disorders.

Treat	Black cold	Dist. end brown	Pulp spot	Grey pulp	Vasc. brown	Anth.	Stem end rot	Firmo*
1	1.5	5.9	4.4	12.5	14.7	2.9	11.8	27.6
2	0.8	6.9	8.4	9.9	21.4	0.8	8.4	26.3
3	0.7	4.3	9.4	12.3	18.1	2.2	9.4	26.6
4	0	1.4	9.2	14.8	17.6	3.5	14.8	24.7
5	0	1.5	10.3	13.2	26.5	3.7	12.5	27.1
6	0.7	1.5	4.4	10.9	24.1	2.9	11.7	24.9
7	1.4	10.3	10.3	12.3	17.8	4.1	4.8	25.3
8	0.7	0.7	8.2	8.2	24.0	4.8	13.7	26.3
9	0.7	5.7	2.1	11.4	22.1	0	11.4	25.8

*LSD (5%) = 2.0

1994 Trial J (Table 3.21)

There were significant differences between treatments in the incidence of black cold, distal end browning pulp spot and stem end rot (Table 3.25). The incidence of black cold was generally very low with a maximum of 1.5% in treatment 1. Treatment 7 had the highest incidence of distal end browning which was once again associated with high incidences of pulp spot, grey pulp and vascular browning.

Comparing treatment 6 to treatments 1 to 5, treatment 1 had a significantly higher incidence of black cold, and treatment 2 had a significantly higher incidence of distal end browning.

Table 3.22 Percentage 'Fuerte' fruit with lenticel damage in 1993 temperature regime trials.

Trial	Treatment								
	1	2	3	4	5	6	7	8	9
A	13.9	8.2	23.0	32.8	31.2	31.2	32.0	6.0	26.4
B	60.5	84.0	11.5	36.9	20.6	71.4	29.6	-	-
C	96.4	80.0	60.7	77.9	50.0	77.1	51.4	74.3	92.9
D	95.0	91.5	96.4	100	94.3	89.3	84.3	80.0	3.6
E	15.0	12.1	17.9	13.6	25.3	18.6	12.9	81.4	22.9
F	4.3	6.4	11.4	10.0	10.7	6.4	2.9	12.9	4.3
G	17.1	27.9	17.1	15.7	7.1	36.4	5.7	7.1	21.4
H	2.9	11.4	12.1	10.0	2.1	11.4	4.3	5.0	0.7
I	15.0	1.4	17.1	0	8.6	14.3	0.7	8.5	0.7

Table 3.23 Percentage 'Fuerte' fruit with lenticel damage in 1994 temperature regime trials.

Trial	Treatment								
	1	2	3	4	5	6	7	8	9
A	73.6	95.0	92.1	99.3	92.1	94.3	97.1	53.6	86.4
B	50.7	84.8	48.5	42.6	54.3	85.8	82.6	97.1	45.1
C	56.4	36.8	81.0	56.9	63.6	51.6	34.1	90.7	17.5
D	19.6	34.5	24.1	33.7	7.3	28.2	38.2	42.1	17.0
E	23.6	22.1	25.0	60.0	32.1	57.9	26.2	50.7	30.0
F	7.9	7.9	36.4	10.0	40.7	16.4	29.3	10.7	7.1
G	9.3	20.0	42.8	15.9	11.9	15.7	52.7	15.7	24.7
H	5.7	12.9	20.7	35.7	12.1	18.6	18.6	25.0	22.9
I	20.7	30.1	23.3	32.6	28.9	27.4	45.1	18.2	14.7
J	26.5	42.7	16.7	12.7	23.5	29.9	13.7	14.4	22.9

Table 3.24 Statistical differences between all treatments in 1993 temperature regime trials.

Trial	Black cold	Pulp spot	Grey pulp	Vasc. brown	Anth.	Stem end rot	Lenti-cel damage
A	**	NS	NS	NS	NS	NS	**
B	**	**	**	NS	**	*	**
C	**	**	**	*	**	**	**
D	**	**	**	**	**	**	**
E	NS	*	**	*	NS	NS	**
F	**	**	NS	**	**	**	**
G	*	**	**	**	NS	NS	**
H	NS	NS	*	*	**	NS	**
I	NS	**	**	**	**	**	**

* = significantly different at 95% level. ** = significantly different at 99% level
NS = not significantly different.

Table 3.25 Statistical differences between all treatments in 1994 temperature regime trials.

Trial	Black cold	distal end brown.	Pulp spot	Grey pulp	Vasc. brown.	Anth.	Stem end rot	Lenti-cel damage
A	**	**	*	NS	*	NS	**	**
B	**	**	**	**	*	NS	**	**
C	**	**	**	**	**	NS	**	**
D	**	**	*	NS	**	**	*	**
E	**	NS	NS	**	NS	*	**	**
F	**	**	NS	**	NS	**	**	NS
G	*	NS	NS	NS	NS	**	*	**
H	**	**	**	**	**	**	**	**
I	NS	*	**	**	**	**	**	**
J	*	**	*	NS	NS	NS	**	**

* = significantly different at 95% level. ** = significantly different at 99% level
NS = not significantly different.

Table 3.26 Summary of analyses of treatments 1-6 of 1993 temperature regime trials showing those treatments which were different fom treatment 6 (control) for the various disorders.

Trial	Blac k cold	Pulp spot	Grey pulp	Vasc. brown	Anth .	Stem end rot	Lenti- cel damage
A	$1^{h}3^{h}$	-	-	-	-	-	$1^{l}2^{l}$
B	$(1\text{-}5)^{l}$	-	-	-	-	-	$2^{h}(3\text{-}5)^{l}$
C	-	-	-	-	-	4^{l}	-
D	$2^{h}3^{h}5$ h	-	-	-	-	-	-
E	-	-	-	-	-	-	-
F	-	4^{h}	-	-	-	-	-
G	-	$2^{h}4^{h}$	-	$1^{h}4^{h}$	-	-	$1^{l}3^{l}4^{l}5^{l}$
H	-	-	-	-	-	-	$1^{l}5^{l}$
I	-	-	-	3^{h}	-	-	$2^{l}4^{l}$

[h]Significantly higher incidence of disorder at 95% level.
[l]Significantly lower incidence of disorder at 95% level.

Table 3.27 Summary of analyses of treatments 1-6 of 1994 temperature regime trials showing those treatments which were different fom treatment 6 (control) for the various disorders.

Trial	Black cold	Distal end brown	Pulp spot	Grey pulp	Vasc. brown	Anth.	Stem end rot	Lenti-cel damage
A	-	-	-	-	-	-	2^{h}	$1^{l}4^{h}$
B	-	-	-	-	-	-	$3^{l}4^{l}5^{l}$	$1^{l}3^{l}4^{l}5^{l}$
C	-	-	-	-	1^{l}	-	-	$2^{l}3^{h}5^{h}$
D	-	2^{h}	-	-	4^{l}	-	-	5^{l}
E	-	-	-	-	4^{h}	-	-	$1^{l}2^{l}3^{l}5^{l}$
F	$(1\text{-}5)^{l}$	$4^{l}5^{l}$	-	3^{l}	3^{l}	-	$1^{h}3^{l}$	$1^{l}2^{l}3^{l}5^{l}$
G	-	-	-	$2^{l}3^{l}$	-	-	2^{l}	3^{h}
H	-	5^{h}	$2^{l}3^{l}$	5^{h}	$2^{l}5^{l}$	$1^{l}5^{l}$	$1^{l}4^{l}5^{l}$	$1^{l}4^{h}$
I	-	-	$2^{h}5^{l}$	2^{h}	$2^{h}5^{h}$	4^{h}	$1^{h}3^{h}$	1^{l}
J	1^{h}	2^{h}	-	-	-	-	-	$3^{l}4^{l}$

[h]Significantly higher incidence of disorder at 95% level.
[l]Significantly lower incidence of disorder at 95% level.

Although treatments 1 to 5 showed significantly less lenticel damage than treatment 6 in a number of trials in both the 1993 and 1994 seasons (Tables 3.26 and 3.27), this disorder was not used as a measure of the effect of temperature regime on cold storage disorders as treatment 9 often had a lower incidence of lenticel damage than treatment 4. For example in the 1993 trial D (Table 3.22) and 1993 trial E (Table 3.23), yet the first four weeks of treatment 9 were identical to treatment 4 (Table 3.1). Lenticel damage is probably as a result of pre-harvest factors or rough handling rather than low storage temperatures. In addition, a "presence or absence" rating of this disorder does not take into account the severity of the disorder and consequently a fruit with only a few damaged lenticels will receive the same rating as a fruit where the majority of lenticels are damaged.

In 1993, treatments 1 to 5 of trial B and treatment 4 of trial E had a lower incidence of black cold and stem end rot than treatment 6 (Table 3.26). In all the other cases where there were significant differences between treatment 6 and the other five treatments the incidence of those disorders was higher than those in treatment 6. The incidence of black cold in 1993 trial B was much higher in treatment 6 (39.7% as opposed to 2.3% for treatments 4 and 5) (Table 3.4), but in trial D of the same year, treatments 2, 3 and 5 had a higher incidence of this disorder then treatment 6 (Tables 3.6 and 3.26). From this, it is obvious that one cannot conclude that the stepped down temperature regimes of treatments 2, 3 and 5 reduced the incidence of black cold. However one can more easily conclude that stepped down temperature regimes were not more effective than 5.5°C throughout the storage period for 'Fuerte' fruit from Everdon Estate in 1993. In some cases, e.g. 1994 trail G (Table 3.26), the stepped down regimes produced fruit with a higher incidence of vascular browning and grey pulp.

For the first 5 weeks of the 'Fuerte' harvesting season (trials A - E), treatments 1 - 5 seldom produced a lower incidence of internal physiological disorders (Table 3.26), which is contrary to the findings of Vorster *et al.* (1990) where higher temperatures during the earlier stages of the season reduced the incidence of internal physiological disorders in Transvaal fruit. It must be borne in mind however, that fruit from different geographic locations may react differently to cold storage (Rowell, 1988).

During the 6th to 8th weeks of the 1994 season (trials F - H), some of the stepped down temperature regimes showed a lower incidence of pathological and physiological disorders than treatment 6, although trials I and J proved the stepped down temperature regimes in some cases to be worse (Table 3.27). As was the case with the 1993 trials, higher storage temperatures during the earlier part of the 1994 season did not reduce the incidence of internal physiological disorders. The lower incidence of disorders in some of the stepped down temperature regimes in trials F - H cannot be explained by fruit maturity as there was very little difference in fruit maturity between trials H and I based on fruit moisture content (Table 3.28).

Table 3.28 Moisture content (% by mass) of 'Fuerte' fruit used in the 1993 and 1994 temperature regime trials.

Trial	1993	1994
A	76,6	77.8
B	74.2	75.7
C	73.8	76.3
D	73.8	73.7
E	72.9	71.5
F	69.8	72.4
G	69.3	74.1
H	65.4	68.1
I	64.5	67.2
J	-	66.8

Fruit firmness on arrival in Europe was identified by Bezuidenhout and Eksteen (1994) as an important quality determining factor. Fruit with firmometer readings of < 35 are classified as acceptably firm by the South African Avocado Growers' Association and fruit with a firmometer reading of < 30 are classified as "hard" (J.F. Hardy, 1994[5]). With the

[5]Hardy, J.F. 1994. Pers. comm. S. Afr. Avocado Grw. Assoc., Tzaneen.

exception of treatment 7 in 1993 trial B (Table 3.4), all treatments in the 1993 and 1994 trials yielded fruit with a firmometer reading of < 30.

In order to observe seasonal trends in the occurrence of physiological disorders, the occurrence of each disorder in treatment 6 was plotted for both the 1993 and 1994 seasons (Figs 3.1 to 3.5). The incidence of black cold was generally low in both seasons except in trial B of 1993 (Fig. 3.1), however the incidence of black cold in treatments 1 - 5 for that trial was very low or non-existent (Table 3.4). As it seems that fruit moisture loss plays a role in causing black cold (see Chapter 4), the higher incidence of this disorder in this treatment may have been due a higher percentage of water-stressed fruit in that treatment. Because the fruit used in these trials was taken from a commercial packline, it was not possible to determine whether all the fruit had undergone exactly the same treatment from the time of harvest to cold storage, which makes it difficult to pinpiont the cause of the higher incidence of black cold in treatment 6.

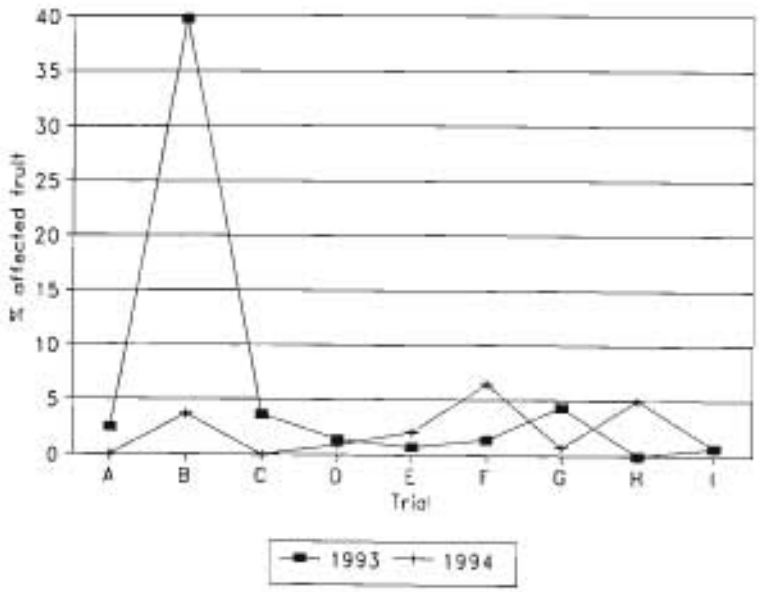

Fig. 3.1 Incidence of black cold in treatment 6 during the 1993 and 1994 'Fuerte' harvesting seasons.

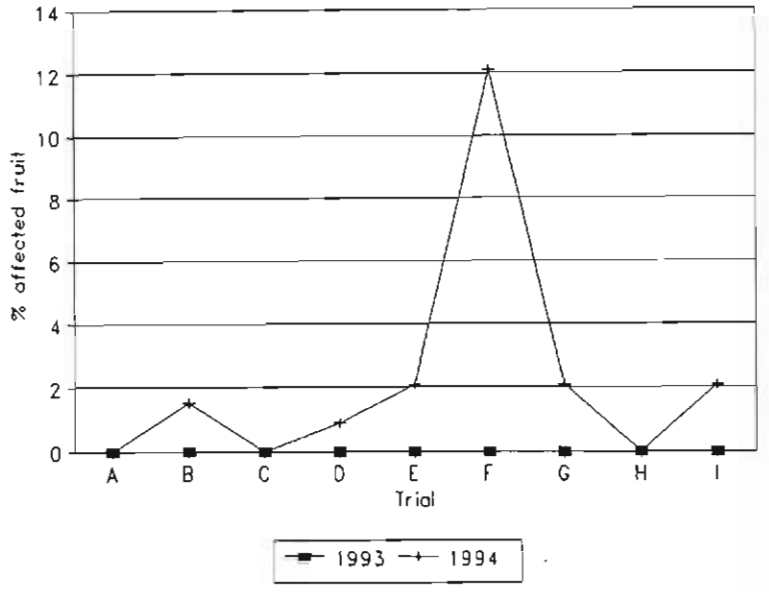

Fig. 3.2 Incidence of distal end browning in treatment 6 during the 1993 and 1994 harvesting seasons.

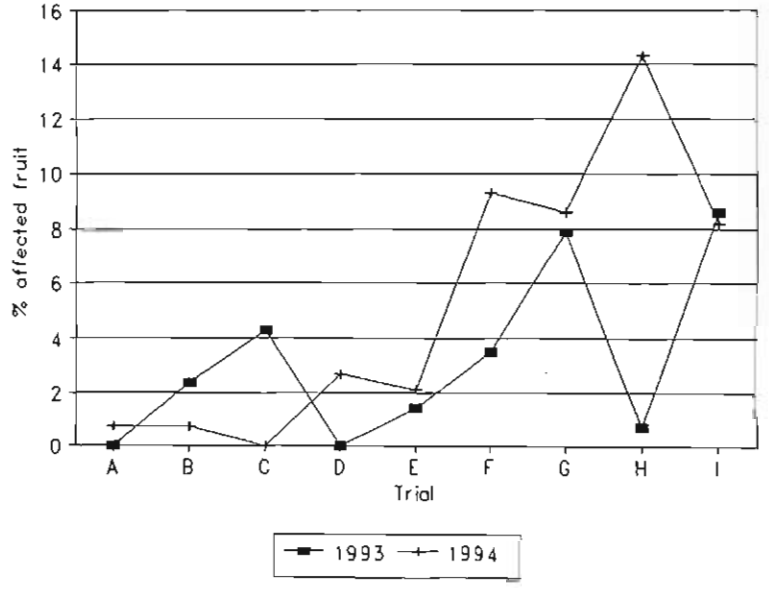

Fig. 3.3 Incidence of pulp spot in treatment 6 during the 1993 and 1994 'Fuerte' harvesting seasons.

Distal end browning (Fig 3.2) was only observed in a few fruits in the 1993 season and was not recorded as a disorder when evaluating the ripe fruit, but was more prevalent in the 1994 season where the disorder occurred in between 0 and 12% of the fruit (Fig. 3.2). Not all treatments had as high an incidence of the disorder in trial F of 1994. The incidence in treatments 4 and 5 was significantly lower (Table 3.27). The incidence of pulp spot (Fig. 3.3), grey pulp (Fig. 3.4) and vascular browning (Fig. 3.5) was generally higher in 1994 than in 1993. This indicates that pre-harvest factors play an important role in determining the quality of cold stored avocados. At Everdon Estate, the 1993 crop was heavier than the 1994 crop with less vegetative growth and the incidence of internal physiological disorders was higher in 1994, confirming industry experience that fruit physiological disorders are worse in seasons characterised by high tree vigour, usually associated with light cropping (Wolstenholme, 1994[6]). It seems that the vegetative-reproductive balance in avocado trees is crucial in determining tolerance of the fruit to low storage temperatures. Cultural practices should be employed to reduce the vigour of the spring flush.

[6]Wolstenholme, B.N. 1994. Pers. comm. Dept. Hort. Sci. University of Natal, Pietermaritzburg.

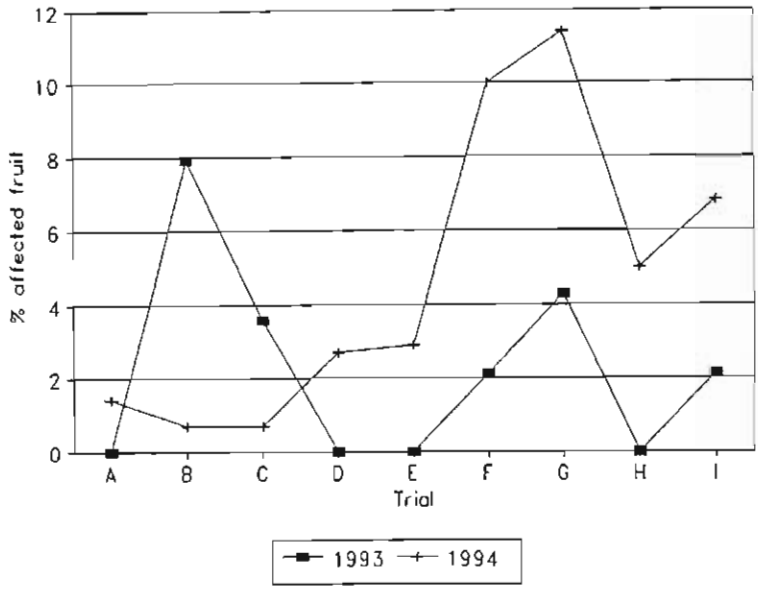

Fig. 3.4 Incidence of grey pulp in treatment 6 during the 1993 and 1994 'Fuerte' harvesting seasons.

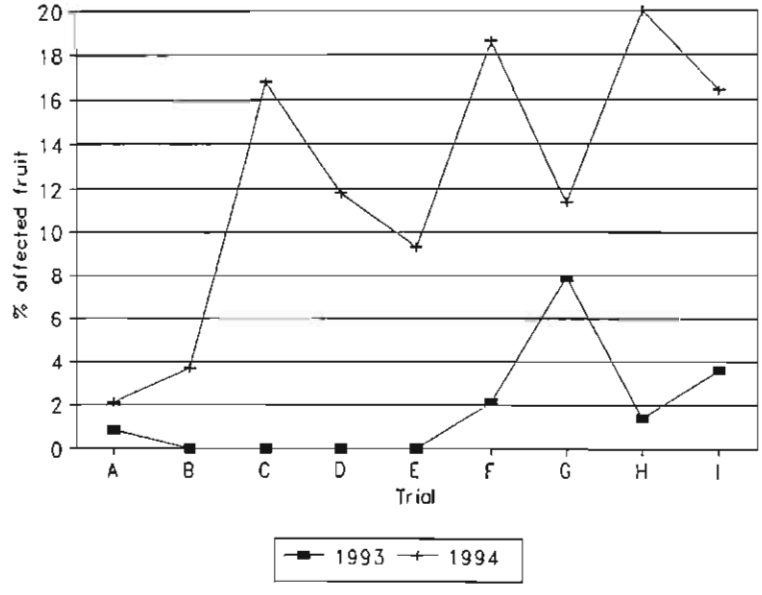

Fig. 3.5 Incidence of vascular browning in treatment 6 during the 1993 and 1994 'Fuerte' harvesting seasons.

Fig. 3.6 'Fuerte' fruit showing distal end browning and associated pulp spot, grey pulp and vascular browning.

3.3.2 Total phenolic quantification

There was no significant difference between total phenolic concentration in the fruit mesocarp during the 1994 'Fuerte' harvesting season ($P < 0.05$) (Fig. 3.7). Trials F, G and H of 1994 showed an increase in the incidence of pulp spot (Fig. 3.3) and grey pulp (Fig. 3.4) compared the previous 5 weeks of the harvesting season. This increase in browning disorders was not accompanied by an increase in total phenolic levels. The results obtained in this experiment are in agreement with those of Cutting *et al.* (1992) where there was no marked increase in phenolic levels from May to July in 'Fuerte' fruit harvested at Everdon Estate. Their work only showed an increase in total phenolics in September and October which falls outside of the 'Fuerte' harvesting season at Everdon Estate.

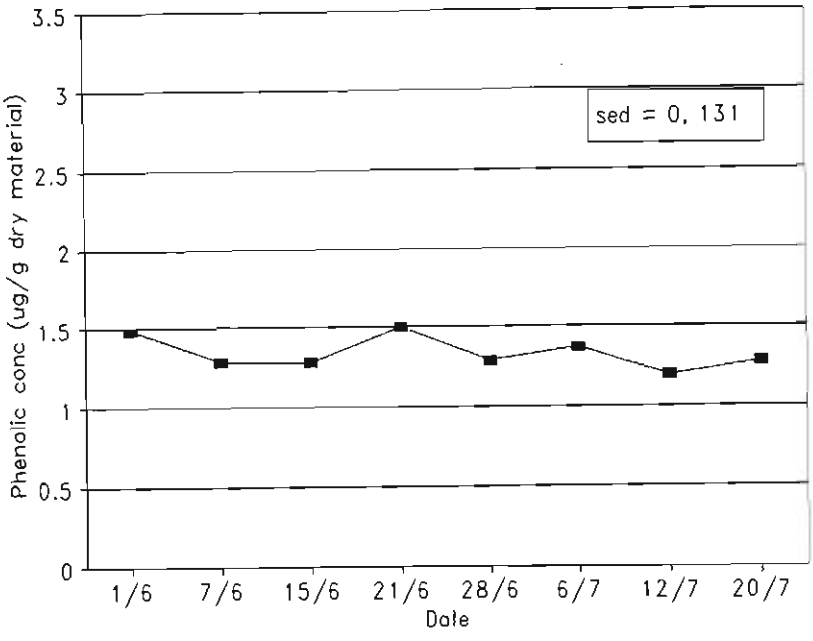

Fig. 3.7 Total phenolic concentration in 'Fuerte' avocado mesocarp of fruit harvested at weekly intervals during the 1994 'Fuerte' harvesting season.

3.3.3 Ethylene evolution

The levels of ethylene production during storage at 5.5°C and 7.5°C were very low viz. mostly between 0 and 5 $\mu l.kg^{-1}.h^{-1}$. On removal from cold storage, the fruit stored at 7.5°C had a higher rate of ethylene production, peaking at 109 $\mu l.kg^{-1}.h^{-1}$ as opposed to 75 $\mu l.kg^{-1}.h^{-1}$ for the fruit stored at 5.5°C (Fig. 3.8). An increase in the levels of ethylene production in both the 7.5°C and 5.5°C treatments occurred on day nine of cold storage, but it is not known if the levels continued to increase on day ten as no samlpes were taken on that day. Increased duration of storage also reduces the amount of ethylene produced at the climacteric peak after removal from cold storage in 'Hass' fruit (Eaks, 1983). The degree of cold stress therefore seems to have a negative effect on the levels of ethylene produced after cold storage. Similar results were obtained by Zamorano *et al.* (1994) who found that avocado fruit softening was delayed at 7°C but was inhibited at 3°C during a storage period of 55 days and that ethylene production rates were drastically reduced by cold storage. Although

ethylene production was inhibited, CO_2 production increased after 25 days of storage at 7°C which coincided with softening.

Sanxter *et al.* (1994) found that ethylene scrubs did not influence the development of chilling injury in cold stored 'Sharwil' avocados. This is probably because levels of ethylene produced during cold storage are very low. It is also noteworthy that the rise in ethylene production associated with the respiratory climacteric, occurred after removal from cold storage, not during cold storage, contrary to a common industry perception that this occurs during the sea voyage to Europe.

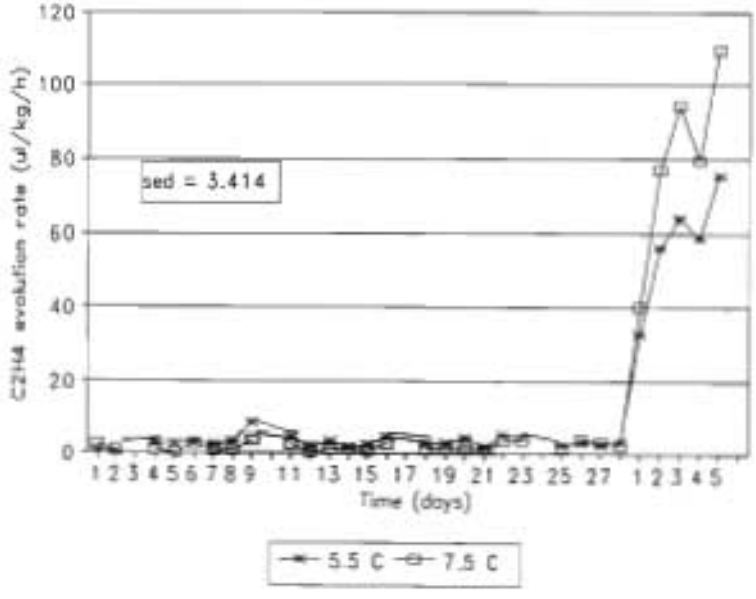

Fig. 3.8 Ethylene evolution rate of 'Fuerte' avocado fruit stored at 5.5°C and 7.5°C for 28 days and then allowed to ripen at room temperature.

3.4 CONCLUSIONS

It seems that industry experience of soft fruit on arrival in Europe is due to a break or breaks in the cold chain from the time the fruit leaves the packhouse to when it arrives at its destination, as all the fruit in the 1993 and 1994 trials, with the exception of 1993 Trial 2 treatment 7, had average firmometer readings of < 35 (which is considered acceptable), and the majority of the trials produced fruit with firmometer readings of < 30. Higher storage temperatures during the earlier part of the season do not seem to have an effect on the incidence of black cold or internal physiological disorders. It appears that in Natal , 'Fuerte' fruit harvested in the first 6 weeks of the season has a lower incidence of grey pulp, pulp spot and vascular browning. From the moisture contents of fruit in the 1993 and 1994 trials, it appears that the incidence of these disorders increases at a moisture content of < ca. 73% (Table 3.28). Export of 'Fuerte' fruit before this stage of maturity would probably result in a lower incidence of these physiological disorders.

The absence of distal end browning and lower incidences of vascular browning, grey pulp and pulp spot in the latter part of the 'Fuerte' harvesting season of 1993 as opposed to the 1994 season shows that fruit quality and susceptibility to physiological disorders is seasonally dependent. The high quality fruit of the 1993 season was associated with a heavy crop and low tree vegetative vigour. In contrast, the fruit of relatively poorer quality in the 1994 season was associated with greater tree vegetative vigour and a lighter crop. Mesocarp discolouration has been associated with lower fruit calcium levels (Resnisky and Sive, 1993) and the spring vegetative flush (which coincides to varying extents with fruit set), is a stronger sink for calcium than setting fruit (Witney *et al*, 1990). In seasons where there is a heavier crop and less vigourous vegetative growth (such as 1993), export of 'Fuerte' fruit harvested over a longer period is probably possible without a higher incidence of internal physiological disorders in the more mature fruit. A system would have to be developed however, where one could determine the potential for physiological disorders using factors such as vigour of the spring flush, fruit calcium concentration and crop load.

Stepped down temperature regimes did not show a trend of reducing the incidence of physiological disorders compared to 4 weeks of storage at 5.5°C. Although there were some stepped down temperatures which produced lower incidences of physiological disorders in

some trials, there were other trials where the incidence of some disorders was lower in fruit stored continually at 5.5°C. No trends were evident w.r.t. fruit maturity and different temperature regimes producing the best quality fruit. Stepped down temperature regimes appear therefore to be no better than storage at 5.5°C throughout the entire storage period for 'Fuerte' avocados grown in the Natal Midlands.

Although total phenolic concentration has been implicated as a factor causing mesocarp discolouration (Golan *et al.*, 1977), the increase in vascular browning, pulp spot and grey pulp from the 6th week in the 1994 season (Trial F) was not accompanied by an increase in fruit phenolic concentration. Fruit phenolic levels do not appear therefore, to have an influence on the incidence of mesocarp discolouration during the normal harvesting season at Everdon Estate.

Ethylene evolution levels of 'Fuerte' avocados showed only slight fluctuations during cold storage and it is certain that the climacteric rise in ethylene production associated with the respiratory climacteric does not occur during cold storage, but occurs on removal from cold storage. In addition, there does not seem to be a stage during cold storage at which 'Fuerte' avocado fruit become less sensitive to chilling temperatures, as stepped down temperature regimes appear to have been no more effective than a constant 5.5°C in reducing the incidence of disorders, even in the 1994 season where the fruit was more susceptible to physiological disorders than in 1993.

It appears therefore, that prompt cooling after harvest and strict maintenance of the cold chain until the fruit reaches the market, is the best strategy for landing hard fruit of high quality in Europe. Fruit should be cooled to the desired pulp temperature in the packhouse cooling facilities and only be transported to the docks once that temperature is reached, as refrigerated trucks do not have the capacity to cool fruit efficiently. Fruit temperatures above 7°C during this time may initiate ripening, resulting in soft fruit. As room cooling, which is currently used in the South African avocado industry is not as efficient in removing field heat with minimal moisture loss as methods such as forced air cooling and hydrocooling (Mitchell, 1992), research in this area would be advantageous to the industry.

3.5 SUMMARY

Avocados grown in the Natal Midlands mature approximately 2 months later than the same cultivars grown in the Northern and Eastern Transvaal, and the stepped down temperature regimes used for cold storing Transvaal fruit during export are not as effective for Natal fruit. Nine different temperature regimes were tested on 'Fuerte' fruit from Everdon Estate in the Natal Midlands during the 1993 and 1994 seasons. The 1993 fruit showed a lower incidence of internal physiological disorders than the 1994 fruit, associated with a heavier crop an lower tree vegetative vigour. Stepped down temperature regimes (Beginning at around 7.5°C and ending at around 4.5°C) appeared to be no more effective than continual storage at 5.5°C and generally did not reduce the incidence of physiological disorders associated with cold storage. During 4 weeks of cold storage at 5.5°C or 7.5°C, levels of ethylene evolution remained low, with a slight increase on day nine. The rise in ethylene production associated with the respiratory climacteric occurred after removal from cold storage. Total fruit phenolic levels did not change significantly during the 1994 harvesting season at Everdon Estate. It appears that prompt removal of field heat and strict maintenance of the cold chain is the best strategy for cold storage of 'Fuerte' fruit grown in the Natal Midlands.

CHAPTER 4

MOISTURE LOSS AND CHILLING SENSITIVITY IN 'FUERTE' FRUIT

4.1 INTRODUCTION

Mesocarp discolouration is a symptom of chilling injury in avocados (Chaplin and Scott, 1980; Eaks, 1976). However, it may occur in fruit which not been cold stored (Vakis, 1982) and is often associated with more mature fruit (Swarts, 1984). Tree moisture stress was found by Bower (1988) to influence fruit polyphenol oxidase (PPO) activity which has been associated with mesocarp discolouration in ripe avocados. Cold storage of avocado fruit in a humidified atmosphere to minimise moisture loss reduced the incidence of both pathological disorders and mesocarp discolouration (Bower *et al.* (1989). Pitting and blackening of the exocarp is a symptom of chilling injury known as "cold damage" in the South African avocado industry, and is caused by low storage temperatures. The symptoms of this disorder are described as clearly defined, sunken, darkened patches on the rind, which appear after a few days in cold storage, darkening with time until they become black, and are confined to the rind, not permeating the flesh. The severity of this disorder is proportional to the severity of the low temperature to which the fruit is exposed, and its duration (Swarts, 1984). Grapefruit (Purvis, 1984) and eggplant fruit (Abe *et al.*) also develop rind pitting as a symptom of chilling injury and in both of these fruits, shrink wrapping to reduce moisture loss during cold storage reduced the severity of chilling injury symptoms.

Because rind pitting in grapefruit was reduced by reducing fruit moisture loss during cold storage, and black cold in symptoms in avocados included rind pitting, it was hypothesised that frit moisture loss after harvest may have an effect on fruit sensitivity to black cold. This experiment was conducted to determine the effect of rapid moisture loss after harvest on the sensitivity of 'Fuerte' avocado fruit to black cold.

4.2 MATERIALS AND METHODS

Late season 'Fuerte' avocados were harvested in mid-September from 7 year old trees on 'Duke 7' rootstock at Everdon Estate, Howick. The normal harvesting season for 'Fuerte' at Everdon Estate runs from late May to mid-July. The fruit was picked in the early morning to ensure that it was turgid and transported to the laboratory in Pietermaritzburg. Each fruit was weighed individually and the average fruit mass was 226g. The fruits were placed in 2 l glass Consul® jars, sealed, and connected to a vacuum pump and refrigerated condensation trap (Fig. 4.1). A vacuum of -90 kPa was applied which facilitated rapid moisture removal from the fruit. Three different amounts of moisture were removed, viz. 2, 4, and 6% of the initial fruit mass. After moisture removal, the fruits were placed in cold storage at 5.5°C. A temperature of 5.5°C should not produce cold damage symptoms in late season 'Fuerte' fruit as sensitivity to this disorder is reduced with acclimation to colder winter temperatures on the tree (Swarts, 1982) Each treatment had 20 single fruit replicates. A further 20 fruits were placed directly into cold storage and served as a control. The approximate times taken to remove 2, 4 and 6% of the initial fruit mass were 2, 5 and 6h respectively. A moderate sized avocado left at room temperature in the laboratory loses about 1% of its fresh mass daily (Wolstenholme, 1994[7]). Although some of the mass loss during the moisture extraction at a temperature of +/- 20°C would be due to respiration, this was considered to be negligible for a time period of 2 to 6 h.

After 14 days in cold storage, at 5.5°C, the fruits were rated for severity of cold damage on a scale of 1 to 3 (1 = slight (< 10% of the fruit surface affected); 2 = moderate (10 to 30% of the fruit surface affected) and 3 = severe (> 30% of the fruit surface affected)). A weighted average for a chilling injury index was calculated by multiplying the number of fruit in each rating by the designated number and taking an average (McCornack, 1976). After 28 days the fruit was removed from cold storage and was allowed to ripen. When eating-ripe as determined by a firmometer (Swarts, 1981), the fruit was rated for pathological and internal physiological disorders.

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[7]Wolstenholme, B.N. 1994. Pers. Comm. Dept. Hort. Sci. University of Natal, Pietermaritzburg.

4.3 RESULTS AND DISCUSSION

Rapid moisture removal from the harvested fruit prior to cold storage produced pitting and blackening of the rind (Figs 4.2 & 4.3), whereas the control fruit showed no such symptoms during the four week storage period at 5.5°C (Fig. 4.3). Cold damage developed within 48h of treated fruit being placed in cold storage, the severity of which was related to the amount of water lost prior to cold storage, with 2% loss being the least severe and 6% loss the most severe (Figs 4.3 and 4.4). The 2% treatment caused pitting and blackening only around the individual lenticels. Removal of 4% and 6% moisture resulted in the spread of the blackened areas so that they coalesced, indicating that moisture loss aggravates the symptoms of black cold in cold storage. The severity of the "black cold" symptoms increased gradually during storage. It is interesting to note that some fruit developed brown pits around the lenticels before being placed into cold storage, when more than 4% moisture had been removed. This was probably due to damage of cells due to rapid moisture loss resulting in a breakdown of cell compartmentation and consequent browning reactions. Ripe fruit with cold damage did not show internal physiological disorders such as mesocarp discolouration. The incidence of anthracnose in the treated fruit was high. A loss of cell integrity due to moisture loss making the fruit tissue more susceptible to pathogenic attack would explain this phenomenon. Vascular discolouration associated with stem-end rot occurred in some fruits.

Chilling injury occurs in two stages: a primary event occurs, which is then followed by a series of secondary events. A number of possible primary events have been put forward e.g. a change in membrane lipid structure (Raison, 1974), or a conformational change in some structural proteins or regulatory enzymes (Graham and Patterson, 1982) or cytoskeletal changes in the cell (Patterson *et al.*, 1979). The primary event is thought to occur instantaneously once the plant reaches its critical chilling temperature and is reversible if the chilling temperature is of short duration (Raison and Lyons, 1986). Secondary events include cessation of cytoplasmic streaming, impairment of ion movement through membranes;

Fig. 4.1 Vacuum pump and refrigerated condensation trap used to rapidly extract water from 'Fuerte' avocado fruit.

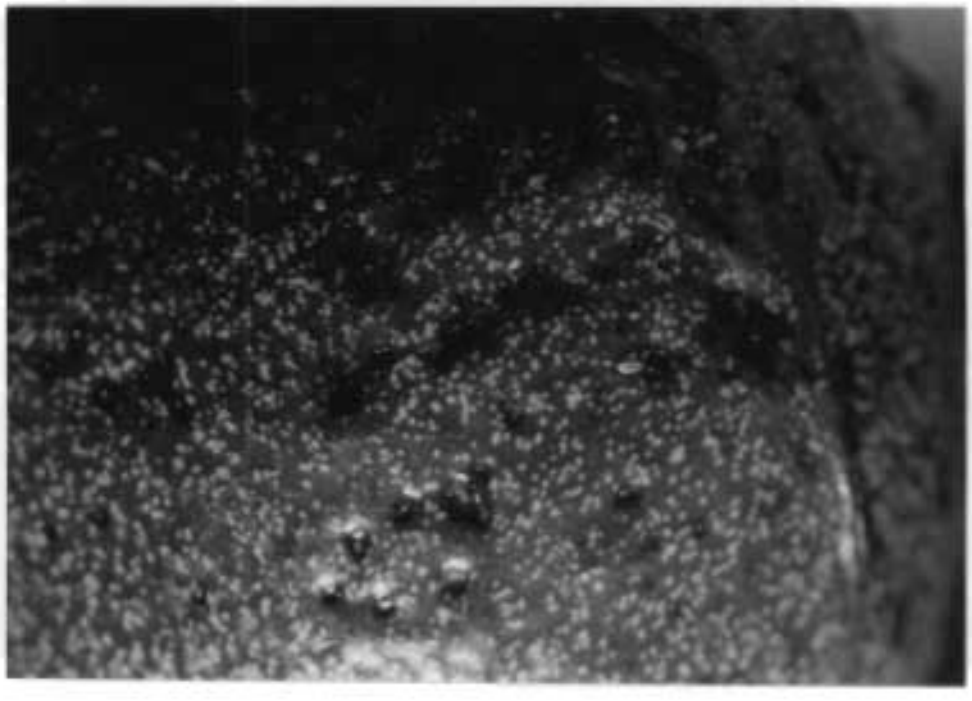

Fig. 4.2 Cold damage symptoms on the exocarp of a 'Fuerte' avocado. Note that pitting initially occurs around the lenticels (mag. 3 X).

respiration, photosynthesis and protein synthesis - all of which give rise to membrane breakdown and the resultant visual symptoms of chilling injury (Wills *et al.*, 1989).

Moisture loss from the fruit could aid membrane breakdown, due to reduced turgor, resulting in a loss of cell compartmentation which gives rise to visual chilling injury symptoms, but has seldom been implicated as a cause of chilling injury. Rind pitting which is a symptom of chilling injury in grapefruit was reduced by wrapping the fruit in polyethylene shrink film to reduce water loss, although it was concluded from this work that moisture loss was not the primary event causing chilling injury (Purvis, 1985). Pitting was also found to occur in areas of the grapefruit rind where diffusive resistance was lowest (Purvis, 1984). Whether or not moisture loss is the primary event causing external and internal chilling injury symptoms in avocados is of academic interest only as it seems that moisture loss aggravates these symptoms and should therefore be minimised.

This experiment shows that the severity of cold damage was related to the degree of moisture loss from the harvested fruit. Cutting and Wolstenholme (1992) found that fruits not cold stored lost more moisture during ripening than those cold stored. However, symptoms of cold damage only occur in cold stored avocados, indicating that moisture loss increases the susceptibility to this disorder. The very rapid loss of moisture under vacuum however, may have caused damage to the cells in the rind which could have caused blackening even in fruit not cold stored. Fruit treated in this manner should have been allowed to ripen at room temperature immediately after treatment to see whether they developed "black cold" symptoms or not.

Rapid water loss followed by cold storage induced cold damage but did not cause internal discolouration, probably because most of the moisture lost was from the rind. Bower et al. (1989) found that humidification of the storage atmosphere to reduce evaporative demand during cooling reduced the incidence of pathological and internal physiological disorders in avocado fruit. Scott and Chaplin (1978) reported a reduction of mesocarp discolouration in 'Hass' and 'Fuerte' avocados stored in sealed polyethylene bags. They attributed this to a modified atmosphere in the bags with CO_2 concentrations ranging from 3 to 7 % and 2 to 6 % O_2. In the light of the results obtained by Bower *et al.* (1989) it seems that a reduction of water loss also played a role in reducing mesocarp discolouration. Passive water infusion

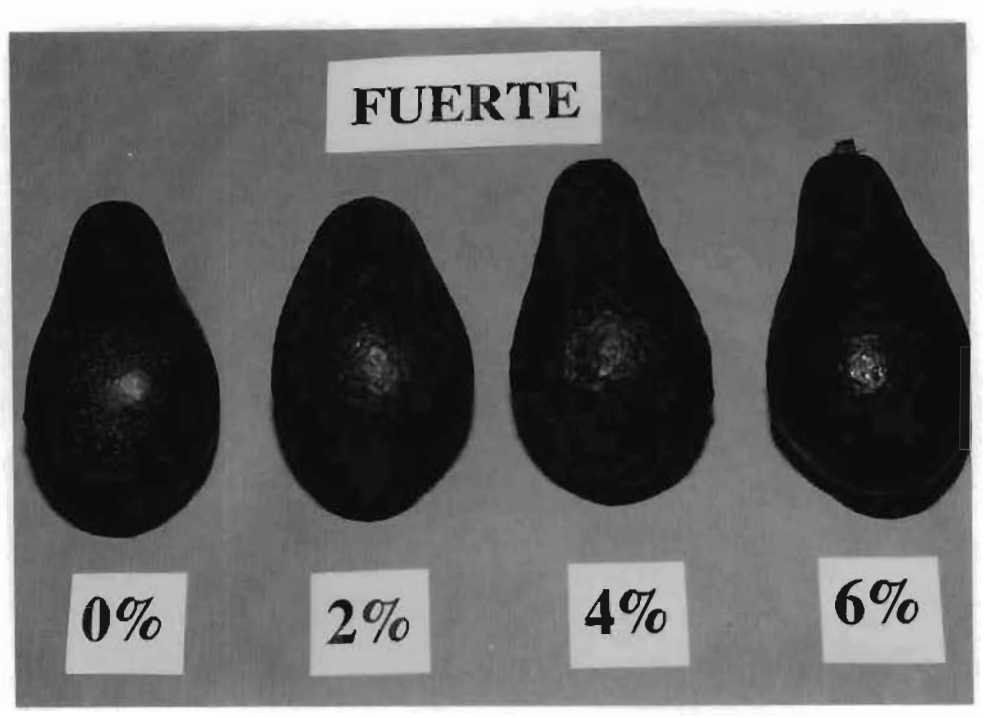

Fig. 4.3 'Fuerte' fruit after 14 days at 5.5°C after different levels of moisture loss before storage, showing cold damage symptoms.

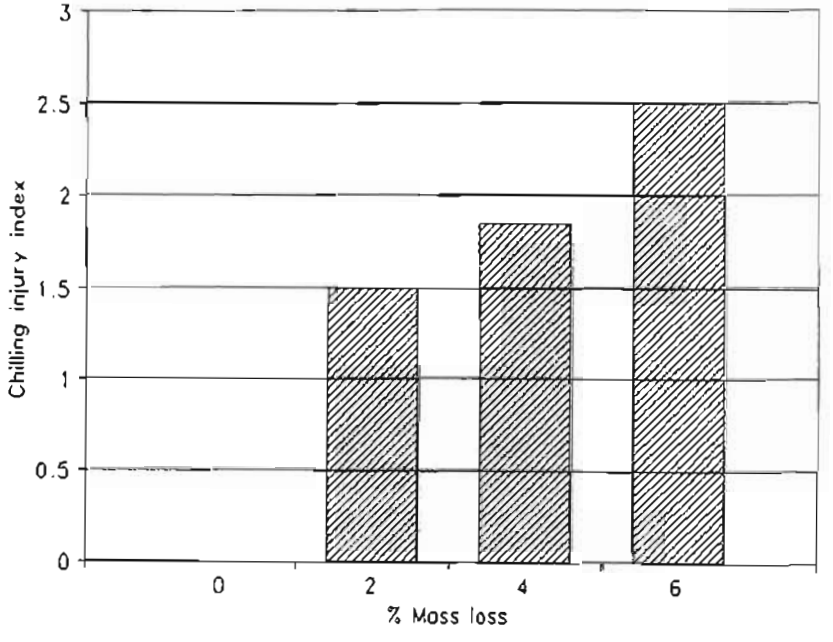

Fig. 4.4 Chilling injury indices of 'Fuerte' fruit after 14 days at 5.5°C after different levels of moisture loss before storage.

through the pedicel of avocado fruit during cold storage at 5.5°C for 28 days totally inhibited mesocarp discolouration (Cutting and Wolstenholme, 1992). It therefore seems that moisture loss through the pedicel and especially through the rind during cold storage may the cause of- or increase susceptibility to mesocarp discolouration. Judicious waxing of the fruit should greatly reduce the incidence of cold damage, but cold damage occurs fairly commonly in waxed export fruit, indicating that it is necessary to maintain fruit turgidity right from the time of harvest. Practical measures such as pre harvest irrigation, minimisation of fruit moisture loss in transit to- and in the packhouse, and during cold storage are necessary to reduce cold damage. Delay of harvest during hot dry periods (e.g. during spring berg winds) to make sure that turgid fruit arrives at he packhouse may reduce black cold but is probably not practical as fruit for export has to be packed according to the arrival dates of the ships on which the fruit is transported at the ports. Restricting harvesting to the cooler parts of the day will also help reduce moisture loss, but may be counter productive if wet fruit are harvested due to greater post harvest disease problems during and after cold storage (Darvas and Kotzé, 1979)

4.4 CONCLUSIONS

Rapid moisture loss from 'Fuerte' fruit after harvest and before cold storage caused cold damage symptoms, but it cannot be concluded that fruit moisture loss is the cause of this disorder under normal circumstances, but probably increases fruit susceptibility to this disorder. The severity of black cold may be related to the water status of the fruit at harvest and the amount of moisture lost after harvest. Pre- and postharvest practices should be aimed at harvest of turgid fruit and minimal fruit moisture loss from the time of harvest to arrival at the market.

This work should be repeated with the inclusion of control fruits which have had moisture rapidly removed and are not cold stored, to determine whether "black cold" symptoms will develop in fruits which have had moisture removed but have not been cold stored. If these symptoms do develop in treated fruit which has not been cold stored, the similarity of the symptoms obtained in this work to black cold would be in question.

Cooling systems in the South African avocado industry should be reviewed. Room cooling, which is presently used results in high fruit moisture loss. Hydrocooling and forced air cooling which are more efficient in removing field heat with less water loss should be looked into. Forced air cooling would probably be the more practical of these two methods as the cooling rooms presently used in packhouses could be modified to carry out this type of cooling. Although forced air cooling was found to cause cold damage in avocados Slabbert and Toerien, 1984), further research into air flow rate and temperature relationships may solve this problem. Installation of hydrocooling systems in packhouses would require extra space. Rate of cooling is higher and moisture loss is negligible compared to forced air cooling, the process is however carried out prior to packing which means there will be an increase in fruit temperature of varying extents during packing, depending on the ambient temperature and the time taken to pack the fruit after cooling.

GENERAL DISCUSSION AND CONCLUSIONS

From the temperature regime trials on 'Fuerte fruit run at Everdon Estate in 1993 and 1994, it seems that stepping down of storage temperatures from around 7.5°C to around 4.5°C during the period of cold storage is no more effective than storage throughout at 5.5°C. 'Fuerte' fruit appear to be less sensitive to cold storage disorders in the earlier part of the season, with the exception of cold damage. Stepped down temperature regimes did not have a consistent effect on cold damage. In some cases stepped down temperature regimes had a lower incidence of cold damage, and in other cases the incidence was lower than in fruit stored continuously at 5.5°C. Further research into storage temperature regimes for 'Fuerte' fruit grown in the Natal Midlands does not seem necessary.

The 1994 season produced 'Fuerte' fruit with a higher incidence of grey pulp, pulp spot and vascular browning than the 1993 season, especially in the last 4 weeks of harvesting, indicating that fruit susceptibility to cold storage disorders varies from season to season. Tree vegetative / reproductive balance, or leaf to fruit ratio appears to play a large role in determining fruit quality as the 1993 crop was heavier than the 1994 crop with less vigorous vegetative growth. Correct orchard management according to phenological events will help to maintain the correct vegetative-reproductive balance in the tree, but cannot be totally effective in doing so as alternating heavy and light crops to varying extents seem to be the norm. Pruning is not only useful in maintaining tree size and architecture, but also in the control of vegetative vigour. Summer pruning of vigorous vegetative growth e.g. watershoots may reduce the incidence of physiological disorders by increasing the fruit sink strength for minerals and assimilates, as is the case in apples. Pruning is a management tool which is as yet not used in the avocado industry and research in this field is necessary.

In a "good" season when the fruit is of high quality (e.g. 1993), 'Fuerte' fruit can be harvested for a longer period without an increase in physiological disorders, than in a "bad" season such as 1994. In 1994, the incidence of internal physiological disorders increased when fruit moisture content was lower than ± 73%. In a bad year, 'Fuerte' fruit harvested before this stage of maturity will probably have a low incidence of physiological disorders. Research to determine to what extent the season will be "good" or "bad" would be useful in

determining a maximum maturity before which the fruit should be harvested to avoid a high incidence of internal physiological disorders after cold storage. A model determining a fruit quality index as a function of factors such as the extent of vegetative growth during the spring flush, crop load and fruit calcium content would be useful in predicting the maximum exportable maturity in a specific season. The length of the harvesting season could then be determined before harvesting began and picking and packing schedules determined accordingly. Data of fruit calcium levels, vegetative vigour, crop load, fruit moisture content and the incidence of physiological disorders as a result of cold storage, would have to be collected over a number of seasons in order to develop such a model.

Postharvest heat treatments to reduce chilling injury is presently receiving much attention from postharvest physiologists. Heat treatment using dry heat and warm water baths always caused rind blackening in 'Fuerte' fruit, although the severity differed with different time - temperature relationships. Only three vapour heat treatments viz. 1.5 h and 3 h at 40°C and 10 min at 48°C produced fruit with less cold damage and lower incidences of grey pulp than untreated fruit after cold storage. As replication was insufficient to prove these results statistically, further trials using these time - temperature relationships need to be run throughout an entire 'Fuerte' harvesting season as changing fruit maturity may cause different reactions to heat treatment. However, in the light of the results obtained in the temperature regime trials, where fruit quality was generally very good after storage a t 5.5°C for four weeks, it does not seem necessary to improve fruit tolerance to cold temperatures. If 'Fuerte" fruit is harvested before a stage of critical maximum maturity, in seasons of poor quality, the incidence of cold storage disorders should be minimised. Heat treatments may be useful if they are able to reduce internal physiological disorders associated with more mature fruit in a season of poor fruit quality, in order to extend the export season.

Moisture loss from harvested 'Fuerte' fruit appears to play a role in determining fruit susceptibility to cold damage minimisation of fruit water stress from the time of harvesting right through to arrival at the market in Europe should improve fruit quality. The problem of soft fruit arriving in Europe seems to be as a result of breaks in the cold chain or inefficient initial cooling as the temperature regime trials run in 1993 and 1994 always produced hard fruit with the exception if one treatment in one of the trials in 1993. Research into methods of rapid cooling such as hydrocooling and forced air cooling will enable rapid

removal of field heat and make sure that the fruit has reached the desired temperature before it is trucked to the docks as the refrigeration units on cold trucks do not have the capacity to cool the fruit, and differences in temperature between delivery and return air of greater than 1°C result in a large vapour pressure deficit which causes desiccation (Woods, 1990). Forced air cooling has been found to increase the incidence of cold damage in avocados (Slabbert and Toerien, 1984), but experimentation with air flow rates and temperatures may eliminate this problem. Hydrocooling for avocados should also be researched as well as sanitation procedures which would be necessary in controlling pathogens spread in the water used for this purpose.

In conclusion, it seems that postharvest handling of 'Fuerte' avocados should be according to the general principles used in the handling of most other fresh produce, namely, harvest a turgid product, keep it as cool as possible, and minimise moisture loss in transit to the packhouse, during packing, and remove the field heat from the fruit as soon as possible, but taking care not to cause damage as a result of cooling too rapidly, and maintain the cold chain until the fruit arrives at the market.

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