

**FACTORS AFFECTING MESOCARP DISCOLOURATION SEVERITY IN
'PINKERTON' AVOCADOS (*Persea americana* MILL.).**

By

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DECLARATION

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

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ABSTRACT

The susceptibility of the 'Pinkerton' avocado cultivar to mesocarp discoloration, after storage, has seriously threatened its export from South Africa. This disorder has proven to be complex, requiring a better understanding of the fruit's physiology. The purposes of this study were to identify the role of pre- and postharvest factors, or their interactions, in the development of the problem. This was done by obtaining fruit from several production areas of varying mesocarp discoloration histories (referred to as "high", "medium" or "low risk" areas) during the 2000 and 2001 seasons. Fruit were stored at 8, 5.5 and 2°C for 30 days, as well as ambient (20°C). Evaluations of fruit quality were made before and after storage, as well as after softening. Once removed from storage the weight loss (during storage) was determined, and fruit firmness and carbon dioxide (CO₂) production rates monitored daily. It was found that temperatures below the recommended shipping temperature of 5.5°C, i.e. 2°C, produced the best internal fruit quality. This was supported by the membrane integrity studies that showed less membrane stability at the warmer storage temperature of 8°C. Furthermore, remained hard during storage and subsequently had an extended shelf life.

Fruit origin was also found to play a major role in browning potential, with discoloration being consistently more severe in fruit from "high risk" areas and increasing in severity as the season progressed. The rate of CO₂ production was found to follow a similar trend, with rates increasing as the season progressed, and also being slightly higher in fruit from "high risk" areas. The higher CO₂ production rates were thought to be related to a decrease in membrane integrity as the season progressed. While storage temperature was not found to have a significant effect on the rate of CO₂ production after storage, it did affect the time taken to reach the maximum rate, with fruit stored at 2°C taking longer.

Biochemical analyses to determine the concentration of total phenolics and the activity of the enzyme polyphenol oxidase (PPO) also showed that the potential for browning was initiated by preharvest conditions. While no significant differences were found between growers with regards to total phenol concentrations, the PPO activity was found to be higher in fruit from poor quality areas, and subsequently browning potential was expected to be higher in these fruit. It was, however, found that the potential for browning could be reduced by storing fruit at 2°C, as this decreased the total phenolics concentration. This evidence further emphasized the idea that storage at 2°C could be highly advantageous.

Fruit mineral analysis showed that certain key elements played a significant role in the severity of mesocarp discolouration, with excessive fruit nitrogen and decreasing copper and manganese concentrations appearing to play major roles. The high fruit nitrogen concentrations were suspected to reflect fruit grown on very vigorous trees, resulting in shoots competing with fruit for available reserves. It is suggested that 'Pinkerton' of a quality acceptable to the market, can be produced by manipulating source:sink relationships, particularly through decreasing the availability of nitrogen, followed by low temperature (2-4°C) shipping. Future work should concentrate on manipulation of source:sink relationships, to take account of both climatic conditions and leaf to fruit ratios.

The evaluation of chlorophyll fluorescence as a tool for predicting mesocarp discolouration potential in 'Pinkerton' proved to be unsuccessful in this study and future studies may require modifications to the current technique. It is suspected that differences in chlorophyll content, for example, between fruit from different origins, will have to be taken into account when interpreting results.

The success of using 2°C storage to improve the internal quality on 'Pinkerton' fruit prompted further studies, during 2004, to ensure that the development of external chilling injury would not decrease the marketability of the cultivar. Low temperature conditioning treatments, prior to storage, proved to be highly successful in reducing the development of external chilling injury, thus further improving fruit quality as a whole. Preconditioning treatments consisted of fruit that were kept at either 10°C, 15°C or 20°C for 1 or 2 days before being placed into storage for 30 days at 2°C or 5.5°C. All preconditioning treatments were compared to fruit that were placed directly into storage. The effect of fruit packaging on moisture loss (as determined by weight loss) and chilling injury was also investigated using unwaxed fruit, commercially waxed and unwaxed fruit individually sealed in micro-perforated polypropylene bags with an anti-mist coating on the inside (polybags). Holding 'Pinkerton' fruit, regardless of packaging treatment, at 10°C for 2 days prior to storage at 2°C or 5.5°C significantly decreased the severity of external chilling injury. The use of polybags during preconditioning and storage showed potential in further reducing the development of external chilling injury, although the higher incidence of fungal infections in these fruit needs to be addressed. The determination of proline concentrations in fruit exocarp tissue after storage was helpful in determining the level of stress experienced by fruit that were subjected to different packaging

and preconditioning treatments. In this study waxed fruit subjected to 1 d preconditioning at 10°C, 15°C or 20°C or placed directly into storage at 2°C showed very high proline concentrations and also displayed more severe external chilling injury, despite unwaxed fruit losing more weight during these treatments. The role of moisture loss thus needs further investigation. The thickness and method of wax application was thought to play an important role in the higher external chilling injury ratings in this study as waxed fruit often developed chilling injury symptoms around the lenticels and it was suspected that either the lenticels were damaged by the brushes used to apply the wax or that the lenticels became clogged thus resulting in reduced gaseous exchange. Nevertheless, the success of low temperature conditioning in reducing external chilling injury, while maintaining sound internal quality, may enable storage temperatures to be dropped even further, thus enabling South Africa to export avocados to countries that require a cold disinfestation period prior to entry to eliminate quarantine pests (e.g. fruit fly).

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A hundred times every day, I remind
Myself that my inner and outer life
Are based on the labours of other
Men, living and dead, and that I
Must exert myself in order to give in
The same measure as I have received.

- Albert Einstein

“I can do everything through Him who gives me strength”.

- Philippians 4: 13

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INTRODUCTION

The South African avocado industry is highly export driven and during 2005 an estimated total of 11.5 million, 4 kg cartons (46 000 tons), were expected to be exported to France, Germany, the U.K. and the rest of Europe. Of the exported crop almost 51% are made up of greenskins and 49% of 'Hass'. During 2002 the 'Pinkerton' cultivar only made up 3% of the total value, however current figures reflect an estimated value of up to 7.1% of the final total (3 272 tons).

The avocado (*Persea americana* Mill.) belongs to the family Lauraceae and has been classified into three botanical races viz. *Persea americana* var. *americana*, the West Indian types; *Persea americana* var. *drymifolia*, of Mexican origin; and *Persea americana* var. *guatemalensis*, of Guatemalan origin (Bergh and Ellstrand, 1986). The 'Pinkerton' cultivar originated in 1959 as a 'Hass' X 'Rincon' hybrid, showing mostly Guatemalan characteristics (Wood, 1984). The tree has an irregular, moderately spreading growth habit and is a very precocious and consistent bearer.

Transport to European markets is mainly done by sea, which necessitates storage of up to 28 days. Due to the highly climacteric and rapidly softening nature of the fruit the only way to ensure that fruit arrive at their market with a reasonable shelf life is to ship the fruit at low temperatures. Unfortunately this low temperature storage may result in the development of certain physiological disorders (Eaks, 1976; Chaplin *et al.*, 1982; Swarts, 1984). Physiological disorders refer to the breakdown of tissue that is not caused by either the invasion of pathogens or by mechanical damage (Wills *et al.*, 1989).

Mesocarp discolouration

The export of the 'Pinkerton' cultivar, in particular, has been seriously threatened by the development of a disorder that is known as mesocarp discolouration, grey pulp or chilling injury, with the last name reflecting what is thought to be the cause of the disorder (Chaplin *et al.*, 1982; Couey, 1982). The disorder is characterized by a discolouration of the mesocarp on cutting the fruit in half, with exposure to the atmosphere causing further blackening. This disorder is not unique to the 'Pinkerton' cultivar, however, and studies trying to ascertain the

cause have been conducted over many years, and in fact for similar symptoms in many other fruit types as well (Ferguson and Watkins, 1989; Crisosto *et al.*, 1997).

Chilling injury may manifest itself in many ways; for example, surface lesions on fruits, water soaking of tissues, internal discolouration (including vascular browning), increased susceptibility to decay, and failure to ripen normally (Saltveit and Morris, 1990). Studies on avocados have, however, found that the mesocarp discolouration may also develop in unstored fruit (Vakis, 1982). This has resulted in some confusion as to the role of storage temperature in disorder development. Studies, done specifically on 'Pinkerton', have shown inconsistent results with some research showing that this cultivar should be shipped at 7°C to reduce mesocarp discolouration (Sippel *et al.*, 1994; Kruger *et al.*, 2000), while yet other research showed that 5.5°C rendered better quality (Schutte, 1994). Zauberman and Jobin-Décor (1995) attributed mesocarp discolouration in 'Hass' to being the result of ripening occurring during cold storage, and suggested fruit could be stored at 2°C. However, Kritzinger *et al.* (1998) found that storage at 2°C in 'Pinkerton' resulted in a significantly higher incidence of mesocarp discolouration than fruit stored at 5°C. Shipping 'Pinkerton' in controlled atmosphere (CA) storage has also not alleviated the problem, with mesocarp discolouration even reported to being higher in CA than regular atmosphere (RA) (Eksteen *et al.*, 1998).

Matters are further complicated by the fact that the severity of mesocarp discolouration differs between fruit origins (Kruger *et al.*, 2000) and also between seasons, which has led to preharvest factors being thought to play a role (Rowell and Durand, 1982; Bezuidenhout and Kuschke, 1982; Bezuidenhout, 1983; Bower, 1984). Mineral nutrition is known to play a large role in tree health and also in fruit quality. Calcium has been associated with more physiological disorders than any other mineral (Bangerth, 1979; Wills *et al.*, 1989) and many studies have investigated the role of calcium in mesocarp discolouration. In addition, fruit firmness, after storage, was thought to be related to disorder potential (Eksteen *et al.*, 1998) and calcium is known to be important for membrane integrity (Kremer-Köhne *et al.*, 1993). While ensuring high calcium concentrations appeared to solve the problem in many of the other avocado cultivars (Chaplin and Scott, 1980; Cutting *et al.*, 1992; Hofman *et al.*, 2002; Thorp *et al.*, 1997), calcium applications to 'Pinkerton' rendered inconsistent results (Penter *et al.*, 2001).

Fruit maturity, prior to cold storage, has also been found to play an important role in the severity of mesocarp discolouration. In South Africa an attempt was made to identify which growing areas showed a higher potential for mesocarp discolouration development in 'Pinkerton' fruit. The affect of fruit maturity was then investigated and it was found that the severity of the disorder could be reduced by assigning various "cut-off" maturities for fruit from the respective areas based on their potential for disorder development (Kruger *et al.*, 2000). The determination of fruit maturity is, however, complicated by the fact that the 'Pinkerton' cultivar has an extended flower period resulting in differences in fruit maturity at the harvesting stage. Late set fruit have a much faster growth rate than early set fruit, resulting in fruit being picked prematurely if fruit size is taken as a maturity index (Sippel *et al.*, 1994). Tree yield also affects final fruit quality with high yielding trees producing fruit with a shorter shelf life and more internal browning (Cutting and Vorster, 1991). Similarly, the percentage of fruit with internal disorders can be higher in fruit from low yielding trees (Köhne *et al.*, 1992). Internal disorders and external skin discolouration due to chilling injury may also be greater in fruit from more vigorous tress (Vorster *et al.*, 1989).

In summary, the factors that contribute to mesocarp discolouration development in 'Pinkerton' avocados appear to be complex, and the lack of clear guidelines to both grower and packer, have resulted in quality so poor that by 1999, exporters were warning producers that they were finding it difficult to market the cultivar. In fact, the situation has become so serious that the future of 'Pinkerton' is threatened. The main purpose of this research was, thus, to identify possible pre- and postharvest factors, or interactions, which would contribute to mesocarp discolouration development. Postharvest studies would concentrate on elucidating the role of shipment temperature on the incidence of the internal disorder, and would include evaluations of membrane integrity (DeEll *et al.*, 1999; Thompson, 1988; Stanley, 1991) and fruit respiration (Bain and Mercer, 1964). Fruit from different fruit origins, varying in mesocarp discolouration histories, would be obtained, throughout the harvest season, to determine the effect of these variables on temperature response. Fruit weight loss during storage, and fruit firmness after storage would also be evaluated as they are thought to be related to membrane integrity. Furthermore, the effects of temperature and fruit origin on internal browning potential would also be investigated by determining the total phenolics concentration and the activity of the enzyme polyphenol oxidase (PPO) in fruit. The quantity of either total phenolics and/or the PPO enzyme in the avocado fruit flesh has been found to have an effect on the severity of discolouration (Golan *et al.*, 1977).

To determine possible preharvest differences between fruit from the various origins, fruit mineral concentrations would be determined to evaluate what role they play in postharvest fruit quality. Previous studies raised some questions as to the role of calcium in disorder development and thus other elements would be included in the study. Elements with known roles in avocado fruit quality include nitrogen (Arpaia *et al.*, 1996), boron (Smith *et al.*, 1997), magnesium, potassium (Koen *et al.*, 1990; Witney *et al.*, 1990), and zinc (Vorster and Bezuidenhout, 1988).

External chilling injury

Horticulturalists are faced with many challenges when trying to reduce the development of a certain disorder, as the factors contributing to a decrease in the severity of that disorder may aggravate and or lead to the development of another. Furthermore, the search for new export markets requires that fruit from certain areas be subjected to periods of cold disinfestations before fruit are allowed to enter the country. While the main aim is to eliminate the potential for insect infestations in the fruit, the mandatory cold temperature treatment gives rise to the development of skin damage (external chilling injury). In South Africa external chilling injury is also known as cold damage, and typical symptoms may include the formation of black areas on the fruit, which become slightly sunken, and is thus called "pitting".

While external chilling injury has not been the limiting factor in exporting 'Pinkerton' fruit in the past it still affects overall fruit quality, and symptoms may become aggravated if cold disinfestations proves to be a successful way of eliminating quarantine pests. As with mesocarp discolouration, external chilling severity can also be affected by preharvest orchard conditions (Bower, 1988; Kremer-Köhne *et al.*, 1993; Kruger *et al.*, 2004). However, the use of certain postharvest techniques, that have proven to be successful in alleviating the severity of the disorder, may prove to be preferable in terms of logistics as it may to enable fruit from different areas to receive the same postharvest handling. Many studies have demonstrated that low temperature conditioning of fruit and seedlings can alleviate chilling injury symptoms (Wang, 1993), and more specifically in avocados (Woolf *et al.*, 2003). Fruit packaging, for example the use of waxes and film packaging, have also shown varying degrees of success in reducing chilling severity (Ben-Yehoshua *et al.*, 1981; Forney and Lipton, 1990; Wang,

1993). The packaging treatments were thought to modify gaseous exchange and reduce moisture loss (as determined by weight loss), which is thought to prevent the collapse of membranes (Forney and Lipton, 1990; Wang, 1993).

In an attempt to improve overall fruit quality in 'Pinkerton' avocados the affect of low temperature conditioning and the application of various packaging treatments would be investigated in the second half of this study, as these treatments are fairly simple to implement and don't necessitate major modifications to the current cold chain system. To determine the role of moisture loss on external chilling injury development three packaging treatments would be used in the study, i.e., unwaxed, waxed and fruit sealed in polypropylene bags. Throughout the preconditioning and storage treatments the weight loss of fruit would be recorded and compared to external chilling injury ratings, as well as other fruit quality determinants (such as days to ripening, mesocarp discolouration severity and the presence of pathogenic infections). To establish the level of stress experienced by the fruit, during the preconditioning treatments, the proline concentrations of the fruit exocarp would be determined.

It was hoped that the results of the study would give a more holistic understanding of which factors, or interactions, play a significant role in mesocarp discolouration severity in 'Pinkerton', and possibly the mechanisms involved, so that corrective measures could be taken to avoid, or at least minimize, mesocarp discolouration development. This, together with determining a successful postharvest technique of reducing external chilling injury, will enable the South African avocado industry to produce fruit of a high quality and make the goal of entering new markets a reality.

CHAPTER 1

LITERATURE REVIEW

Wherever plants grow they will be subject to a great variety of stresses, which will restrict their chances of development and survival. Organisms will, naturally, respond differently to a particular stress and the nature and intensity of the response of individual plants may vary considerably, depending upon age, degree of adaptation, and on seasonal and even diurnal activity. Harvested products should, therefore, also not be expected to respond uniformly. Specific mechanisms of adaptation involve all functional levels; in many cases they are elicited by differential gene activation. Also characteristic for a state of stress are non-specific manifestations, which are primarily an expression of the degree of severity of the disturbance. Examples are alterations in membrane properties, increased respiration, inhibition of photosynthesis, reduced dry matter production, growth disturbances and premature senescence. Further non-specific effects of stress are changes in enzyme activities; *de novo* synthesis and accumulation of antioxidants, or stress metabolites, as well as numerous secondary plant substances (Larcher, 1995).

Mesocarp discolouration and external chilling injury will therefore, likely be the result of some kind of stress(es) inflicted on the fruit, which have a cumulative effect of tissue damage.

1.1. FRUIT RIPENING

The avocado is unlike most other fruit in that ripening does not normally take place on the tree and only commences after harvest (Schroeder, 1953). The reason for this phenomenon is not fully understood, but Tingwa and Young (1975) postulated that a ripening inhibitor, possibly an anion, moves either to or from the fruit pedicel once detached from the tree thus preventing on-tree ripening. More, recently Liu *et al.* (2002) suspected that seven-carbon (C7) sugars could control the ripening process.

Ripening may be considered as the first stage in the senescence of fruits in which the characteristic changes in structure and composition occur that make the fruit acceptable to eat (Rhodes, 1980). Ripening, thus, imparts value to fruit as agricultural commodities. It has been suggested that deterioration of fruits, vegetables and other plant materials, either by

natural senescence or by aging due to physiological damage, share a common mechanism (McKersie *et al.*, 1988; Palta, 1990; Stanley, 1991). It will thus be highly advantageous to gain an understanding of the ripening process to aid our manipulation of the process for extending storage and shelf life.

1.1.1 Structural changes

Ripening of avocados is not simply a degradative process and involves many catabolic and anabolic changes (Seymour and Tucker, 1993), requiring large amounts of energy as well as prolonged integrity of membranes (Bruinsma, 1981). The cell membrane system, and in particular the plasma membrane, make up an important aspect of fruit ripening (Bower and Cutting, 1988). In avocados, Golgi bodies and plasma membranes were reported to show increased buoyant density during ripening, while thylakoids and mitochondrial membranes showed no change (Dallman *et al.*, 1988). Generally, during ripening, anabolic changes would include the production of new flavour volatiles or new pigments while catabolic changes would include the breakdown of chloroplast thylakoids or in the breakdown of cell wall constituents during tissue softening. In avocados lipid peroxidation may be regarded as one of the earliest detectable processes in fruit ripening (Meir *et al.*, 1991). Ultrastructural changes observed in avocados during ripening by Platt-Aloia and Thomson (1981) included a loosening and eventual breakdown of the cell wall, and swelling and vesiculation of the rough endoplasmic reticulum. Montoya *et al.*, (1994) also noticed some changes in electrical conductivity. Ultimately in senescence, catabolic processes become dominant (Rhodes, 1980).

There have been numerous reports of changes in activity of various metabolic pathways during the ripening process, with these being expressed as the activation, inhibition, synthesis, or release of rate-limiting enzymes of these pathways (Rhodes, 1980). Blackman and Parija (1928) thought that the activation of enzymes was as a result of changes in cell compartmentation occurring during ripening. In fact many authors have found that a common feature accompanying ripening and senescence, is increased membrane permeability, expressed as increasing leakage of ions (Bain and Mercer, 1964; Thompson, 1988; Stanley, 1991); with similar results in avocado fruit (Ben-Yehoshua, 1964). In apples this leakage correlated with increased membrane viscosity and decreased degree of fatty acid unsaturation (Lurie and Ben-Arie, 1983; Lurie *et al.*, 1987). The same occurred in potatoes

(Knowles and Knowles, 1989). Increased phase transition temperatures of membrane lipids and a decline in fluidity have been described in the senescence of flowers; these events preceded enhanced ethylene production and ion leakage (Faragher *et al.*, 1986). Together these findings suggest that compositional changes that determine the decreased fluidity of membranes are translated into leakage of ions, and therefore reduced functionality of membranes.

However, Palma *et al.* (1995) reported that the increases in ion leakage observed in senescing fruit were significantly correlated to losses in microsomal membrane K⁺-stimulated H⁺-ATPase activity and not to the saturation index of membrane lipids. Therefore, in that study at least, degradation of membrane lipids was not the mechanism by which increased ion leakage occurred. However, ion leakage and ATPase activity could be correlated to linolenic acid, a fatty acid particularly prone to oxidation, and which is found in avocado fruit (Seymour and Tucker, 1993). Peroxidation of fatty acids with resulting free radical formation has been described as one of the major deteriorative processes of membranes (McKersie *et al.*, 1988; Thompson, 1988; Stanley, 1991; Voisine *et al.*, 1991; Voisine *et al.*, 1993) and increased free radical production has been observed in a variety of senescent tissues. It is a common belief that changes in membrane lipids resulting in decreased fluidity will affect the functionality of the associated proteins as well. There is some evidence that elevated membrane viscosity is associated with lowered ATPase-specific activity. Furthermore, Vickery and Bruinsma (1973) studied changes in the passive efflux of K⁺ under isotonic conditions from slices of tomato pericarp taken from fruit at different stages in ripening and they concluded that the permeability of neither the plasmalemma nor the tonoplast changed during ripening. They suggested that active transport remains under metabolic control throughout the period of ripening. Both Burg (1968) and Vickery and Bruinsma (1973) were of the opinion that changes in cell leakage often reflect changes in the total concentration of the solute available for leakage rather than changes in membrane permeability. There are thus conflicting views on the occurrence or importance of changes in cell permeability during ripening.

1.1.2 Respiration

The avocado is a climacteric fruit and displays a characteristic peak of respiratory activity during ripening, termed the respiratory climacteric. Fruit with high respiratory rates, such as

banana and avocado, tend to ripen very rapidly and hence are more perishable. This has led to the regulation of respiration as a possible mechanism for the biochemical manipulation of shelf life (Tucker, 1993). The substrate for respiration in avocados is not well defined, but the respiratory quotient (RQ) (the ratio of carbon dioxide (CO₂) produced to oxygen (O₂) consumed) remains at around 1 during the climacteric, indicating that the substrate during this period is carbohydrate rather than lipid (Blanke, 1991). Liu *et al.* (2002) reported that the increase in respiration, associated with the onset of ripening, was not initiated until a drop in C7 sugar levels occurred. However, there are also indications that some degradation of the lipid reserve does occur during ripening (Kikuta and Erickson, 1968).

The respiratory pathways utilized by fruit for the oxidation of sugars are those common to all plant tissues, namely glycolysis, pentose phosphate pathway and the tricarboxylic acid pathway (TCA) (Tucker, 1993). The increased respiration of sugars in climacteric fruit seems to be mediated largely by the increased flux through glycolysis (Solomos and Laties, 1974). Studies with inhibitors and isolated mitochondria, in avocados, suggested that the TCA cycle was operating during the climacteric to bring about oxidation of the respiratory substrate, and that this oxidation was coupled to the production of adenosine triphosphate (ATP), probably by electron transport through a cytochrome-mediated pathway (Biale and Young, 1971).

The respiratory climacteric is common to a wide range of fruit, yet its role in ripening is still unclear (Tucker, 1993). In avocados, it has been proposed that the respiratory climacteric represents "maintenance metabolism" of mitochondria in senescent fruit cells (Seymour and Tucker, 1993). Studies with avocado and banana tissue found an increase in leakage of solutes attended the onset of the climacteric, and that the rate of leakage increased exponentially during the respiratory rise (Sacher, 1962). The increase in membrane permeability during ripening would expose mitochondria to harmful substances, and there is evidence to suggest that to retain respiratory control, the mitochondria respond by increasing ATP synthesis (Huang and Romani, 1991). The large increase in ATP synthesis (Solomos and Laties, 1976; Bennett *et al.*, 1987) is accompanied by a decrease in the ADP/ATP ratio with the transition from the preclimacteric to the climacteric, indicating a high-energy charge, but low ATP demand. The climacteric may, thus, not be the result of an increased energy demand, but could be a response to changes in the cytosol (availability of substrates, cofactors, activators and inhibitors) or the mitochondria (Blanke, 1991).

Furthermore, excess glucose is believed to cause modification in the structure and function of proteins, some of which may be critical to cellular function (Sharon, 1980). Release of compartmentalised amino or organic acids may lead to pH changes in tissues, with detrimental consequences to a host of metabolic paths (Davies, 1973). Analogous damaging effects could also be elicited by the release into the cytosolic environment of other endogenous compounds, notably phenolics (Frenkel, 1987).

1.1.3 Plant growth regulators

Plant growth regulators (PGRs) have been found to play a large role in avocado fruit ripening (Bower and Cutting, 1988). As the purpose of this research was not to evaluate the role of the PGRs the following information will serve only to acknowledge that there are many components involved in the ripening process. In avocados the onset of ripening is marked by a large increase in ethylene production. The precise role of ethylene is still uncertain, but it appears to be involved in the initiation and coordination of ripening in fruits (Seymour and Tucker, 1993). Increased ethylene biosynthesis is, however, not unique to the ripening of climacteric fruit. Most plant tissues will respond to wounding with an increase in ethylene production. Preharvest water stress has been found to alter the ethylene evolution pattern resulting in uneven ripening and poor fruit quality (Cutting *et al.*, 1986). Like ethylene, abscisic acid (ABA) also appears to be a ripening promoter, while auxins, cytokinins and gibberellins, are inhibitors of fruit ripening (Rhodes, 1981). Exogenously applied ABA has been shown to stimulate ethylene biosynthesis and fruit ripening (Vendrell and Palomer, 1997). Similarly, ethylene induces a rise in ABA levels in immature melons (Guillen *et al.*, 1998).

Stress is a major factor affecting ABA levels. Hiron and Wright (1973), for example, found a strong relationship between water stress and ABA synthesis. Adato and Gazit (1974) found that the greater the daily water loss from harvested avocado fruits the more rapid the ripening. Infusion of water delayed ripening, and it was thus concluded that moisture stress could be an important factor in ripening. Furthermore, temperature can also affect both ABA levels and the rate of ripening. Wang *et al.* (1972) found that pear fruits subjected to low orchard temperatures ripened faster and also had higher ABA levels. High levels of ABA were also found to significantly increase PPO activity in avocados, reducing the internal fruit quality and time to ripeness (Cutting *et al.*, 1988).

1.2 FACTORS AFFECTING FRUIT QUALITY

Successful marketing of horticultural products requires predictability and consistency of quality and subsequently an understanding of the basis of quality at the cellular and molecular level. It is important to consider that harvested fruit are 'living' structures, which continue to perform the metabolic reactions and maintain the physiological systems, which were present while the fruit were attached to the tree. Respiration and transpiration continue after harvest and, since the fruit is now removed from its normal source of water, photosynthates and minerals, the produce is dependent entirely on its own reserves and moisture content. As the losses of respirable substrates and moisture are not made up after harvest, fruit deterioration starts to occur, making fruit highly perishable commodities (Tucker, 1993). It is this perishability, and inherent short shelf life, that presents the greatest problem to the successful transportation and marketing of fresh fruit. Solutions to the postharvest problems of fruit, as well as improvements in the handling procedures, may therefore come from a better understanding of factors affecting fruit quality. A number of factors can affect fruit quality, but in terms of this study, perhaps the most important, is that of fruit browning.

1.2.1 Fruit browning

The browning potential of various fruits has been directly related to the phenol level, the polyphenol oxidase (PPO) activity, or a combination of these factors (Mapson *et al.*, 1963; Ranadive and Haard, 1971; Golan *et al.*, 1977). These two components will thus play an important role in discolouration development and this section will give a brief overview of their function and location in the fruit.

1.2.1.1 Total phenolics

Plant phenolics include a variety of compounds such as simple phenols, phenolic acids, coumarins, flavonoids, tannins, and lignins. Phenolic acids have a benzene ring, a carboxylic acid, and one or more phenyl hydroxyl groups that may become methylated to produce methoxy groups (Torres *et al.*, 1987). Phenolic acids commonly occur as esters and/or ethers in combination with various sugars and aliphatic or aromatic acids and hydroxy acids. Phenolic acids make up significant components of taste and odour, are involved in the browning reaction, and are thought to be involved in growth regulation and in disease and

herbivore resistance. Phenolic acids are generally found in the cell vacuole or in special tissues, and are precursors of many other compounds. When membrane integrity is lost the phenols are released and become oxidized, atmospherically and enzymatically, to quinones (Torres *et al.*, 1987). The quinones are irreversibly oxidised to melanin pigments that are brown in colour (Bower and Cutting, 1988).

Total phenolic content can generally vary in different species of the same genus (Gartlan *et al.*, 1980), in the same species at different times of year, and in the same tissue at different stages of growth (Lowman and Box, 1983). This was confirmed in avocados where certain cultivars were found to have significantly higher total phenolics than others and where a difference in phenol content was found between the proximal and distal ends of the mesocarp of the same fruit (Golan *et al.*, 1977). In the same study a positive correlation was found between total phenolic concentration and fruit browning. Cutting *et al.* (1992) also found that total phenolics in avocado increased with increasing maturity, as did mesocarp discolouration after cold storage. No difference in phenolic concentration was, however, found between cold- and non-stored fruit. Conversely, Graham and Patterson (1982) found that the concentration of fruit phenolics increased at low temperatures. This was attributed to the increased production of two enzymes concerned with the synthesis of phenolic compounds, phenylalanine ammonia lyase (PAL) and hydroxycinnamoyl CoA quinate hydroxycinnamoyl transferase (CQT), increasing at low temperatures.

1.2.1.2 Polyphenol oxidase

Polyphenol oxidase (PPO) is a copper-containing enzyme complex which catalyses the conversion of monophenols and *o*-dihydroxyphenols to *o*-quinones in higher plants. The *o*-quinones produced by this reaction can undergo polymerisation and bind covalently to nucleophilic amino acids to form black or brown pigments which cause the characteristic postharvest browning of fruit and vegetables (Mayer and Harel, 1979; 1991; Stewart *et al.*, 2001). PPO is a nuclear-encoded protein (Lax *et al.*, 1984) that is transported to the plastids, where it is associated with the internal thylakoid membranes (Vaughn *et al.*, 1988). As mentioned previously the phenolic substrates for PPO are located in the vacuole, and therefore the enzymic browning reaction only occurs when subcellular compartmentation is lost following tissue damage. PPO gene expression is usually highest in developing tissues and meristematic regions, and decreases during tissue maturation (Hunt *et al.*, 1993; Dry and

Robinson, 1994; Boss *et al.*, 1995). Generally, PPO can exist in a soluble and an insoluble form (Kahn, 1977a). It is possible to solubilise and release additional PPO activity from the insoluble fraction, and in some cases, soluble PPO can exist in a latent form that is activated by storage, temperature, detergents or denaturing agents (Kahn, 1977a). In avocados an anionic detergent, sodium dodecylsulphate (SDS), has been successfully used to activate latent PPO (Kahn, 1977a). Low temperature storage (Sharon and Kahn, 1979), for increasing lengths of time (Golan and Sadovski, 1977), has also been reported to result in an increase in PPO activity in avocados.

Although the physiological function of PPO in higher plants has not been “unequivocally determined” to date there is evidence to suggest that the enzyme plays a role in plant defence (Vaughn *et al.*, 1988). This is to be expected as cellular damage can result in the loss of PPO latency, decompartmentalization of the phenols and thus interaction of PPO with these phenolic substrates, with the production of bactericidal and fungicidal hydroxyphenolics and quinines which may polymerise to seal off infected tissues. There are, however, studies in which no correlations or negative correlations have been obtained between PPO activity and disease resistance (Brune and Van Lelyveld, 1982).

The relationship between PPO activity and postharvest fruit quality has also received much attention. In pineapple fruit a correlation was found between PPO activity and the development of the internal browning disorder known as blackheart (Van Lelyveld and De Bruyn, 1977), which is thought to be a chilling disorder. Furthermore, cultivars of grapes with low PPO levels were found to be superior because the wine from these grapes did not turn brown as easily as from varieties having higher PPO levels (Vaughn *et al.*, 1988). The possible involvement of PPO in the development of mesocarp discolouration in avocado fruit was also suggested by Engelbrecht (1987). Similarly, Kahn (1975) found that avocado cultivars, which were more susceptible to mesocarp discolouration, had higher PPO activity than those less susceptible. PPO activity has also been linked to preharvest water stress, storage container ventilation (Bower and Van Lelyveld, 1985) and moisture loss during cold storage (Bower and Cutting, 1987).

Senescence is also thought to result in decompartmentalization of PPO and its substrates. This, together with the fact that total phenolics and PPO have been found to increase as the season progresses, may imply browning potential would be higher during this time. However,

a strict correlation between PPO activity and browning capacity does not necessarily exist (Sharon-Raber and Kahn, 1983) as the phenomenon is also both qualitatively and quantitatively substrate dependent.

Some studies have found correlations between the presence of high levels of phenolic compounds and PPO (Golan *et al.*, 1977) or low levels of phenolics and no PPO in some tissue types (Vaughn *et al.*, 1988). However, these correlations do not always exist. Kahn (1977b) found that in some cases a relatively high substrate concentration inhibited avocado PPO activity. Mueller and Bechman (1978) noted that, although some cell types with vacuolar phenolic depositions had plastids with PPO, other layers of tissue had plastids that lacked PPO activity but had phenolic depositions in the vacuole. While studies found that PPO is not involved in the synthesis of phenolic compounds in healthy, intact cells (Vaughn *et al.*, 1988; Strack *et al.*, 1986), it is not thought to be totally unrelated to phenolic metabolism.

The fact that PPO is located on the thylakoids of mature chloroplasts, and that it is a redox enzyme, has also led to investigations of a possible role of PPO in energy transduction of the chloroplast. Vaughn and Duke (1984) suggested that PPO might act in mediating photoreduction of molecular oxygen (the Mehler reaction) by photosystem I (PSI). PPO was found to have a low affinity for molecular oxygen and be to a large degree latent on the thylakoid membrane, increasing in its activity during membrane damage. It was thought, therefore, that the regulation of the Mehler reaction by PPO might be modulated by oxygen levels and any factor that might alter PPO latency.

1.2.2 Temperature

1.2.2.1 Factors affecting chilling injury

Several factors influence the incidence of chilling injury. The great diversity in shape, size, and physiology of various fruits and vegetables contributes to the variations in chilling tolerance. Substantial differences in the degree of chilling sensitivity also exist among cultivars and species. In addition, environmental conditions under which the crops are grown have great influence on the susceptibility to chilling injury. Considering all of these factors, it is not hard to understand why we still do not have a universal method, which could be effective in reducing chilling injury in all crops under any circumstances. The degree of

chilling injury incurred by a plant or plant part depends on the temperature to which it is exposed, the duration of exposure, and the species sensitivity to chilling temperatures. The lower the temperature to which a product is exposed below its threshold chilling temperature, the greater the severity of the eventual injury. The rate of development of injury symptoms in storage is also generally decreased with temperature; however, upon removal to non-chilling conditions the full manifestation of the stress becomes apparent (Kays, 1991). Chilling stress and injury does not just occur during storage. Chilling temperatures may be encountered in the field, during handling or transit, during wholesale distribution, in the retail store, and in the home.

1.2.2.2 Symptoms of low temperature disorders

The onset of symptom expression due to chilling injury in plants varies visually and temporally among plant species. In general, greater injury occurs in plants exposed to lower chilling temperatures for longer periods of time. In some plants, injury may be expressed during the chilling period or after the plant tissue has been re-warmed. Symptoms of chilling injury include cellular damage (changes in membrane structure and composition, decreased protoplasmic streaming, electrolyte leakage, and plasmolysis), altered metabolism (increased or reduced respiration, production of abnormal metabolites due to anaerobic conditions), surface lesions on fruits, water soaking of tissues, internal discolouration (including vascular browning), increased susceptibility to decay, and failure to ripen normally (Saltveit and Morris, 1990). Some of the more common symptoms of chilling stress are rapid wilting followed by water-soaked patches which develop into sunken pits that reflect cell and tissue collapse. Following warming the sunken pits usually dry up, leaving necrotic patches of tissue.

1.2.2.3 Primary versus secondary chilling injury lesions

It has long been debated whether there is some primary event that leads to chilling injury in plants, and if so what the event is. Evidence has accumulated that chilling stress affects several functions in plants, including biochemical and biophysical structure of membranes, nucleic acid synthesis, changes in protein synthesis, enzyme conformation, affinities and activation energies, water and nutrient (particularly calcium) balances, cellular cytoskeletal structure, and photosynthetic and respiratory function. Which of these represent the primary event and which are secondary events leading to symptom expression is the basis for the

debate. Raison and Orr (1990) propose a single primary event based on temperature transition in the molecular ordering of membrane lipids, which was initially expressed by Lyons and Raison in 1970. This model suggests that chilling injury can be divided into a single primary event and several secondary events (Figure 1). The primary event (probably, membrane phase transition) is initiated when temperature drops below a certain critical temperature. This temperature will vary with species and conditions under which the plant is grown. The primary event is then responsible for initiating numerous secondary events, but the order of initiation, if any, is not clear. If the level of chilling is not too great or too long and the plant is returned to warmer temperatures, the process can be reversed and the plant does not sustain injury. However, if the stress is maintained at too low a temperature for too long, and then returned to warmer temperatures, injury and cellular degradation are accelerated.

Naylor (1983) argues against a single primary lesion as being responsible for initiating the cascade of events that lead to injury and symptom development. Instead it is envisioned that chilling stress affects the physical concepts of coordination of metabolic pathways (rate effects) and the stability of complex biological molecules (weak bond effects), either or both of which may be affected at different levels of organization and expression. In the case of rate effects, sensitivity of any one metabolic process, or enzyme within that process, to chilling temperatures could have a significant impact on the functioning of other enzymes in that process or substantially alter the balance among multiple metabolic processes. Naylor (1983) further points out that biochemical structure, including protein configuration, membrane structure, nucleic acid structure, and most biochemical interactions, require a high degree of specificity, which, if not totally, is highly dependent on weak chemical bonds. Thus when considering the importance of just hydrogen bonding (with bonding energies 10 to 12 times weaker than covalent bonds) in protein configuration, the structure and stability of membranes, and protein-membrane and protein-substrate interactions, it becomes clear that there may be numerous opportunities where biochemical functions could be affected simultaneously by chilling stress, making the identification of a single primary lesion difficult if not impossible. The ability of organisms to cope with chilling temperatures and other environmental stresses then lies with the development of physical and/or biochemical strategies that protect the organism against breaking of weak bonds.

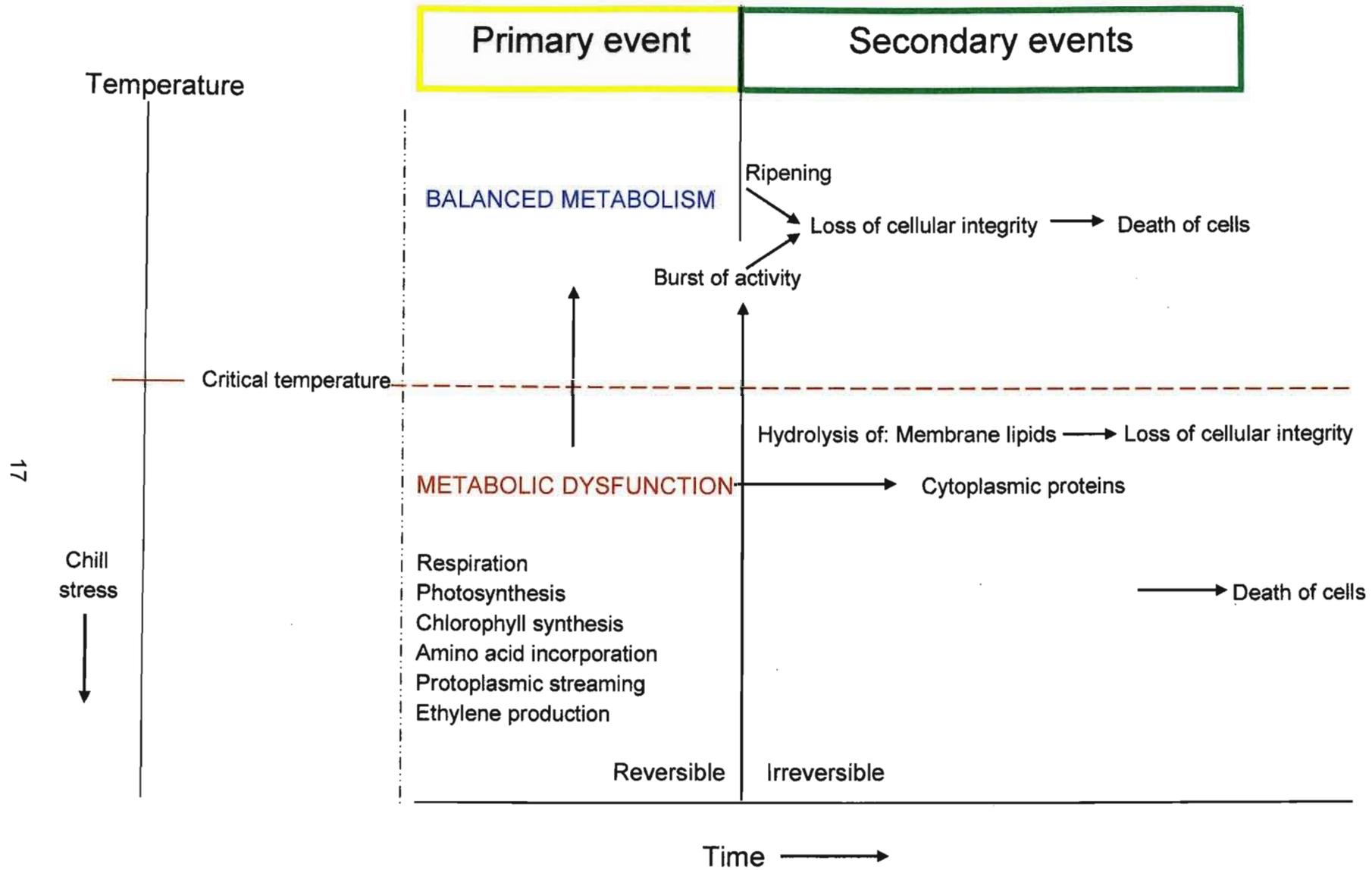


Figure 1. Relationship between "primary" and "secondary" events during chilling injury. (Modified from Raison and Orr, 1990).

1.2.2.4 Physiological and biochemical effects of chilling stress

A number of mechanisms have been proposed to accommodate the physiological and biochemical changes associated with acclimation and adaptation of plant cell membranes to different environmental temperatures. A review of these changes indicates that chilling changes the physical stability of the membrane and its ability to function under different conditions (Lyons, 1973; Pantastico *et al.*, 1975; Quinn, 1988; Nilsen and Orcutt, 1996). Plants of tropical and subtropical origin are generally assumed to contain more highly saturated fatty acids than species growing in cold regions (Pantastico *et al.*, 1975). In these plants, phase transitions in membrane lipids have been proposed as the primary event in physiological disorders such as "chilling injury" (Lyons, 1973; Raison, 1973). While this hypothesis received initial support, further studies found that only a small proportion (<10%) of the membrane lipids underwent phase transition at physiological temperatures (Wills *et al.*, 1989). A refinement to the lipid-phase transition theory has been based on the presence of heterogenous lipid domains in biological membranes undergoing liquid-crystalline to gel-phase transitions (Stanley, 1991). The membranes of chilling-injured avocado fruit were found by Platt-Aloia and Thomson (1987) to contain particle-depleted regions in the plasmalemma. These particle-depleted microdomains were suspected to be due to lateral phase separations of the membrane components, possibly due to an increase in the viscosity of some membrane lipids, leading to the formation of microdomains of gel phase lipid in the plane of the membrane. This has the potential of forming inverted micelles in the membrane, which could facilitate nutrient or ion leakage. In addition, such restructuring could influence the configuration or positioning of proteins (enzymes) within the membrane altering their functions (Nilsen and Orcutt, 1996). Thus, membranes may not respond to environmental perturbations in a general way as previously thought, but rather, may influence specific organelle membrane lipids and/or affect isolated domains in those membranes, resulting in localized changes in fluidity or perhaps inducing pores as a result of lateral phase transitions resulting from homologous lipid aggregations. Chilling sensitivity was recently correlated with the degree of unsaturation of fatty acids in phosphatidylglycerol of chloroplast membranes (Murata *et al.*, 1992).

Thus plants have a mechanism by which they can maintain membrane fluidity by changing the saturation and unsaturation of fatty acyl groups of glycerolipids. It is reported that as temperature increases the degree of unsaturation is reduced, while as temperature

decreases, unsaturation increases. Insertion and removal of sterol from membranes may also be part of the mechanism for membrane fluidity adjustment as temperatures change (Nilsen and Orcutt, 1996). As mentioned in the previous section the physical change in the membrane lipids, with the lowering of temperature, is thought to cause changes in the properties of the membranes (Wills *et al.*, 1989). Generally, chilling stress conditions weaken hydrophobic interactions, expose sulfhydryl groups, and can alter the lipid environment surrounding membrane-associated proteins which can ultimately lead to changes in the configuration of proteins and possibly enzyme kinetics (Nilsen and Orcutt, 1996). Similarly, early studies found that an increase in membrane permeability would lead to an upset in ion balance and also to increases in the activation energy (E_a) of membrane-bound enzyme systems, leading to a suppressed reaction rate and establishing an imbalance with non-membrane-bound enzyme systems (Lyons, 1973). For example, below a critical temperature, at which phase transition occurs, the E_a for membrane-bound mitochondrial respiration increases while the E_a for soluble enzyme systems, such as glycolysis, decreases causing an imbalance in the two systems. Enzymes of photosynthetic metabolism are similarly affected. The consequences of such changes in enzyme activities are thought to be imbalances in metabolism, which eventually lead to cell death. Plank (1938) assumed that, under chilling stress conditions, two main types of reactions were involved in the cells – one leading to the accumulation of toxins and the other to their removal. By selecting values for the temperature coefficient used in his equations, he was able to show the critical temperature at which the production and removal of toxins are in equilibrium and below which cell toxins would accumulate, causing chilling injury. Other workers have also accepted the toxin hypothesis of chilling injury, in which toxic products of metabolism accumulate (Eaks and Morris, 1957; Hulme *et al.*, 1964; Wills *et al.*, 1989). Apparent differences among species or cultivars in chilling sensitivity are suspected to be related to different tolerances in withstanding or metabolising the resulting toxic compounds (Lyons, 1973).

Furthermore, a progressive decline in the capacity of the fruit for oxidative phosphorylation occurs with exposure to low temperature. This could lead to a shortage of high energy, typically ATP, needed for the maintenance of cell organization in the presence of enzymatic processes, constantly tending to disrupt the system. A net breakdown of complex cellular components follows because of the resultant shortage of energy (Pantastico *et al.*, 1975).

The temperature-induced phase change in the lipid portion of the membranes is completely reversible, though the effect on the whole organism is reversible only until the system incurs some degenerative injury. Thus, with a short chilling treatment followed by a warmer temperature, respiration increases sharply but only transiently, with the normal metabolism soon re-established. If chilling temperatures continue long enough for degenerative changes to occur, however, the respiration rate remains elevated, reflecting a disrupted metabolism (Lyons, 1973). The effect of temperature on respiration rates (Gonzalez-Meler *et al.*, 1999) could, in the short term, be due to the kinetics of most metabolic reactions being highly temperature dependent (Raison, 1980). For most plants respiratory activity at low temperatures remains in balance with glycolysis and other closely related reactions. In contrast, the respiratory activity of many tropical and sub-tropical plants decreases more than other reactions when the temperature is lowered, and this leads to imbalances in metabolism (Raison, 1980). Furthermore, increasing respiratory rates during chilling, accelerated respiration following chilling, and altered respiratory quotients have all been used as indices of chilling sensitivity (Pantastico *et al.*, 1975).

In addition, structural proteins of the cell's cytoskeleton, such as tubulin, are cold-labile and undergo dissociation at low temperatures. This is thought to account for the effect of low temperatures on protoplasmic streaming which is especially sensitive in chilling sensitive plants (Wills *et al.*, 1989). Degradation of cell walls plays an obvious role in the development of the visible symptoms of stress response. Localized softening associated with fruit bruising is the most obvious example of enzymatic breakdown of cell walls. Examples of cell-wall breakdown during chilling injury include the development of pitting (Shewfelt, 1993).

The mechanism of low temperature disorders thus encompasses several elements operating independently or simultaneously: imbalances in metabolism, accumulation of toxic compounds, dis-equilibrium of reactions and increased permeability. Levitt (1972), however, proposed that all types of chilling injury can be the result of a change in cell permeability and many studies have in fact concentrated on the effect of membrane permeability, as determined by increased electrolyte leakage, on low temperature disorders (Lyons, 1973; Pantastico *et al.*, 1975; Levitt, 1980; Bramlage, 1982; Morris, 1982) and also specifically on avocados (Platt-Aloia and Thomson, 1992; Woolf *et al.*, 2000).

1.2.2.5 *Effect on other fruit*

In mango studies, where fruit have a critical storage temperature of about 10°C, a marked increase in sucrose degradation was found when fruit were exposed to temperatures of 2-5°C, which was thought to be indicative of an increase in glycolysis (Chattpar *et al.*, 1971). In bananas the respiratory enzymes were found to decrease or lose their activity after exposure to low temperatures (Murata, 1969), with the polyphenol substances, in severely chilled fruits, being oxidised instead of the regular respiratory substrates. Jones (1942) observed that CO₂ production by papaya fruits was higher than expected at chilling temperatures. Miller and Heilman (1952) proposed that in pineapples, the destruction of ascorbic acid constituted the first phase in the development of chilling injury. They proposed that interference in some specific steps in the respiratory process caused quinines to accumulate because of their failure to be converted back to phenols by ascorbic acid, and that this accumulation of quinines resulted in the discolouration noted in many kinds of chilled fruits. In grapefruit, intermediate temperatures were found in some instances to cause greater chilling injury than either higher or lower temperatures (Pantastico *et al.*, 1975). It was thought that the greater injuries noted at the intermediate temperatures were possibly restricted to a specific time period (Ulrich, 1958). Preharvest environment is also thought to influence chilling susceptibility (Lyons, 1973). Palmer (1971) cited studies indicating that banana fruits maturing at higher field temperatures were more susceptible than those maturing in a cooler climate.

1.2.2.6 *Effect on avocado fruit quality*

The critical temperature for cold storage of unripe avocados has been reported to be 8°C (Lyons, 1973). Storage at below optimal temperatures has been found to affect fruit ripening (Biale, 1941; Eaks, 1976; 1983; Cutting and Wolstenholme, 1992). Unripe 'Hass' exposed to temperatures of 0°C and 5°C for 4-6 weeks displayed chilling injury symptoms, abnormal ripening, atypical respiratory rate patterns, and greatly reduced ethylene peaks when ripened at 20°C (Eaks, 1983).

Studies have, however, found that the optimum temperature and storage period of avocados depends on a number of factors. For example, not all cultivars are equally sensitive to low temperatures (Vakis, 1982; Bezuidenhout, 1983; Vorster *et al.*, 1987; Eksteen *et al.*, 1998).

The stage of fruit development can also be important as sensitivity in 'Hass' was found to be relatively high in the preclimacteric stage, increasing to a maximum at the climacteric peak, and decreasing rapidly as the fruit ripens, and finally reaching a minimum about two days past the climacteric (Kosiyachinda and Young, 1976). Similarly, Bezuidenhout (1983) found that excessive cold prior to the climacteric, in 'Fuerte' fruit, was favourable for chilling injury and pulp spot development. The changes in chilling sensitivity were suspected to be related to changes in the activity of regulatory enzymes such that intermediates accumulated to levels that were toxic to the cells (Kosiyachinda and Young, 1976). These levels of intermediates were found to be lower after the climacteric peak had been reached and thus the storage life of avocados could be extended by transferring the fruit to 2°C after the climacteric peak (Kosiyachinda & Young, 1976). Zauberman and Jobin-Décor (1995) found that unripened 'Hass' could also be stored successfully for four to five weeks at 2°C without developing injury or abnormal ripening. These fruit were still hard after removal from storage and were thus still suspected to be in the preclimacteric stage.

The potential for physiological disorders and cold damage varies throughout the season (Swarts, 1980; Bower *et al.*, 1986; Vorster *et al.*, 1987). Early season avocado fruit have been found to be more susceptible to chilling injury than fruit picked later in the season (Swarts, 1980; Smith and Lunt, 1984; Kritzinger and Kruger, 1997). Swarts (1980), however, proved that this was not the effect of picking maturity, but rather the result of a drop in the preharvest ambient orchard temperature to below 17°C. Based on this evidence it was suggested that storage temperature during the early parts of the season should be higher than later in the season. Application of this concept resulted in Vorster *et al.* (1987) finding that 'Fuerte' avocados picked later in the season could be stored at 3.5°C without danger of chilling injury. Zauberman and Schiffman-Nadel (1977) also found that preharvest temperature could have an adverse effect on fruit ripening, *viz.* temperatures above 30°C. Bezuidenhout (1983) attributed up to 50% of the probable causes of internal disorders to "unknown orchard factors". This was confirmed by Rowell (1988) who found that there was a marked variation in the way in which fruit from different areas reacted to temperatures.

1.2.3 Water content

1.2.3.1 Maturity

In South Africa the moisture and oil content of avocado fruit are used as maturity parameters (Swarts, 1982; Kruger *et al.*, 1999). Oil content is known to increase and water content to decrease with increasing maturity (Pearson, 1975). In recent years maturity has been considered to be the main causative factor of mesocarp discolouration development in South Africa (Snijder *et al.*, 2002; Snijder *et al.*, 2003; Kruger *et al.*, 2004). In South Africa the moisture content of 'Pinkerton' avocados, being considered for export, should be between 80% (Thompson, 1996) and 73% (Snijder *et al.*, 2003). Postharvest time to ripening is thought to be a function of fruit maturity, less time being required with increasing maturity (Zauberman and Schiffman-Nadel, 1972; Adato and Gazit, 1974). Similarly Cutting and Wolstenholme (1992) found that increasing maturity (on-tree storage) decreased the time taken for avocado fruit to ripen after harvest. This is to be expected, as there is a decreasing water percentage with increasing maturity at harvest.

Stage of fruit ripeness, at the time of low-temperature storage, has also been reported to play a role in determining the sensitivity of 'Hass' and 'Fuerte' avocados to chilling injury (Kosiyachinda and Young, 1976). In bananas ripe fruit, regardless of variety, are reportedly less sensitive than green fruit. Among the various varieties of citrus, grapefruit harvested early in the season are found to be more susceptible to pitting with the susceptibility decreasing as fruit became more mature. However, differences in grapefruit susceptibility are also related to season and variety. Tomato fruit are also particularly susceptible to chilling injury at the mature-green stage when they are normally harvested and shipped (Saltveit, 1991).

1.2.3.2 Preharvest orchard conditions

Preharvest water relations also affect the rate of fruit ripening. Bower (1984) found that more negative avocado fruit water potentials at harvest, caused faster ripening after storage. Long term preharvest stress (particularly during the first 3 months after fruit set) also caused an altered ethylene evolution pattern (Cutting *et al.*, 1986). Furthermore, ripening was uneven, and fruit quality poor. Excessive irrigation was also found to reduce calcium uptake as well

as increase levels of ABA in avocado fruit (Bower *et al.*, 1986; Bower, 1987). In 'Pinkerton' it was also found that storage potential was drastically reduced during a wet season if there was a very low crop load (Kruger *et al.*, 2004).

1.2.3.3 Postharvest handling

The potential for water stress does not stop at harvest; rather in most postharvest products the stress potential increases sharply. When individual plant parts are severed from the parent plant at harvest, their ability to replace water lost through transpiration is eliminated, making them much more susceptible to water stress. Fruit firmness and membrane integrity, during storage, are thought to be affected by relative humidity (Ben-Yehoshua, 1985). When relative humidity is too low, transpiration is enhanced, resulting in loss of moisture. The primary factor controlling the rate of moisture loss is actually the water vapour pressure deficit, which reflects the difference between the humidity in the tissue and the humidity of the air in the storage room or container (Kays, 1991). As water evaporates from the tissue, turgor pressure decreases and the cells begin to shrink and collapse. In studies on avocados Bower and Cutting (1987) also found that the rate of fruit moisture loss during storage was associated with fruit quality. Furthermore, the infusion of avocado fruit with water during ripening (Cutting and Wolstenholme, 1992), as well as storage of fruit under high relative humidity (Bower, 1988), was found to reduce the incidence of physiological postharvest disorders. Water stress caused by prolonged storage can result in increased and early ethylene production, which in turn may enhance ripening processes (Adato and Gazit, 1974). Water status thus plays a very important role in maintaining membrane integrity and slowing down ripening. Storage at very high relative humidity (close to 100%) also can result in adverse effects. Free water on the plant tissue provides an excellent environment for decay microorganisms. Although healthy plant tissue is resistant to decay, cuts, bruises, or additional stress can weaken the endogenous resistance of the tissue.

High relative humidity storage combined with low temperatures has been suggested as a means of maintaining quality of bulk products for extended periods of time. Humidity close to 100% has been found to ameliorate chilling injury in some instances, with low humidity aggravating symptoms. Morris and Platenius (1938) showed that, although the severity of pitting was directly correlated with the rate of transpiration, very rapid rate of water loss did not result in pitting if the fruit had not been exposed to low relative humidity at the same time.

The reduction of chilling injury in citrus by seal-packaging was not attributed to the inhibition of transpiration by the sealing, since the cooling was thought to inhibit transpiration, and chilling injury could be reduced even more effectively by curing fruit for a week at 21°C before exposing it to the sensitive low temperatures. During this curing period, the fruit transpired rapidly (Ben-Yehoshua, 1985).

1.2.3.4 Effect of hydration on membranes

Degree of hydration is very important in affecting the fluidity of plant membranes. In general, if cell water percentage falls to a level of 20% or less of the cell dry weight, this is considered to be a critical level relative to maintaining homeostatic viscosity of the membrane and may even affect the thermodynamic stability of the membrane (Nilsen and Orcutt, 1996). As with temperature stress, lateral phase transitions may occur in which homologous lipids aggregate into different regions or domains of a membrane, resulting in ion leakage. Varying lipid components in membranes exhibit differing degrees of hydration or affinity for water. Glycolipids apparently have a very high affinity for water, which reflects the carbohydrate component of these lipids. Carbohydrates, particularly non-reducing disaccharides such as trehalose and sucrose, appear to be important in organisms capable of survival after complete dehydration. It appears that many organisms produce high levels of these carbohydrates when exposed to dehydration stress, and it is hypothesised that the carbohydrates interact with cellular membranes to increase the stability of the lipid bilayers. The protective mechanism is uncertain, but it has been suggested that under stress, water molecules normally associated with the phospholipid head-groups are replaced with sugars, which prevent lateral phase transition and the formation of lipid domains (Nilsen and Orcutt, 1996).

1.2.4 Mineral nutrition

Mineral nutrition plays a very important role in the growth and development of any fruit, and subsequently determines fruit quality and storage life (Ginsberg, 1985). A number of nutrient elements are of importance and also the balances of these elements in relation to each other. Divalent and monovalent cations are known to have a considerable influence on the fluid nature of biomembranes (Nilsen and Orcutt, 1996). How these ions interact with membranes depends on the type and abundance of membrane components present, as well as how they

are arranged in the bilayer. The phospholipids appear to be the most drastically affected components of the membrane; however, interactions with proteins can also occur. Many disorders are prevented by the addition of a specific mineral either during growth or postharvest, although for most disorders the actual role of the mineral in preventing the disorder has not been established (Wills *et al.*, 1989). Generally, all plants require a balanced mineral uptake for proper development. However, while leaf and soil norms have been established for optimum avocado production (Koen and Du Plessis, 1991; Köhne *et al.*, 1990), there are very few studies that have been conducted to evaluate and establish fruit norms with respect to fruit quality (Cutting *et al.*, 1992; Thorp *et al.*, 1997; Hofman *et al.*, 2002; Snijder *et al.*, 2002). Some studies have related fruit quality to soil mineral concentrations (Kremer-Köhne *et al.*, 1993), however there are studies that have found no correlation between soil, leaf and fruit concentrations (Thorp *et al.*, 1997). Furthermore, there appear to be very few studies that evaluate a wide range of both macro- and micro-elements.

1.2.4.1 Calcium

Calcium has been associated with more physiological disorders than any other mineral (Bangerth, 1979; Wills *et al.*, 1989). It is thought to play a significant role in the rate of fruit softening with high endogenous levels of calcium (Tingwa and Young, 1974; Cutting *et al.*, 1992) or postharvest calcium applications being found to delay the overall softening process during ripening (Wills and Tirmazi, 1982; Ferguson, 1984). Furthermore, the infiltration of calcium into avocados has been shown to greatly reduce the ethylene peak and respiration rate, resulting in an extended shelf life (Tingwa and Young, 1974; Eaks, 1985; Yuen *et al.*, 1994).

Fruit quality is also thought to be affected by fruit calcium concentrations, with higher concentrations resulting in fewer disorders in many avocado cultivars (Chaplin and Scott, 1980; Eaks, 1985; Cutting *et al.*, 1992; Hofman *et al.*, 2002). The fact that mesocarp discolouration appears first in the distal end of fruit has also been correlated with lower fruit mesocarp calcium concentrations in this part of the fruit (Chaplin and Scott, 1980). The development of mesocarp discolouration, resulting from increased PPO activity, is also thought to be the end result of a calcium deficiency (Kirkby and Pilbeam, 1984). Calcium applications were found to reduce PPO activity and total phenol content in avocado fruit (Van Rensburg and Engelbrecht, 1985), which are related to browning. However, not all studies

regarding postharvest calcium applications have proved to be successful in reducing internal disorder severity (Penter *et al.*, 2001). Snijder *et al.* (2002) evaluated the effect of ensuring sufficient calcium concentrations during specific periods of fruit growth and found that a calcium content of between 1000 and 1200 ppm during November (in South Africa) reduced the potential for mesocarp discolouration development.

1.2.4.1.1 *Mode of action*

Calcium plays an important role in cell division and cell development, especially in root tips, leaves, and fruit (Marschner, 1995). Calcium is thought to play a significant role in membrane stabilisation (Battey, 1990) and in cell wall structure (Ferguson, 1984). It appears that calcium affects membrane permeability by virtue of its intermolecular bridging of phosphate head groups in membranes (Ferguson and Drobak, 1988; Nilsen and Orcutt, 1996). Calcium-deficient tissues are, therefore, less resistant to electrolyte leakage (Simon, 1978) resulting in the leakage of phenols from the vacuole into other cellular compartments where they undergo oxidation (Van Rensburg and Engelbrecht, 1985). Calcium also plays an important role in cell walls, and 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 that appear to provide stability and mechanical strength (Bangerth, 1979; DeMarty *et al.*, 1984; Burns and Pressey, 1987). The extensive cross-linking not only facilitates packing of pectic polymers in the middle lamella, but also reduces the accessibility of enzymes that contribute to the breakdown of cell walls and softening (Glenn and Poovaiah, 1990; Conway *et al.*, 1992).

Calcium deficiency disorders are believed to be due to the inefficient distribution of calcium rather than poor calcium uptake (Thorp *et al.*, 1997), and are restricted to organs and tissues that have low transpiration rates. Most of the calcium taken up by the roots is transported by mass flow in the xylem. 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. However, because of their greater physiological activity and transpiration rate, leaves and shoots are stronger sinks for calcium than fruit (Witney *et al.*, 1990). Fruit borne on vigorously growing trees will, therefore, have a lower calcium content, especially during the early stages of fruit growth.

1.2.4.2 Nitrogen

Typically, crops that contain high levels of nitrogen have poorer keeping qualities than the same variety of crop with lower levels (Thompson, 1996). High nitrogen applications have been found to result in faster ripening and more internal disorders in 'Hass' avocado fruit (Arpaia *et al.*, 1996), and in some pome and stone fruits (Link, 1980). Similarly Kremer-Köhne *et al.* (1993) found that the percentage of avocado fruit free of physiological disorders was considerably decreased by nitrogen applications. High fruit nitrogen concentrations were also found to increase the severity of mesocarp discolouration in 'Fuerte' fruit after cold storage (Koen *et al.*, 1990). In fact in South Africa it was suggested that fruit nitrogen content, especially for 'Pinkerton', be less than 1% by March to reduce mesocarp discolouration development (Snijder *et al.*, 2002); and less than 1 % by January to reduce external chilling/black cold injury. Excessive and deficient nitrogen concentrations have also been found to affect chilling injury (Bramlage, 1982). Thus, nitrogen management is important as the type, rate and timing of fertilizer applications have been found to significantly affect fruit yield and quality (Lovatt, 2001).

1.2.4.2.1 Mode of action

Nitrogen is a key component of chlorophyll and is required for the synthesis of plant hormones, which control plant growth. Nitrogen is considered to be very mobile within the plant (Marschner, 1995), and its status often reflects the vigour of the plant. Nitrogen absorption during early fruit development tends to stimulate shoot growth, which acts as a preferential sink for calcium (as well as water and other metabolites) and further reduces calcium transport into fruit (Witney *et al.*, 1990). Adding nitrogen to trees also tends to increase fruit nitrogen significantly (Arpaia *et al.*, 1996) and creates a high N/Ca ratio, which has been associated with the development of certain internal disorders in apple (Ferguson and Watkins, 1989) and pear (Curtis *et al.*, 1990).

Superficial scald in apples (Emonger *et al.*, 1994) and translucence in pineapple has been related to high nitrogen (Soler, 1994; Paull and Reyes, 1996). It is also more common in large fruit, which suggests that the disorder is related to fruit growth rates, and water and carbohydrate supply (Ferguson *et al.*, 1999). Higher nitrogen concentrations in plant tissues may result in increased ABA production (Nilsen and Orcutt, 1996), weaker cell walls and the

activity of key enzymes involved in phenol metabolism may be reduced, thus decreasing host resistance to fungal attack (Matsuyama and Dimond, 1973).

1.2.4.3 Boron

Boron has been found to be the most common nutrient deficiency in avocado trees (Whiley *et al.*, 1996), with soil applications being reported to improve fruit quality (Smith *et al.*, 1997). Furthermore, boron has a major effect on avocado fruit shape, as its deficiency causes uneven cell division within the first six weeks of fruit growth (Whiley *et al.*, 1996). The exact function of boron is, however, still uncertain and renders it one of the least understood of all plant nutrients (Hu and Brown, 1994; Marschner, 1995). It has, however, been found to be an essential micronutrient in the normal development of root and shoot tips, flowers and fruit, being directly involved in cell division and cell growth (Whiley *et al.*, 1996). It has also been proposed that boron plays key roles in cell wall structure and metabolism, plasma membrane integrity, and in phenol metabolism and lignin biosynthesis, which are important processes in plant defence mechanisms against physiological disorders and pathogenic infections (Marschner, 1995; Match, 1997).

Boron deficient plants have been found to contain higher calcium, nitrogen, magnesium and iron than boron fed plants (Lal and Subba Rao, 1954). In fact, boron has been closely linked to calcium in plant nutrition (Shear, 1975), probably due to its effect on diffusible auxin (IAA) (Marschner, 1995). In apples boron can enhance calcium transport in apple trees, and help maintain more plant calcium in soluble forms, which may enhance calcium absorption (Faust and Shear, 1968). It is considered to play some part in the regulation of water relations of plasma colloids and helps carbohydrate transformation and utilization (Lal and Subba Rao, 1954).

Boron deficiency initially tends to increase respiration, however a decline is noted as deficiency symptoms become evident (Shelp, 1993). Phenol accumulation, resulting in browning reactions, has also been observed in plants growing in conditions of long-term boron deficiency (Shkolnik, 1984). Marschner (1995) states that under boron deficiency phenols accumulate and polyphenol oxidase activity is increased which leads to highly reactive intermediates such as caffeic quinone in the cell walls. These quinines, as well as light activated phenols, are very effective in producing superoxide radicals potentially capable

of damaging membranes by lipid peroxidation. Where boron is not limiting, it forms complexes with many phenolic compounds thereby reducing phenolic concentration (Cakmak *et al.*, 1995).

1.2.4.4 Magnesium

Magnesium is an essential component of chlorophyll, regulates the absorption of other plant nutrients, and is essential for many cellular biochemical functions (Marschner, 1995). Excessive magnesium concentrations in the soil have been found to suppress calcium absorption by roots (Himelrick and McDuffie, 1983). However, an adequate supply of magnesium is important, as the mobility of calcium in the xylem is improved by the presence of divalent cations, such as magnesium, which are also adsorbed onto exchange sites (Himelrick and McDuffie, 1983). A study done in avocados showed that the magnesium concentration decreases as fruit increase in relative maturity (Cutting *et al.*, 1992).

1.2.4.5 Potassium

Potassium has several important roles in plants, such as the regulation of water balance through controlling the opening and closing of stomata on leaves and the synthesis and movements of starches, sugars, and oils, which may have a direct affect on fruit quality. Potassium also has a prominent effect on the conformation of enzymes and thus regulates the activities of a large number of enzymes (Marschner, 1995). Both high and low levels of potassium have been associated with abnormal metabolism (Wills *et al.*, 1989). It is a relatively mobile mineral in the plant (Terblanche, 1972) and its concentration has been shown to have a distinct fluctuating trend with increasing maturity in avocados (Cutting *et al.*, 1992). Like magnesium, very high soil levels of potassium may suppress calcium absorption by roots (Himelrick and McDuffie, 1983). In plants potassium may increase phloem transport, which can further depress the calcium status of fruit because the ratio of xylem to phloem transport into fruit is reduced (Bangerth, 1979).

In avocado, the balance of fruit magnesium, potassium and calcium concentrations has proven to be important in internal disorder severity. Strong negative correlations were found between mesocarp discolouration severity in 'Hass' fruit, and mesocarp calcium, magnesium and (Ca+Mg)/K ratio (Hofman *et al.*, 2002). Cutting and Bower (1990) also indicated that

high magnesium levels and a high (Ca+Mg)/K ratio were positively related to a high PPO activity, which is involved in the browning reaction. Marschner (1995) also stated that potassium deficient plants exhibit a much higher activity of oxidases such as polyphenol oxidase than do tissues of normal plants. Koen *et al.* (1990) found a positive relationship between proximal fruit potassium concentrations and mesocarp discoloration, but a strong negative correlation between leaf and soil (Ca+Mg)/K and grey pulp (mesocarp discoloration). Du Plessis and Koen (1992) also found that the incidence of mesocarp discoloration was strongly correlated with the subsoil (Ca+Mg)/K ratio. They also reported a significant reduction in the incidence of pulp spot with high levels of subsoil potassium, which, conversely, was found to aggravate mesocarp discoloration. In a study by Thorp *et al.* (1997), however, no relationship was found between leaf, soil and fruit mineral concentrations.

1.2.4.6 Zinc

Zinc is taken up predominantly as a divalent cation. High concentrations of other divalent cations, such as calcium, inhibit zinc uptake to a certain extent (Marschner, 1995). Zinc acts either as a metal component of enzymes or as a functional, structural, or regulatory cofactor of a large number of enzymes. Zinc also plays an essential role in the production of certain plant hormones, such as auxins, although the mode of action is still obscure. Zinc-deficient plants have been found to exhibit lower levels of indoleacetic acid and ABA (Nilsen and Orcutt, 1996). Zinc also has a regulatory role in the absorption of water, and is necessary for normal chlorophyll production (Marschner, 1995). Zinc plays a part in cellular oxidation and the fundamental processes involved in cellular metabolism and respiration. Zinc is also considered to act as a catalytic agent on some essential reactions bringing about inhibition of carbohydrate transformation. It is also a component of a catalytic system necessary for the phosphorylation of glucose (Lal and Subba Rao, 1954). Deficient plants contain more reducing sugar but less sucrose and starch than healthy plants. It is not very mobile either in the soil or in the tree and it tends to accumulate in roots (Marschner, 1995).

Zinc is thought to have a synergistic effect on the entry and accumulation of calcium in plant tissues (Testoni and Pizzocaro, 1980). Zinc applications to apples, were found to increase fruit calcium concentrations and reduced the severity of the internal disorder bitter pit (Shear, 1980). The higher zinc concentrations were thought to result in more bound calcium being

released from various chelating and complexing agents (such as lignin, organic acids, and proteins) for transport to the fruit.

Zinc and copper are both important components of superoxide dismutase (SOD). Under zinc deficiency the level of toxic oxygen species is high because of both depressed SOD activity and lower export rates of carbohydrates as a result of low sink activity (Marschner, 1995). This results in the peroxidation of membrane lipids and an increase in membrane permeability. Chvapil (1973) reported that zinc has the greatest affinity for membranes followed by copper, iron and calcium and found considerable evidence that zinc may increase the stability of membranes. Shear (1980) speculated that increases in zinc status in fruit may increase fruit quality.

Vorster and Bezuidenhout (1988) found that 'Fuerte' fruit with poor internal quality had significantly lower fruit zinc and calcium concentrations than unaffected fruit. Furthermore, high leaf zinc concentrations were associated with less pulp spot in fruit after cold storage (Bezuidenhout and Vorster, 1991). Zinc has been found to have a direct role on avocado fruit size and shape, with fruit from zinc deficient trees tending to be smaller and rounder than fruit from trees of the same cultivar with adequate zinc concentrations (Kadman and Cohen, 1977; Crowley *et al.*, 1996).

1.2.4.7 *Phosphorus*

Phosphorus is important for energy metabolism in maintenance and overall fruit growth, and can also be involved in membrane stability and carbon partitioning (Marschner, 1995). Phosphorus also controls some key enzyme reactions. It is incorporated in membrane lipids and nucleic acids and affects the photosynthetic efficiency of chlorophyll molecules. Studies have found that high concentrations of phosphorus in cells, specifically chloroplasts, can lead to the inhibition of starch synthesis. Low phosphorus concentrations have also been associated with chilling injury in certain fruit (Bramlage, 1982).

1.2.4.8 *Copper*

Copper is known to activate a group of oxidising enzymes – polyphenol oxidase, monophenol oxidase, laccase and systems of oxidising ascorbic acid. It acts as a catalyst for the enzymic

systems which lead to enzymic browning, the browning of cut or damaged tissues that are exposed to air. In copper deficient leaves the activity of polyphenol oxidase has been found to be almost absent (Marschner, 1995). Copper is also an important constituent of tyrosinase. Tyrosinase plays a part as a terminal oxidase in respiration of plants. Absence of copper also reduces apparent photosynthesis (Lal and Subba Rao, 1954).

1.2.4.9 *Iron*

All known enzymatic systems depending on iron involve porphyrin molecules. Enzymes like polyphenol oxidase and succinic dehydrogenase have also been known to be interrelated in effect with iron (Lal and Subba Rao, 1954). Injections of iron have been found to induce symptoms similar to low temperature breakdown and superficial scald in apple, but whether it has a direct role is still uncertain (Wills *et al.*, 1989). Certain respiratory enzymes required for salt accumulation are mediated by iron respiratory enzymes. The role of iron in chlorophyll production and its relation to other metals is of great significance (Lal and Subba Rao, 1954). It is suggested that potassium may displace iron from the enzymes involved in chlorophyll formation.

1.2.4.10 *Manganese*

Bezuidenhout and Vorster (1991) previously suspected that manganese could play a role in fruit quality, although the exact mechanism by which it did was uncertain. Manganese plays an important role in chlorophyll formation (Marschner, 1995). Generally zinc, copper, iron and manganese are all important components of detoxifying enzymes, being directly involved in the photosynthetic electron transport chain. The photolysis of water is mediated by a manganese-containing enzyme attached to photosystem II (Marschner, 1995). In this water-splitting system manganese clusters act as a device for storing energy prior to the oxidation of the two water molecules. Manganese also presumably acts as the binding site for the water molecules that are oxidised. Furthermore, manganese is thought to play an important role in sugar formation and sugar metabolism (Lal and Subba Rao, 1954). It is involved in respiration in that small doses increase oxygen intake. It is also suggested to catalyse aerobic respiration (Lal and Subba Rao, 1954).

1.2.5 Plant growth regulators

Ismail and Grierson (1977) reported that growth regulators applied preharvest, as well as postharvest, alter the susceptibility of the fruit to chilling injury. All the classical plant growth regulators (auxin, gibberellins, cytokinins, abscisic acid (ABA), and ethylene) have been shown to influence biological responses through changes in membrane characteristics (Nilsen and Orcutt, 1996), generally by increasing membrane permeability.

1.2.5.1 Abscisic acid

Abscisic acid (ABA) has been found to help in the prevention of chilling injury in cucumber seedlings and cotton plants (Rikin and Richmond, 1976; Rikin *et al.*, 1979), grapefruit (Kawada *et al.*, 1979) and zucchini squash (Wang, 1991). ABA may protect plants against chilling injury through its action as an anti-transpirant agent (Christiansen and Ashworth, 1978) and membrane stabilizer (Markhart, 1986). ABA was also found to induce protein synthesis, which may be associated with increased chilling tolerance (Xin and Li, 1991). Cold, drought and salinity stress can all cause a reduction in tissue water content. Many studies have shown an endogenous increase in ABA during cold stress. Furthermore, a cause-and-effect relationship between cold tolerance and endogenous ABA has been established by Heino *et al.*, (1990). Therefore, it is likely that desiccation stress caused by cold shock or water stress influences the activity of ABA, which regulates genes that code for stress-induced proteins. Terpenoid analogues of ABA have been shown to retard chilling-induced electrolyte leakage and phospholipid loss and to reduce chilling injury in cucumber seedlings (Flores *et al.*, 1988). Stressful temperatures can alter the internal water status and thereby ABA production (Walton, 1980), or they can directly affect ABA levels. It is, thus, sometimes difficult to separate the direct effects of temperature on ABA levels from the effects of temperature on plant water relations and subsequent ABA levels. In avocados, Cutting *et al.* (1988) showed that high levels of ABA significantly increased PPO activity, reduced internal fruit quality and reduced time to ripeness.

1.2.5.2 Ethylene

Ethylene had been described as a ripening hormone. It can thus be envisaged that fruits that increase their resistance to chilling injury with ripening would benefit from prestorage ethylene

treatment, for example tomatoes and papayas. On the contrary, chilling injury in some fruit becomes more severe after exposure to ethylene. Advancing the ripeness of avocados with ethylene increases anthracnose infection and chilling injury, and reduces shelf life (Chaplin *et al.*, 1983). Avocado fruit stored in an atmosphere containing high ethylene levels also showed more chilling injury than those kept in air at the same temperatures (Chaplin *et al.*, 1983; Lee and Young, 1984). Similarly “wild-type” melons develop more chilling injury when exposed to ethylene during cold storage than do “antisense” 1-aminocyclopropane-1-carboxylic acid (ACC) oxidase melons in which ethylene production is greatly suppressed (Jones *et al.*, 2001). Ethylene has been shown to increase the rate of respiration, alter the cellular compartmentalization, and alter auxin transport and/or metabolism (Pratt and Goeschl, 1969).

Biale (1941) was unable to detect a climacteric rise in ‘Fuerte’ fruit stored at 4-5°C for 5 weeks. In ‘Pinkerton’ fruit, no ethylene was detected during a cold storage period of 16 days (Fuchs *et al.*, 1986). In ‘Hass’ and ‘Fuerte’ fruit, chilling sensitivity was found to be dependant on the stage of the ethylene climacteric, with fruit at the climacteric rise being less sensitive than those at the climacteric peak. It was thought that the periods of sensitivity were correlated with periods of high metabolic activity. Kosiyachinda and Young (1976) proposed that a change in the activity of regulatory enzymes during low temperature storage could lead to the accumulation of intermediates to levels that become toxic to cells.

1.3 PREVENTION OF CHILLING INJURY

One of the main goals of research on chilling injury in horticultural commodities is to find effective methods to reduce the injury induced by chilling. Chilling stress is usually limited to plants native to or growing in tropical or subtropical regions of the world. The temperature range for chilling stress in such plants ranges from just above freezing to 15 to 20°C in some chilling-sensitive plants. Generally, plants are more sensitive to chilling under non-dormant conditions (high metabolic activity), during younger stages of development, under drought stress, and when nutrients are limiting. Thus, fresh produce sensitive to chilling cannot receive the full advantage of cold storage but deteriorates rapidly if not refrigerated. If the tolerance to chilling in these sensitive tissues can be increased, or if the development of chilling injury can be delayed, then it would be feasible to store these commodities at lower temperatures to reduce the rate of deterioration. Furthermore, cold treatment is an approved quarantine treatment. During the past 60 years numerous techniques have been used to

lessen injury during the postharvest period, with results varying between different crops and different times in the season. Thus the search for new techniques continues.

1.3.1 Methods used to prevent external chilling injury

To a horticulturist, perhaps the most important aspect of studying chilling injury is to develop a means of alleviating it during both the preharvest and postharvest periods. Postharvest techniques which have been shown to alleviate low temperature injury include temperature preconditioning, heat treatment, intermittent warming, controlled atmosphere storage, treatments with calcium or other chemicals, waxing, film packaging, genetic modification, and applications with ethylene, abscisic acid, methyl jasmonate, polyamines, or other natural compounds

1.3.1.1 Temperature pre-conditioning

Prestorage temperature significantly affects the susceptibility of commodities to chilling injury (Saltveit, 1991). Low temperature conditioning involves holding cold-sensitive tissue at temperatures just above those at which injury occurs before placing them in storage to induce tolerance to these normally damaging low temperatures and delay the development of injury symptoms (Wheaton and Morris, 1967; Hatton, 1990; Woolf *et al.*, 2003). This form of conditioning has been very successful in a wide range of fruits and vegetables (Wang, 1993). In zucchini squash temperature preconditioning treatment at 15°C for 2 days before storage at 5°C was effective in reducing chilling injury, not only in delaying the onset of chilling injury but also in reducing the rate of the development of injury symptoms (Wang *et al.*, 1992). Exposure of papaya fruit for 4 days at 12.5°C prior to storage at 2°C reduced chilling damage (Chen and Paull, 1986). Hatton and Cubbedge (1982) found that preconditioning of grapefruit at 10°C or 15°C for 7 days reduced chilling injury at 0°C or 1°C. Temperature conditioning is also effective in alleviating chilling injury in other citrus fruits (Houck *et al.*, 1990; Spalding and Reeder, 1983). In nectarines preconditioning fruit for 2 days at 20°C, before storage at 0°C, also substantially reduced chilling injury (Zhou *et al.*, 2000). The most effective conditioning period for mature green bell peppers was 5 days at 10°C before storage at 1°C (Risse and Chun, 1987).

1.3.1.1.1 *Physiological changes induced by temperature conditioning*

Temperature-conditioning treatments are thought to induce an adaptive response in fruits and vegetables to chilling stress. This adaptation to lower temperatures is the result of various physiological modifications induced by the conditioning treatment. Many biochemical and physiological alterations in chilling-sensitive plants have been associated with temperature conditioning or hardening treatments. For example, in cotton plants increases in sugar and starch, and decreases in RNA, protein, and lipid-soluble phosphate occur during exposure to 15°C for 2 days, which also reduces leakage of metabolites and prevents subsequent chilling injury at 5°C (Guinn, 1971). In citrus cold hardening was initially characterized by light-enhanced and cool temperature-induced accumulation of carbohydrates (Yelenosky, 1978). Chilling degrades lipids in cucumber fruit and tomato pericarp and temperature conditioning reduces the loss of lipids. A transition in the molecular ordering and fluidity of membrane lipids is thought to be the primary event causing chilling injury (Raison and Orr, 1990). The fluidity of the lipid bilayer is determined, to a large extent, by the fatty acid composition of the phospholipids. The flexibility of the membranes is associated with the relative proportion of saturated and unsaturated fatty acids in membrane glycerolipids. Temperature conditioning has been reported to increase the degree of unsaturation of fatty acids in phospholipids and to prevent chilling injury. The temperature conditioning also suppresses the increase of sterol/phospholipid ratio during chilling. This ratio is closely associated with membrane viscosity and permeability. It also affects the fluidity of membranes and, in turn, influences the capacity of tissue to withstand chilling stress. The decline in the ratio of unsaturated to saturated fatty acids may indicate an increase in lipid peroxidation during chilling (Wang *et al.*, 1992), which is thought to contribute to the development of chilling injury.

Sugar content has been correlated with changes in chilling sensitivity (Purvis, 1990). It has also been shown that low temperature acclimation increases the levels of proline and reducing sugars in grapefruit (Purvis and Yelenosky, 1983a). The high concentration of proline in the peel of grapefruit was reported to enhance its resistance to chilling temperatures in storage (Purvis, 1981). Other changes that take place inside the plant tissues in response to low temperature hardening include reduced leakage of electrolytes, a decrease in ATP during subsequent chilling, increases in the phosphatidylcholine and phosphatidylethanolamine, increases in unsaturation of fatty acids and increases in the degree of unsaturation of fatty acids.

In nectarines the delayed storage treatment (2 days at 20°C) was thought to prevent the occurrence of the physiological disorder 'wooliness' by allowing softening to continue during the 0°C storage (Zhou *et al.*, 2000). The 2 days delayed-storage fruit softened more during storage than control fruit. This difference in firmness was reflected by the differences in cell wall components of delayed-storage fruit compared to control fruit, and was thought to be reminiscent of changes found in fruit from intermittent warming.

1.3.1.1.2 Quarantine

A quarantine treatment must satisfy two conflicting goals: kill all quarantined insects present and prevent significant damage to the commodity, which, in the case of fresh produce, is also alive. The success of preconditioning, in alleviating chilling injury, has become very important to countries who need to meet certain quarantine regulations in order to export their fruit and who therefore have to subject their fruit to cold treatment. Cold treatment is an approved quarantine treatment for citrus grown in areas infested with a number of tropical fruit flies and involves storage of fruit below 2.2°C for specified periods. Preconditioning grapefruit, which are susceptible to chilling injury during extended cold storage, has helped to maintain fruit quality while meeting quarantine requirements (Hatton and Cubbedge, 1983). In studies on 'Hass' avocado fruit low temperature conditioning, at 6°C for 3 days, before cold disinfestation was found to effectively eliminate external chilling injury as well as improve internal mesocarp quality (Hofman *et al.*, 2003).

1.3.1.2 Packaging and waxing

The use of semi-permeable films, to package fruit and vegetables, which are sensitive to chilling temperatures, is a simple and inexpensive method to increase CO₂ and lower O₂ concentrations and to maintain a high humidity in the atmosphere. Under these modified atmosphere conditions the respiration rate of the fruit is decreased and the ethylene climacteric rise is delayed (Meir *et al.*, 1995). The reduction of water loss from the tissues under high humidity apparently inhibits the collapse of epidermal and underlying cells. These factors are believed to be responsible for the reduction of chilling injury by packaging films (Forney and Lipton, 1990; Wang, 1993). Studies using perforated bags have found that the O₂ and CO₂ concentrations changed very little from the ambient atmosphere (Wang and Qi,

1997). The value of high humidity in suppressing chilling injury was recognized as early as the 1930's (Morris and Platenius, 1938), with the development of chilling injury also being delayed by decreasing the vapour pressure deficit and thus reducing moisture loss from commodities (Wardowski *et al.*, 1973). Film packaging has been reported to be successful in preventing or reducing chilling injury in many crops including avocados and various citrus cultivars (Wang, 1993). In grapefruit and lemons, stored at 5°C and 2°C respectively, high-density polyethylene film seal-packaging was found to create a water-saturated atmosphere around fruit and inhibit chilling injury (Ben-Yehoshua *et al.*, 1981). In some commodities, the reduction of chilling injury by film packaging is attributed largely to the modification of the microenvironment within the package and to the alleviation of water stress (Ben-Yehoshua *et al.*, 1983a). The modified atmosphere was found to extend ripening time, improve firmness, and maintain fruit quality (Ben-Yehoshua *et al.*, 1983a; Wang, 1993). Studies of 'Fuerte' avocados packed in polyethylene bags, and stored at 5.5°C for 33 days before ripening at 20°C, showed that these fruit exhibited no chilling injury and had an extended shelf life, compared to control fruit, although this treatment resulted in an increase in anthracnose rot (Eksteen and Truter, 1985). This was attributed to the higher relative humidity within the bag and the longer period taken to ripen once removed from storage. Storage of 'Hass' avocados was also extended by packing individual fruit in sealed polyethylene bags, especially when an ethylene absorbent was included in the bag (Oudit and Scott, 1973). The use of micro-perforated polypropylene bags, with anti mist coatings, have been successful in reducing moisture stress in 'Pinkerton' avocados while modifying respiration rates. This, in combination with low temperature storage (2°C), was found to significantly reduce external chilling injury, preserve internal quality and decrease the rate of softening, after storage (Bower and Jackson, 2003).

Waxing of fruits and vegetables restricts gas exchange and transpiration of the fresh produce, and thus has effects similar to film packaging. Waxing grapefruit was found to significantly decrease the incidence of chilling injury (Chalutz *et al.*, 1985). Waxing has also been reported to reduce moisture loss, delay softening and improve appearance of 'Fuerte' avocados (Lunt *et al.*, 1981). Unfortunately, in some cases waxing has been reported to lead to the increased incidence of mesocarp discolouration in avocados (Cutting *et al.*, 1989). Ben-Yehoshua *et al.* (1987) found that film wrapping drastically reduced chilling injury (pitting) in citrus, compared to waxed fruit subjected to the same conditions. Similarly, Bower and Magwaza (2004) found that polypropylene packaging in 'Fuerte' avocados was more effective in reducing external

chilling injury than waxing. In 'Fuerte' avocados waxing was found to cause a slight build-up of CO₂ and a possible reduction in internal O₂ concentrations during the climacteric. Furthermore, waxing caused a one day delay in fruit softening under extended cold storage (Durand *et al.*, 1984). The variation in the effectiveness of waxing has been ascribed to differences in the composition of the actual wax formulation that in turn affect the properties of the coating (Amerante and Banks, 2001). In studies on mango and avocado it was found that coating type significantly affected the external damage of fruit (Bower *et al.*, 2003).

1.3.1.2.1 *Effects of individual seal-packaging on gas exchange of fruit*

Ben-Yehoshua *et al.* (1983b) calculated that waxing (1 µm thickness) increases only slightly the resistance of fruit-to water vapour, but raises the resistance to CO₂, O₂ and ethylene by 140%, 250%, and 100%, respectively. Conversely, seal-packaging (10-20 µm) was found to raise the resistance to fruit-to water vapour by 1375%. However, resistance to CO₂, O₂ and ethylene was raised by only 72%, 233%, and 25%, respectively. Studies using a scanning electron microscope found that waxing plugged the stomatal pores of citrus either partially or completely, and this was suspected to restrict CO₂, O₂ and ethylene transport. Furthermore, a wax coating was thought to have a low resistance to water, because the new surface layer, which is formed, has many pits and breaks. The plastic film of high-density polyethylene was not selectively impermeable to water, but the film reduced water loss by 14-times without substantially inhibiting gas exchange, because, initially, the fruit has far less resistance to water than to CO₂, O₂ and ethylene.

1.4 PROLINE

1.4.1 Accumulation of proline

An increase in the concentration of the total free amino acids in plant tissues when the plant is exposed to low temperatures has been frequently recorded (Draper, 1972; Srivastava and Fowden, 1972; Chu *et al.*, 1978). In plants, L-Proline (henceforth referred to as proline) is synthesized from either glutamate or ornithine (Sánchez *et al.*, 2002). However, some studies indicate that most of the proline accumulated in the vegetative tissues of mature plants in response to stress is the result of enhanced synthesis from glutamate (Tayler, 1996; Roosens *et al.*, 1998). Proline accumulation, induced by stress conditions, is thought to be

mediated both by increased synthesis and reduced oxidation of the amino acid (Hare, 1998). Although proline may also be synthesised from ornithine (Roosens *et al.*, 1998), a decrease in proline oxidation frequently accompanies prolonged stress (Madan *et al.*, 1995), although this in itself is unlikely to account for the levels of proline often accumulated (Chiang and Dandekar, 1995).

In establishing why in comparison with other amino acids, proline metabolism appears to be extremely sensitive to adverse environmental conditions, it is useful to compare its metabolism with that of other amino acids. In comparison with most other amino acids, proline has the metabolic advantage of being the terminal product of a relatively short and highly regulated pathway. Proline accumulation therefore affects fewer metabolic reactions than the buildup of multi-use substrates such as glutamate, which are participants in many equilibrium reactions central to intermediary metabolism. Because of the secondary amines of the nitrogens, proline cannot participate in the transamination or decarboxylation reactions common to other amino acids (Phang, 1985).

Proline represents a unique class of molecule among the amino acids. With its peptide bond within the pyrrolidone ring, proline confers rigidity and three-dimensional stability to proteins (Phang, 1985). A specific system of enzymes with special properties has evolved to mediate the metabolism of proline. Another feature of proline as a result of its unusual metabolic feature is that it easily crosses cellular and organellar barriers (Abrahamson *et al.*, 1983).

1.4.2 Role of proline accumulation

Proline has been found to accumulate in plants after they have been exposed to different stresses (Aspinall and Paleg, 1981). Much remains to be understood concerning the mechanisms whereby proline accumulates under stress. Proline accumulation is argued by some researchers to be advantageous to a plant as far as stress tolerance is concerned (Singh *et al.*, 1973). Different roles have, however, been proposed for proline accumulation as an adaptive response; it has been suggested that proline may function as an osmoticum (Wyn Jones *et al.*, 1977), a sink of energy and reducing power (Blum and Ebercon, 1976), a nitrogen-storage compound (Ahmad and Hellebust, 1988), a hydroxy-radical scavenger (Smirnov and Cumbes, 1989), and a compatible solute that protects enzymes (Charest and Chon, 1990). It may also play a role in the regulation of cellular redox potentials. Conversely,

some researchers suggest the opposite to be true, that is, that the mere correlation between the accumulation of proline and the development of stress conditions does not provide sufficient evidence for any adaptive advantage with regards to stress (Kramer, 1983) and that proline accumulation is simply an indication of the damage suffered by the plant during stress conditions (Hanson *et al.*, 1979). Blum and Ebercon (1976) suggested that the positive effect of proline accumulation under stress conditions is that it augments growth upon relief from stress, rather than serving any direct function during the period of exposure to stress.

The proline biosynthetic pathway from glutamate, although short, involves an extremely high rate of consumption of reductants. Furthermore, proline degradation is capable of high-energy output. The accumulation of proline appears to be an excellent means of storing energy since the oxidation of one molecule of proline can yield 30 ATP equivalents (Atkinson, 1977). These two features may have contributed substantially to a role for proline in plants as a resource of value either in the acclimation to stress or in recovery upon relief from stress. The benefit of possessing a metabolic system displaying extreme sensitivity to stress may derive more from its regulatory effects on apparently unrelated pathways than on accumulation of the end product itself (Hare, 1998).

Under stressful conditions proline synthesis may ameliorate the effects of the concomitant reduction of the pyridine nucleotide pools, particularly the accumulation of excessive amounts of nicotinamide adenine dinucleotide hydrogen phosphate (NADPH). The oxidation of NADPH accompanying proline synthesis may assist in restoration of the terminal electron acceptor of the photosynthetic electron transport chain (Berry and Björkman, 1980). High levels of proline during stress play a role in the maintenance of the nicotinamide adenine dinucleotide phosphate (NADP⁺) /NADPH ratio, as even a small increase in the biosynthesis has a large impact on the NADP⁺ pool (Hare and Cress, 1997). This is the result of stomatal closure, which leads to the intercellular decrease of CO₂ as the leaf water stress increases. As the overall protein synthesis declines during drought stress (Van der Mescht and De Ronde, 1993), proline biosynthesis may substitute for protein synthesis in the turnover of ATP and the oxidation of NADP⁺ (Hare, 1995).

The extensive accumulation of active oxygen species and their contribution to cell damage induced by stressful conditions such as temperature extremes and water deficit is well known. In order to deal with this effect, plants have evolved a number of protective, scavenging or

antioxidant defensive mechanisms. Apart from an enzymatic defensive system (Bowler *et al.*, 1992), the accumulation of free-proline may also contribute to the scavenging of these active oxygen species by enhancing photochemical electron transport activities (Alia *et al.*, 1991; Saradhi *et al.*, 1995). It has even been suggested that the accumulation of proline might contribute to the detoxification of the active oxygen species (Floyd and Nagy, 1984).

Many plants accumulate organic osmolytes upon exposure to abiotic stresses that cause depletion of cellular water (drought and temperature extremes) (Aspinall and Paleg, 1981). Of the organic osmolytes, proline is the most widely distributed osmolyte (Tayler, 1996) and extensively studied (Hare, 1998). The proposed role of proline as an osmoregulator (Wyn Jones and Storeys, 1978) can be supported by the involvement of proline in the maintenance of membrane integrity as an adaptation to conditions of reduced water availability (Hare, 1995). The molecules accumulated in the cells during an osmotic stress prevent damage from cellular dehydration by balancing the osmotic strength of the cytoplasm with that of the environment. Proline can affect the solubility of various proteins due to its interaction with hydrophobic residues on the protein surface (Schobert and Tschesche, 1978). The increase in the total hydrophilic area of the protein stabilises it by increasing its solubility in an environment with low water availability (De Ronde, 2000).

1.4.3 Examples and conditions of proline accumulation

Proline accumulation in plants subjected to low temperatures and water stress appears to be a universal phenomenon. The free proline content of the leaves of many species increases with a decrease in leaf water potential (Singh *et al.*, 1973). The accumulation induced by low temperature, however, is not the result of a concomitant decrease in leaf water potential (Chu *et al.*, 1974) and appears to be a more direct response to the decrease in temperature. In citrus the accumulation of free proline is one of the features of water stressed-induced cold hardening (Yelenosky, 1979). A linear relationship was found to exist between free proline concentration and xylem pressure potential in lemon trees (Levy, 1980). Proline accumulation was also found to enhance drought tolerance, and recovery, in sorghum (De Ronde, 2000).

In barley proline accumulation at low temperature was shown to be light-dependent (Chu *et al.*, 1978). A critical temperature was found to exist at which proline accumulation did not occur and it was suggested that the accumulation of proline was a consequence of a specific

metabolic event rather than the result of a continuous spectrum of temperature-affected changes in the total amino acid pool. Proline accumulation in response to water stress was not found to be light-dependent.

Accumulation of sugars and proline in citrus tissues is associated not only with cold hardening of trees, but also with the midseason resistance of grapefruit to chilling injury (Purvis, 1981; Purvis and Grierson, 1982). Grapefruit which have accumulated relatively high concentrations of carbohydrates and proline are less likely to be injured by low, non-freezing temperatures (Purvis, 1981; Purvis and Grierson, 1982). Increases in soluble carbohydrates and proline may be more a consequence of low temperature stress conditions rather than a direct factor in tissue hardening; but, sugars and proline levels, nevertheless, do correlate well with the chilling resistance of grapefruit as well as with cold hardiness of other citrus tissues (Purvis, 1981; Purvis and Grierson, 1982). Grapefruit harvested during midseason are generally found to be more resistant to chilling injury than fruit harvested either earlier or later in the season (Purvis *et al.*, 1979). In addition, unexposed interior canopy fruit are found to be more resistant to chilling injury than exposed exterior canopy fruit (Purvis, 1980). While midseason chilling injury resistance has been related to a high level of reducing sugars, which accumulate in the peel of grapefruit at low orchard temperatures, no differences were observed between sugar levels in the peels of unexposed interior canopy and exposed exterior canopy fruit (Purvis, 1980). Proline contents were, however, found to be higher in interior canopy fruit peels, than exterior canopy fruit, and this correlated well with chilling injury resistance (Purvis, 1981). The accumulation of proline in citrus fruit tissues during stress is thought to result from a reduction in the utilization or oxidation of proline (Stewart and Hanson, 1980), which is translocated into them. Low temperatures may result in decreased proline oxidase activity in mitochondria of citrus fruit tissues (Purvis and Yelenosky, 1983b).

1.5 CHLOROPHYLL FLUORESCENCE

Chlorophyll fluorescence technology has been used to assess the responses of plants to a diverse range of stresses. More recently the application of chlorophyll fluorescence has been extended to studying the response of fruits and vegetables to postharvest stresses (Tijsskens *et al.*, 1994; Woolf and Laing, 1996). DeEll *et al.* (1999) also reported that chlorophyll fluorescence might be useful in evaluating ripening and senescence of fruits. Furthermore,

chlorophyll fluorescence is a non-invasive and non-destructive measurement that can be performed fairly quickly and it is reported to detect cellular damage before the development of visible symptoms.

Chlorophyll fluorescence techniques are based on the theory that light energy is absorbed by chlorophyll molecules within plant tissue and is used to drive photosynthesis. Energy surplus to that utilised in photosynthesis is dissipated as fluorescence or heat. The fluorescence of green plants is almost exclusively emitted by chlorophyll *a* (DeEll *et al.*, 1999). At ambient temperatures the vast majority of emitted fluorescence is derived from processes occurring in Photosystem II (PSII). When healthy plant tissue is suddenly illuminated after a period in darkness, a time-dependent fluorescence induction (Kautsky effect) is observed, the amplitude of which is proportional to the incident light level.

It has been found that a parameter derived from chlorophyll fluorescence, the ratio of variable/maximum fluorescence (F_v/F_m), can be used as a quantitative measure for the basic functioning of photosynthetic electron transport. When F_v/F_m is measured in dark-adapted tissue it is considered to be an indicator of the integrity of the reaction centre and light-harvesting complex of PSII, and thus reflects membrane damage or membrane alterations (DeEll *et al.*, 1999). Lower temperatures can enhance membrane leakage and studies revealed that it appeared to be well correlated with F_v/F_m . DeEll *et al.* (1999) suspected that cold storage induced changes in the thylakoid membranes of plant tissues, result in a decreased exciton transfer efficiency of PSII, which seemed to be temperature dependent.

A few factors have to be considered, however, when interpreting chlorophyll fluorescence data. For example, the quantum yield is both time and temperature dependent. Thus, during ripening there may be a loss of photosynthetic competence per unit chlorophyll, leading to reduced PSII activity. In addition, there may be a decrease in chlorophyll content, which will affect all fluorescence measurements (DeEll *et al.*, 1999). Light, temperature and nitrogen content in the fruit (Kingston, 1991) would also affect chlorophyll content and subsequently fluorescence measurements. Furthermore, it is reported that measurements may be affected by large variations among cultivars and even between fruit of the same cultivar, and thus would be influenced by sample size and ranges of fruit firmness and fluorescence (DeEll *et al.*, 1999).

1.6 CONCLUSION

A review of the literature indicates that there are many pre- and postharvest factors that can contribute to the development of mesocarp discoloration and external chilling injury in fruit. Furthermore, these factors may or may not be related and may affect the physiology of the fruit in different ways. The fact that the severities of these disorders have not been reduced to acceptable levels, despite the volume of work that has already been conducted, is a reflection of the complexity of these problems. It is hoped that a further understanding of how the various factors may contribute to the disorders will aid future research, as well as provide acceptable protocols for shipping and storage, which will decrease the risk of disorders causing losses in the market place.

CHAPTER 2

Effects of storage temperature, harvest date and fruit origin on the post-harvest physiology and internal quality of 'Pinkerton' avocado (*Persea americana* Mill.)

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SUMMARY

The severity of mesocarp discolouration in 'Pinkerton' avocados, a disorder previously suspected to be chilling injury, was found to be decreased by storing fruit below the recommended temperature of 5.5°C. Furthermore, the discolouration was intensified by storage at temperatures above the norm (*viz.* 8°C), and this coincided with higher electrolyte leakage, which was used as a measure of membrane integrity. The disorder was therefore not ascribed to being the result of too low storage temperatures. Fruit firmness and carbon dioxide (CO₂) production rates, monitored daily following storage, showed that fruit harvested later in the season had a slightly higher CO₂ production rate than the fruit picked earlier in the season. Throughout the study, the severity of mesocarp discolouration was affected by fruit origin. The potential for mesocarp discolouration appeared, therefore, to be initiated by pre-harvest factors, although the severity could be modified by storage at 2°C. At 2°C the total phenolics content was found to be significantly ($P < 0.001$) lower and soluble PPO activity was similar to control fruit. Fruit also remained firm during storage at 2°C and electrolyte leakage remained similar to unstored fruit, indicating that membrane integrity was better preserved at this temperature. The role of membrane integrity became more important as the season progressed as total phenolics content increased and as total PPO activity decreased.

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The South African sub-tropical fruit industry is largely export driven and, due to distance from the major markets, successful storage of fruit for extended periods is critical to ensure high fruit quality and optimum returns. Unfortunately, the condition of sea-freighted avocados is often variable upon arrival at destination (Nelson *et al.*, 2001), especially when shipped at low (5.5°C) temperatures. Physiological disorders are a significant contributing factor to the inconsistent quality of avocado fruit. The 'Pinkerton' cultivar, in particular, is severely affected by an internal disorder often referred to as "mesocarp discolouration" or "grey pulp" (Kruger *et al.*, 2000). The disorder is usually prevalent in the distal half of the fruit, but can affect the whole pulp when severe.

Storage temperature has become one of the main methods of slowing down the metabolism of highly climacteric and rapidly-softening avocado fruit, thus also extending its shelf-life. Unfortunately, in the case of 'Pinkerton', the optimum storage temperature has not been determined satisfactorily and there appears to be some confusion resulting from previous studies relating to the disorder. Chaplin *et al.* (1982) attributed symptoms similar to mesocarp discolouration to chilling injury. Vakis (1982) found that although internal darkening of the avocado fruit was indicative of chilling injury, it was also found in control fruit and at non-chilling temperatures (*viz.* 8°C). In addition, increasing the storage temperatures for 'Pinkerton' avocados has not alleviated the problem (Schutte, 1994). An understanding of how temperature affects the physiology and biochemistry of the fruit is therefore necessary to optimise fruit quality and minimise browning.

The integrity of the cell membrane system can play an essential role in the rate of avocado fruit ripening (Sacher, 1976). The deterioration of fruits, vegetables and other plant materials due to physiological damage is also thought to share a common mechanism (Stanley, 1991), with decreased membrane integrity often being expressed as increased ion leakage (Stanley, 1991). One important effect of decreased membrane integrity is the leakage of phenolic compounds from the vacuole into the cytoplasm, with subsequent oxidation by polyphenol oxidase (PPO) resulting in fruit blackening. A close relationship has been demonstrated between PPO activity and avocado mesocarp discolouration (Van Lelyveld and Bower, 1984), although this may not be the only factor involved (Kahn, 1977a). The rate of respiration, as affected by temperature, is also thought to be regulated by the functional integrity of membranes (Nilsen and Orcutt, 1996).

The purpose of the study was to investigate the effects of fruit origin, harvest date and different storage temperatures on mesocarp discolouration severity in 'Pinkerton' avocados. To better understand the mechanisms leading to mesocarp discolouration, membrane

integrity, fruit firmness, days to ripening, fruit respiration, total phenolics content and PPO activity was monitored before and after storage at various temperatures.

MATERIALS AND METHODS

Plant material and treatments

Avocado fruit (*Persea americana* Mill. 'Pinkerton') were obtained throughout the 2000 and 2001 harvest season from three production areas in Mpumalanga Province, South Africa, with varying mesocarp discolouration histories (referred to as "high", "medium" or "low risk" areas). Fruit from the various origins were washed and waxed (Citrashine Pty Ltd., Johannesburg, R.S.A.; 1 t tonne⁻¹ of fruit), at the same packhouse, before being sent by courier to the University of KwaZulu Natal, Pietermaritzburg, South Africa. The delay between harvest and arrival at the University took up to 3 d, with all fruit being transported together under the same conditions.

On arrival, fruit from each origin were divided into the respective storage treatments, with each fruit being numbered, to maintain its individuality. Ten fruit from each consignment acted as controls, with five fruit being sampled immediately on arrival, while five fruit were allowed to ripen at 20°C. The remaining fruit were then placed into storage at 2°C, 5.5°C or 8°C for 30 days, with 10 fruit per storage temperature. After storage five fruit from each temperature treatment were sampled immediately, while five were allowed to ripen. Evaluations of fruit firmness, electrolyte leakage, moisture loss and mesocarp discolouration severity were made before and after storage, as well as after softening when "eating ripeness" was attained. Once removed from storage, fruit firmness and carbon dioxide production rates were monitored daily, and the number of days taken to attain 'eating ripeness' recorded.

On sampling, during the 2001 season, mesocarp tissue from the distal ends, of the individual fruit, were cut into small blocks (1 cm³), flash frozen in liquid nitrogen and stored at -20°C until analysis for total phenolics content and polyphenol oxidase (PPO) activity could be conducted. Selected treatments were then used to determine the effect of fruit origin, harvest date and storage temperature on mesocarp discolouration potential.

Mesocarp discolouration

Fruit were bisected longitudinally and immediately rated visually for mesocarp discolouration using a scale of 0 to 10, where 0 = no discolouration and 10 = 100% of cut surface area black.

Maturity

The maturity of each consignment was ascertained on arrival by determining the moisture content (Kruger *et al.*, 1995) of a sample of mesocarp tissue (20 g). The tissue was cut into small pieces (1 cm³) and immersed in liquid nitrogen. Once frozen, the samples were placed on a freeze drier for 5 d. This was determined to be sufficient time to remove moisture and attain constant mass.

Fruit firmness

Fruit firmness was determined using a hand-held firmness tester (Bareiss, Oberdischingen, Germany). Two readings (on a scale of 100 (hard) to 0 (soft)) were taken per fruit per sampling date. Measurements were taken at the maximum circumference of the intact fruit, turning the fruit 180° after each measurement. The firmness tester measured fruit firmness by means of a metal ball (5 mm diameter) that was pressed onto the fruit. "Eating ripe" was considered to be at a reading of 50 – 55 units.

Electrolyte leakage

The leakage of electrolytes from mesocarp tissue was determined by measuring the electrical conductance of cell effusates using a modified technique of Venkatarayappa *et al.* (1984). A mesocarp plug (1 cm diameter) was taken from the cut-half of each fruit at the distal end, halfway between the seed and the exocarp. Three discs of 2 mm thickness were cut from this plug and rinsed three times in distilled water before being placed in a single boiling tube containing 25 ml distilled water. The tubes were then placed on a shaker for 3 h and the electrical conductivity (EC) measured (Initial EC) using a multi-range conductivity meter (HI 9033, Hanna Instruments, Johannesburg, RSA). The tubes were then placed in a boiling water bath for 20 min; removed and allowed to cool. The EC of each tube was again recorded (Final EC) and the percentage leakage determined as [(Initial EC/Final EC) x 100/1].

Carbon dioxide production

Immediately after the storage period, fruit were allowed to equilibrate to the ambient room temperature, for 8 h, before carbon dioxide production (CO₂), as an indication of respiratory activity, was measured with an environmental gas monitor (EGM-1, PP Systems, Hitchin, Hertfordshire, UK). Subsequent readings were taken at about the same time each day, with fruit being removed from the 20°C chamber and left to equilibrate to ambient room temperature for about 30 min before readings were taken. Each fruit was sealed in a

separate jar for 10 min, after which the headspace CO₂ concentration ($\mu\text{l l}^{-1}$) was determined and the results calculated as a rate of CO₂ production ($\text{m}\ell \text{kg}^{-1}\text{FW hr}^{-1}$), taking into account the fruit mass and volume, free space in the jar and the ambient room CO₂ concentration.

Total phenolics contents

Total phenolics were determined colorimetrically using the method of Donkin (1995), modified from the method of Torres *et al.* (1987). Frozen mesocarp tissue was ground to a powder using a mortar and pestle and liquid nitrogen (to avoid oxidation). A 2 g sample was then transferred into a polypropylene centrifuge tube to which were added 10 m ℓ 100% chloroform and 10 m ℓ 100% hexane. The tube was then shaken on a laboratory shaker for 2 h after which it was centrifuged (Sorvall RC-5C Plus, Newtown, CT, USA.) at 5,000 rpm (2,510 x g) 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 back into the tube and 20 m ℓ 60% methanol in water was added and the tube shaken for an additional 2 h. The extract was filtered through Whatman[®] No. 1 filter paper. Each fruit sample was analysed in duplicate with two replicates each.

A standard curve was prepared using a 160-2.5 $\mu\text{g m}\ell^{-1}$ dilution series of gallic acid. For all the replicates and the standard curve, 0.1 m ℓ aliquots were placed into 20 m ℓ test tubes in duplicates. A spectrophotometer (Anthelie Advanced, Secoman, Domont Cedex, France) was calibrated using a blank of 0.1 m ℓ distilled water. Six m ℓ distilled water and 0.5 m ℓ Folin-Ciocalteu reagent were added to each tube, which was vortexed thoroughly and allowed to stand for 5 min. Sodium carbonate (1.5 m ℓ 20% w/v) was then added, followed by 1.9 m ℓ distilled water to bring the total volume to 10 m ℓ . The solution was mixed thoroughly and incubated in a water bath at 50°C for 2 h. Tubes were then removed and allowed to cool to ambient temperature, before the absorbance at 765 nm was read with a spectrophotometer.

Polyphenol oxidase activities

The method of Bower and Van Lelyveld (1985), with modification, was used to determine soluble PPO activity. Crude extraction for soluble PPO involved grinding 11 g of frozen mesocarp tissue for 7 min using a mortar and pestle and liquid nitrogen (to avoid unnecessary oxidation). One g of insoluble polyvinylpyrrolidone (PVP, Polyclar AT, BDH Laboratories, Poole, England) was added during homogenisation. Two 5 g samples were then weighed out and each transferred separately into polypropylene centrifuge tubes to

which 10 ml cold 10 mM acetate buffer, pH 5.0, was added. The sample solution was then homogenised using an Ultra-Turrax T25 (Janke and Jackson, Staufen, Germany) and allowed to stand for 20 min on ice before the homogenate was centrifuged (Sorvall RC-5C Plus, Newtown, CT, USA) at 18,000 x g for 45 min at 0°C – 4°C. The extract was then filtered through glass wool and the supernatants used immediately to assay for soluble PPO activity. Each extract was assayed in duplicate.

Total PPO was extracted by the same method, except that 0.1% (w/v) sodium dodecylsulphate (SDS) was added to the acetate buffer during extraction.

Total PPO was assayed as described by Van Lelyveld *et al.* (1984) with some modifications to final volumes. Each enzyme extract (2 µl) was added to a mixture of 2 ml 10 mM acetate buffer pH 5.0 and 2 ml 0.02 M 4-methyl-catechol. PPO activity was expressed as the change in optical density (ΔOD) change at 420 nm $\text{min}^{-1} \text{mg}^{-1}$ protein at 24°C.

Protein concentration determination

The total protein concentrations of the extracts used for the soluble PPO assay were determined using the Bradford method (1976). Those of extracts used for the total PPO determination was determined by a modification of Lowry *et al.* (1951) as SDS is incompatible with the dye-binding reagent used in Bradford (1976).

Bradford method: The Bradford Dye-binding reagent was prepared by dissolving 500 mg Coomassie Brilliant Blue G-250 in 250 ml 99.9% ethanol and 500 ml 85% phosphoric acid. The solution was made up to 1 l with distilled water and stirred overnight at 4°C. The resulting solution was filtered through Whatman[®] No. 1 filter paper and stored in an amber-coloured bottle at 4°C for ≤ 6 months. Prior to use, the reagent was diluted five-fold with distilled water.

Protein determination was done by adding 100 µl protein extract to 5 ml dilute Bradford reagent, vortexing the solution and allowing it to stand for 5 min for colour development. Absorbance was then read at 595 nm. Samples were assayed in duplicate and interpolated from a standard curve prepared using bovine serum albumin (BSA; Sigma Chemical Co., St Louis, MO, USA) as a protein standard. Assays for the standard curve were prepared using a dilution series of BSA (0.2 - 1 mg ml^{-1}).

Lowry method: The protein concentrations of the extracts were determined following precipitation of proteins by 10% trichloroacetic acid (TCA), by the method of Lowry *et al.*

(1951), as modified by Leggett-Bailey (1962). Crude protein extracts (0.5 ml) were precipitated with an equal volume of 10% TCA and left for 15 min before centrifuging at 5,000 x g for 10 min at ambient room temperature. The supernatant was discarded and the pellet was redissolved in 100 µl 3% NaOH and after vigorous shaking, 1 ml water was added. To this was added 4 ml Folin A + B reagents in the ratio of 1:30. Folin A consisted of 0.5% CuSO₄.5H₂O in 5% sodium-citrate. Folin B was made up of 2% Na₂CO₃ in 0.1 M NaOH. After 10 min 100 µl Folin Ciocalteu reagent diluted 1:1 with water was added, and the mixture allowed to stand for 15 min. Absorbance at 750 nm was read, and compared with a standard curve obtained using BSA. Each fruit sample was assayed in duplicate with two replicates each.

Statistical Analysis

Data was subjected to analysis of variance (ANOVA) using the GenStat® statistical package (VSN International Ltd, Hemel Hempstead, UK). Least significant difference (LSD) was used to separate treatment means. As fruit were not harvested on the same dates during 2000 and 2001, direct statistical comparisons could not be made between seasons. The strong interactions between factors has resulted in the data being displayed in complex tables indicating the various interactions and their significance.

RESULTS

Mesocarp discolouration

During the 2000 harvest season, mesocarp discolouration severity was commercially acceptable with ratings never exceeding 3 (Table I). The mesocarp discolouration severity, during 2000, was found to be significantly affected by fruit origin ($P < 0.001$), but not harvest date, although there was a significant interaction between these two factors ($P = 0.05$).

During 2001, the mean mesocarp discolouration rating exceeded 5 in the “medium risk” and “high risk” area (Table I). The discolouration severity was more significantly ($P < 0.001$) affected by fruit origin, harvest date and the interaction of these two factors during the season when discolouration was more severe. During 2001, the severity of the disorder was found to be significantly higher at the end of the harvest season (Table I).

During 2000, the mesocarp discolouration ratings were slightly, but not significantly, higher in the 5.5°C storage treatments of fruit from the “low risk” and “medium risk” areas,

while storage at 5.5°C or 8°C gave more severe mesocarp discolouration throughout the season in the “high risk” area.

The effect of storage temperature on mesocarp discolouration was more apparent during 2001, with fruit from all origins exhibiting significantly more severe discolouration when stored at 8°C. Discolouration was evident in fruit cut immediately after removal from storage, although the severity was higher in fruit that were allowed to ripen (Table 1). Storage at 2°C was optimum, in terms of decreasing mesocarp discolouration, throughout 2001. The severity of mesocarp discolouration throughout this study was found to be significantly ($P < 0.001$) affected by strong interactions between storage treatment, fruit origin and harvest date.

Fruit maturity

Physiological maturity, as determined by moisture content, was seen to fluctuate significantly ($P = 0.05$) during both seasons (Table I), but was not found to affect the severity of mesocarp discolouration significantly.

Fruit firmness and days to ripening

During both 2000 and 2001, fruit firmness was significantly ($P < 0.001$) affected by interactions between storage temperature and fruit origin, between storage temperature and harvest date, and between harvest date and fruit origin. The interaction between storage temperature, harvest date and fruit origin did not, however, significantly affect fruit firmness (Table II). During both seasons, fruit from all risk areas were less firm after storage at 8°C than at 2°C (Table II). Storage temperature also had a significant affect on days to ripening ($P = 0.05$) with fruit stored at 8°C taking fewer days to ripen than those fruit stored at 2°C (Table III). During 2000 and 2001, days to ripening was significantly ($P < 0.001$) affected by the interaction between fruit origin and harvest date. Unstored fruit from the “high risk” area took significantly less time ($P < 0.001$) to ripen than unstored fruit from the “low risk” area (Table III). During the 2001 season, the interaction between harvest date and storage treatment was also found to significantly affect ($P < 0.001$) days to ripening, thus indicating a possible change in sensitivity to storage temperature during the harvest season.

Electrolyte leakage

Electrolyte leakage was significantly ($P = 0.05$) affected by the interaction between fruit origin, harvest date and storage temperature. In fruit from each “risk area”, sampled

immediately after storage, electrolyte leakage was significantly ($P = 0.05$) higher at 5.5°C and 8°C than at 2°C, or control fruit (Table IV) for most harvest dates. In fruits stored at 5.5°C and 8°C, the electrolyte leakage was also found to be higher in the second half of each season, irrespective of fruit origin, although more so during 2001.

Carbon dioxide production and days to maximum production

During 2000 and 2001, the maximum CO₂ production rate was significantly ($P < 0.001$) affected by storage treatment, harvest date and fruit origin, with many of these factors having significant interactions. During both seasons, large variations were noticed in the maximum CO₂ production rates of unstored fruit between harvest dates (Table V). Nevertheless, maximum CO₂ production rates of fruit from the “high risk” area were higher ($P = 0.05$), regardless of storage treatment or harvest date, than fruit from the “low risk” area. The role of storage treatment on maximum CO₂ production rate was not consistent, with maximum CO₂ production rates in the unstored fruit generally being lower than fruit that were placed into storage for 30 days, although not for all harvest dates (Table V). Furthermore, no consistent trends were found where found when comparing fruit stored at 8°C to fruit stored at 2°C.

The number of days taken to reach the maximum CO₂ production rate was significantly affected by storage temperature ($P \leq 0.05$) during 2000 and 2001. Storage at any temperature significantly ($P = 0.05$) decreased the number of days taken to reach the maximum CO₂ production rate after storage (Table VI). Storage at 2°C delayed the number of days taken to reach the maximum CO₂ production rate, although not always significantly for all harvest dates.

Total phenolics

Harvest date had a highly significant ($P < 0.001$) affect on total phenolics contents in fruit sampled immediately on arrival (i.e., no storage) from the “high risk” area, with total phenolics contents being higher at the end of the 2001 season (Table VII).

The effect of storage temperature on total phenolics contents was determined using fruit that were most severely affected by mesocarp discolouration (i.e. fruit from the “medium risk” and “high risk” areas). Storage temperature was found to have a significant effect ($P < 0.001$) on total phenolics content, with concentrations being found to be highest at 8°C and lowest at 2°C and unstored fruit (Table VIII). No significant differences were found between control fruit sampled on the same harvest date at different fruit origins (Table IX).

Polyphenol oxidase activities

Harvest date had no significant effect on soluble PPO activities, in control fruit sampled immediately on arrival from the "high risk" area; but harvest date had a significant effect on total PPO activity ($P = 0.05$), which was lower in the second half of the season (Table VII). Fruit origin did not have a significant effect on soluble PPO or total PPO activity in control fruit sampled on the same harvest date (Table IX).

Storage temperature did have a significant effect on soluble PPO activity ($P = 0.05$), with the highest activity at 2°C and in unstored fruit (Table VIII). Storage temperature had the same effect on total PPO activity. No significant interaction was found between fruit origin and storage temperature.

DISCUSSION

Storage temperature

During this study, storage of 'Pinkerton' avocado fruit at 2°C significantly reduced the severity of mesocarp discolouration compared to fruit stored at 8°C and 5.5°C, which is the current industry standard in South Africa. These findings agreed with the work of Zauberman and Jobin-Décor (1995), who found that 'Hass' avocados could be stored at 2°C for up to five weeks without injury, while those stored at 7°C developed significant discolouration, which was suspected to be the consequence of ripening occurring during cold storage. Results from our study confirmed this, with fruit stored at 8°C taking significantly fewer days to ripen ($P = 0.05$) after storage, compared to fruit stored at 2°C (Table VII), and also having more severe mesocarp discolouration (Table I).

As total phenolic contents are involved with the development of mesocarp discolouration, membrane integrity plays a large role in the development of the disorder. High electrolyte leakage is thought to reflect a decrease in membrane integrity (Thompson, 1988). In theory, this indicates a higher potential for PPO to come into contact with its phenolic substrates, resulting in a browning reaction. Lower storage temperatures are thought to be beneficial in slowing down the metabolic rate of the avocados to a greater degree (Bower, 1988), thus preserving membrane integrity (Wills *et al.*, 1989), at least during storage. Nilsen and Orcutt (1996) also suggested that the rate of CO₂ production reflected the energy needed by a plant organ to maintain cell metabolism. While respiration was not monitored during storage, we presume that fruit stored at 8°C would have been more metabolically active than those stored at 2°C, with increased leakage of electrolytes after storage at 8°C (Table IV)

confirming decreased membrane integrity. This could also explain why the time taken to ripen was reduced by storage at 8°C or 5.5°C (Table III).

In addition to decreased electrolyte leakage in fruit stored at 2°C, the lower phenolics contents in these fruit together with the fact that soluble PPO activity remained high, or at least similar to the unstored fruit, gives further evidence that membrane integrity was better preserved at this temperature. Plant tissues are known to respond to damage by metabolising phenolics, which could explain why storage at 5.5°C and 8°C resulted in higher total phenolics contents. While PPO activity is reported to be substrate dependent (Vaughn *et al.*, 1988), PPO activity was found to be lower in fruit stored at these temperatures. Kahn (1977a) reported that plant tissues lose the ability to activate latent PPO after cellular damage occurs, thus, it is suggested that as membrane integrity decreased, in fruit stored at 5.5°C and 8°C, the readily available PPO supply was diminished.

Fruit origin

All fruit in this study were submitted to the same post-harvest conditions within a treatment, thus significant variations in responses between fruit origins could be ascribed, in part, to unidentified pre-harvest conditions. In fruit from the "low risk" area, mesocarp discolouration was significantly less severe than fruit from the "high risk" area (Table I). Electrolyte leakage in unstored fruit from the "low risk" area, while similar to that from the "medium risk" and "high risk" areas, was also significantly less once fruit were placed in storage for 30 d (Table IV). Furthermore, respiration rates were much lower in unstored fruit from the "low risk" area than fruit from the "high risk" area (Table V), which took fewer days to ripen (Table III). During respiration, various substrates are released and consumed to meet the energy demand of the fruit. If membrane integrity was lost during this time, we may assume subsequent release of phenolics into the cytosol.

It is suspected that the differences in CO₂ production rates between fruit from different risk areas are related to pre-harvest orchard conditions. "Medium risk" and "high risk" areas were situated on soils previously planted to banana with high nitrogen contents, consequently the trees are more vigorous. This would result in competition between new vegetative growth and existing fruit for available water, minerals and carbohydrates substrates for respiration and energy (Blanke and Notton, 1991) for fruit growth and maintenance.

Harvest Date

The severity of mesocarp discolouration was found to increase significantly as harvest date was delayed during the 2001 season. Harvest date also had a highly significant ($P < 0.001$) effect on total phenolics contents in unstored fruit sampled on arrival from the "high risk" area (Table VII) and on electrolyte leakage (Table IV), which was higher in the second half of the season. Cutting *et al.* (1992) suggested that decreasing membrane integrity, as the season progressed, would result in a loss of compartmentation of enzymes and substrates. Cellular damage would also result in the ability to activate latent soluble PPO (Kahn, 1977a). It follows, therefore, that maintaining membrane integrity, especially later in the season, could play a large role in reducing the potential for mesocarp discolouration.

The fact that harvest date, and not fruit moisture content, was found to have a significant affect on discolouration severity could indicate that the method currently used to determine physiological maturity, in South Africa, has some deficiencies in that pre-harvest conditions obviously affect fruit moisture content. Thus, maturity, by itself, could not be used as a predictor of the mesocarp discolouration potential.

Harvest season

Harvest season had a large effect on the severity of mesocarp discolouration, with discolouration being more severe during 2001. Pre-harvest factors are considered to play an important role, as the same trend in increased electrolyte leakage at 5.5°C and 8°C was evident during the 2000 season (Table IV), although mesocarp discolouration was less prevalent (Table I). Many avocado cultivars are known to follow an alternate-year bearing pattern, with environmental conditions differing between seasons, resulting in different demands on the tree from season to season. Identifying what these factors are and how they affect fruit quality would thus enable the correct post-harvest management of fruit; for example, selecting the correct storage temperature.

CONCLUSIONS

Few post-harvest disorders of fruit are completely independent of pre-harvest factors. In the case of mesocarp discolouration in 'Pinkerton', however, post-harvest treatments cannot remedy poor quality fruit. Nonetheless, storage at 2°C did prove to be successful in minimising mesocarp discolouration. Thus, the disorder cannot be ascribed to chilling injury, as previously suspected.

Further research should concentrate on measuring the rate of CO₂ production both during and after storage to determine how a low storage temperature affects the metabolic activity of the fruit. Additional work might also include identifying and minimising pre-harvest factors that adversely affect membrane integrity, and increase PPO activity and total phenolics content so that the potential for mesocarp discolouration can be reduced.

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REFERENCES

(See final reference section, pg's 129-155)

Effects of storage temperature, harvest date and fruit origin ("low risk", "medium risk" and "high risk"), on mesocarp discolouration severity in 'Pinkerton' avocado fruit throughout the 2000 and 2001 harvest seasons

Risk area	2000										2001									
	Harvest date	Moisture content (%)	Mesocarp discolouration rating*								Harvest date	Moisture content (%)	Mesocarp discolouration rating							
			Unstored		8°C		5.5°C		2°C				Unstored		8°C		5.5°C		2°C	
SI#	R#	SI	R	SI	R	SI	R	SI	R	SI	R	SI	R	SI	R	SI	R	SI	R	
Low	30/05/00	77.3	0	0	0	0	0	0	0	0.2	11/06/01	71.6	0	0.2	0	0	0.6	0	0	0.4
	21/06/00	75.5	0	0	0	0	0.2	0.2	0	0.2	26/06/01	73.0	0	0	0	0	0	0.8	0	0.2
	06/07/00	75.3	0	0	0	0	0	0	0	0	11/07/01	70.7	0	0	0	0	0	0.2	0	0.2
	18/08/00	73.1	0	0	0	0	0.2	0	0	0	24/07/01	68.7	0	0	0	0	0	0.6	0	0.4
											06/08/01	68.6	0	0	1.2	1.0	0.4	0.2	0.2	0.6
Medium	17/05/00	78.0	0	0.2	0	0.4	0.4	0.6	0	0.6	11/06/01	74.4	0	0	0	0	0	0.0	0	0
	30/05/00	78.7	0	0	0	0.2	3.0	0	0	1.0	26/06/01	72.8	0	0	1.2	0.2	0	0.4	0	0.6
	21/06/00	75.7	0	0	0	0	0	0	0	0.6	11/07/01	71.2	0	0	0	4.0	0	2.0	0.2	1.0
	06/07/00	73.8	0	0	0.4	0	0	0	0	0.6	24/07/01	71.8	0	0	1.6	5.0	0	1.0	0	2.0
											06/08/01	73.7	0	0.2	1.2	3.4	0	3.0	0	1.6
High	17/05/00	78.9	0	0	0	0	0.4	0.6	0	1.0	11/06/01	73.8	0	0	0.4	3.8	0	0.4	0	0.8
	30/05/00	73.6	0	0	0	2.2	1.2	1.2	0	0.2	26/06/01	74.9	0	0	0	0	1.2	1.6	0.2	1.8
	21/06/00	74.3	0	0	0.8	2.8	0.2	1.2	0	2.0	11/07/01	74.0	0	0	0.4	1.8	0	0.6	0	0.8
	06/07/00	76.9	0	0	0.8	0.2	2.2	0.4	0	0	24/07/01	70.5	0	0	3.8	5.6	1.0	6.7	0.4	5.8
											06/08/01	72.2	0.3	0.4	2.8	7.4	2.6	5.6	1.0	1.8
Moisture content	Date = 2.0 *										Date = 2.1 *									
	Risk area = 1.7 n.s										Risk area = 1.6 *									
	Risk area x Date = 3.4 *										Risk area x Date = 3.6 n.s									
	LSD ($P = 0.05$); * = significant; n.s = not significant; n = 5																			
Discolouration	Date = 0.2 n.s										Date = 0.4 *									
	Risk area = 0.2 *										Risk area = 0.3 *									
	Storage treatment = 0.3 *										Storage treatment = 0.5 *									
	Risk area x Date = 0.4 *										Risk area x Date = 0.6 *									
	Date x Storage treatment = 0.6 *										Date x Storage treatment = 1.0 *									
	Risk area x Storage treatment = 0.5 *										Risk area x Storage treatment = 0.8 *									
	Risk area x Storage treatment x Date = 1.1 *										Risk area x Storage treatment x Date = 1.8 *									
	LSD ($P = 0.05$); * = significant; n.s = not significant; n = 5																			

*Discolouration rating 0 – 10, 0 = no discolouration, 10 = 100% of cut fruit surface area black.

*SI = sampled immediately; R = ripened.

Firmness of 'Pinkerton' avocado fruit obtained from different fruit origins ("low risk", "medium risk" and "high risk"), throughout the 2000 and 2001 harvest seasons, immediately after storage at different temperatures for 30 d

Risk area	Harvest date	Fruit firmness (units)				Harvest date	Fruit firmness (units)			
		No storage	8°C	5.5°C	2°C		No storage	8°C	5.5°C	2°C
Low	30/05/00	86.8	80.1	81.2	83.2	11/06/01	88.1	80.0	77.8	81.4
	21/06/00	85.3	75.5	78.0	82.0	26/06/01	85.9	78.2	82.6	86.8
	06/07/00	85.8	81.4	81.4	85.7	11/07/01	83.7	82.9	86.1	86.0
	18/08/00	85.3	76.5	70.6	84.0	24/07/01	83.0	75.7	81.9	87.8
						06/08/01	85.1	73.6	76.5	83.5
Medium	17/05/00	86.3	75.5	79.0	87.2	11/06/01	85.0	73.2	77.1	81.5
	30/05/00	86.6	75.0	79.4	88.0	26/06/01	84.9	72.3	82.1	83.0
	21/06/00	89.6	84.2	84.3	86.6	11/07/01	86.6	80.1	82.1	86.5
	06/07/00	86.7	77.6	73.0	85.9	24/07/01	85.0	70.4	79.3	83.7
						06/08/01	85.5	73.5	75.7	83.8
High	17/05/00	84.9	77.6	73.0	85.9	11/06/01	86.6	76.0	80.0	78.3
	30/05/00	87.4	73.2	71.5	88.3	26/06/01	84.6	73.4	79.9	82.2
	21/06/00	88.7	67.3	72.9	86.2	11/07/01	82.1	68.4	79.0	83.2
	06/07/00	87.2	76.7	79.5	87.0	24/07/01	86.2	67.2	81.7	85.0
						06/08/01	78.4	63.4	73.8	75.0
Date = 1.3 *						Date = 1.6 *				
Risk area = 1.1 *						Risk area = 1.3 *				
Storage treatment = 1.3 *						Storage treatment = 1.5 *				
Risk area x Date = 2.3 *						Risk area x Date = 2.9 *				
Date x Storage treatment = 2.6 *						Date x Storage treatment = 3.3 *				
Risk area x Storage treatment = 2.3 *						Risk area x Storage treatment = 2.6 *				
Risk area x Storage treatment x Date = 4.5 *						Risk area x Storage treatment x Date = 5.7 n.s				
LSD ($P = 0.05$); * = significant; n.s = not significant; n = 5						LSD ($P = 0.05$); * = significant; n.s = not significant; n = 5				

Number of days to reach "eating ripeness" (firmness 50 - 55) in 'Pinkerton' avocado fruit obtained from different fruit origins ("low risk", "medium risk" and "high risk"), throughout the 2000 and 2001 harvest seasons, and stored at different temperatures for 30 d

Risk area	Harvest date	Days to ripeness (d)				Harvest date	Days to ripeness (d)			
		No storage	8°C	5.5°C	2°C		No storage	8°C	5.5°C	2°C
Low	30/05/00	16.4	6.2	6.0	9.0	11/06/01	20.4	13.4	11.8	15.8
	21/06/00	15.8	6.4	4.4	9.6	26/06/01	17.0	10.0	14.4	16.6
	06/07/00	20.0	11.8	9.2	14.0	11/07/01	17.2	9.6	8.0	8.6
	18/08/00	15.2	4.8	4.4	8.2	24/07/01	16.0	6.8	6.8	11.2
						06/08/01	14.0	5.4	13.4	11.2
Medium	17/05/00	16.2	5.2	6.6	13.0	11/06/01	14.2	6.2	6.4	10.8
	30/05/00	16.8	6.8	9.4	11.0	26/06/01	13.4	8.8	6.6	9.4
	21/06/00	15.0	5.4	7.4	10.6	11/07/01	15.8	8.4	8.6	8.4
	06/07/00	18.46	10.8	12.4	13.6	24/07/01	15.0	4.8	4.4	8.0
						06/08/01	9.4	3.2	6.0	8.8
High	17/05/00	14.4	5.8	4.6	9.6	11/06/01	12.6	5.8	7.0	10.4
	30/05/00	14.4	5.6	5.2	8.0	26/06/01	11.6	7.4	8.8	9.6
	21/06/00	12.4	3.0	4.4	8.6	11/07/01	12.4	6.4	5.4	7.8
	06/07/00	14.6	8.4	7.2	9.6	24/07/01	12.8	6.0	7.2	9.2
						06/08/01	6.2	5.0	7.2	8.6
Date = 0.7 *					Date = 0.9 *					
Risk area = 0.6 *					Risk area = 0.7 *					
Storage treatment = 0.7 *					Storage treatment = 0.8 *					
Risk area x Date = 1.2 *					Risk area x Date = 1.6 *					
Date x Storage treatment = 1.4 n.s					Date x Storage treatment = 1.8 *					
Risk area x Storage treatment = 1.2 *					Risk area x Storage treatment = 1.4 *					
Risk area x Storage treatment x Date = 2.4 n.s					Risk area x Storage treatment x Date = 3.1 n.s					
LSD ($P = 0.05$); * = significant; n.s = not significant; n = 5					LSD ($P = 0.05$); * = significant; n.s = not significant; n = 5					

TABLE IV

Electrolyte leakage of 'Pinkerton' avocado fruit obtained from different fruit origins ("low risk", "medium risk" and "high risk") throughout the 2000 and 2001 harvest seasons and stored at different temperatures for 30 d

Risk area	Harvest date	Electrolyte leakage (%)				Harvest date	Electrolyte leakage (%)			
		No storage	8°C	5.5°C	2°C		No storage	8°C	5.5°C	2°C
Low	30/05/00	21.9	17.4	32.1	9.1	11/06/01	26.4	15.9	41.3	20.2
	21/06/00	19.2	31.7	65.4	15.6	26/06/01	25.7	29.0	16.7	16.8
	06/07/00	20.0	34.0	32.4	21.6	11/07/01	24.4	21.0	17.8	15.9
	18/08/00	22.8	63.2	65.8	24.1	24/07/01	21.9	84.6	55.6	16.3
						06/08/01	34.5	61.6	59.5	20.8
Medium	17/05/00	24.2	12.5	72.5	9.7	11/06/01	25.7	22.8	19.6	20.2
	30/05/00	20.7	31.7	65.4	15.6	26/06/01	25.9	46.5	16.8	18.6
	21/06/00	22.8	57.8	78.7	14.6	11/07/01	24.0	66.2	41.9	18.6
	06/07/00	21.1	63.2	23.1	21.1	24/07/01	21.5	83.4	41.8	17.3
						06/08/01	38.6	75.6	38.0	19.2
High	17/05/00	27.8	14.8	66.0	12.8	11/06/01	25.6	92.4	41.5	17.1
	30/05/00	18.2	14.0	46.8	8.0	26/06/01	24.1	49.6	27.5	18.1
	21/06/00	22.8	57.8	78.7	14.6	11/07/01	25.2	92.9	43.7	25.6
	06/07/00	16.0	78.2	76.5	18.9	24/07/01	22.3	90.1	63.3	18.1
						06/08/01	42.3	89.5	63.7	37.8
	Date = 7.2 *					Date = 6.8 *				
	Risk area = 6.2 n.s					Risk area = 5.2 *				
	Storage treatment = 7.2 *					Storage treatment = 6.1 *				
	Risk area x Date = 12.4 *					Risk area x Date = 11.7 *				
	Date x Storage treatment = 14.3 *					Date x Storage treatment = 13.5 *				
	Risk area x Storage treatment = 12.4 *					Risk area x Storage treatment = 10.5 *				
	Risk area x Storage treatment x Date = 24.8 *					Risk area x Storage treatment x Date = 23.4 *				
	LSD (P = 0.05); * = significant; n.s = not significant; n = 5					LSD (P = 0.05); * = significant; n.s = not significant; n = 5				

TABLE V

Maximum CO₂ production rate of 'Pinkerton' avocado fruit obtained from different fruit origins ("low risk", "medium risk" and "high risk"), throughout the 2000 and 2001 harvest seasons, and stored at different temperatures for 30 d

Risk area	Harvest date	Maximum CO ₂ production rate (ml kg ⁻¹ h ⁻¹)				Harvest date	Maximum CO ₂ production rate (ml kg ⁻¹ h ⁻¹)			
		No storage	8°C	5.5°C	2°C		No storage	8°C	5.5°C	2°C
Low	30/05/00	57.0	87.6	81.0	73.0	11/06/01	30.4	55.9	77.1	72.8
	21/06/00	49.9	83.6	84.5	66.3	26/06/01	48.9	67.3	67.9	67.0
	06/07/00	39.4	40.8	52.8	51.0	11/07/01	65.4	68.0	72.2	59.3
	18/08/00	97.4	73.7	86.4	59.0	24/07/01	65.7	94.9	99.4	74.0
						06/08/01	51.5	95.3	97.3	97.6
Medium	17/05/00	36.4	52.1	43.5	38.1	11/06/01	40.6	81.8	104.7	77.9
	30/05/00	50.2	77.2	62.1	56.0	26/06/01	80.7	86.7	107.3	104.1
	21/06/00	81.9	76.9	77.8	82.1	11/07/01	84.5	107.5	111.8	99.9
	06/07/00	95.9	62.0	68.2	76.8	24/07/01	94.8	134.4	138.4	131.1
						06/08/01	77.2	110.8	114.3	121.0
High	17/05/00	31.3	35.4	47.2	36.4	11/06/01	43.4	83.8	86.0	90.9
	30/05/00	64.3	95.8	103.1	98.7	26/06/01	83.3	80.1	91.1	93.7
	21/06/00	89.9	101.4	108.1	113.3	11/07/01	93.5	123.9	132.8	120.4
	06/07/00	82.4	64.9	63.6	79.0	24/07/01	89.3	114.7	140.3	126.0
						06/08/01	90.2	121.8	111.2	116.5
Date = 6.4 *					Date = 7.6 *					
Risk area = 5.5 *					Risk area = 8.9 *					
Storage treatment = 6.4 n.s					Storage treatment = 6.8 *					
Risk area x Date = 11.0 *					Risk area x Date = 13.2 *					
Date x Storage treatment = 12.7 *					Date x Storage treatment = 15.3 *					
Risk area x Storage treatment = 11.0 *					Risk area x Storage treatment = 15.6 n.s					
Risk area x Storage treatment x Date = 22.0 n.s					Risk area x Storage treatment x Date = 26.4 n.s					
LSD (<i>P</i> = 0.05); * = significant; n.s = not significant; n = 5					LSD (<i>P</i> = 0.05); * = significant; n.s = not significant; n = 5					

Days to reach maximum CO₂ production rate in 'Pinkerton' avocado fruit obtained from different fruit origins ("low risk", "medium risk" and "high risk"), throughout the 2000 and 2001 harvest seasons, and stored at different temperatures for 30 d

Risk area	Harvest date	Days to max CO ₂ production rate				Harvest date	Days to max CO ₂ production rate			
		No storage	8°C	5.5°C	2°C		No storage	8°C	5.5°C	2°C
Low	30/05/00	11.4	3.0	2.2	3.6	11/06/01	12.4	4.8	3.0	4.4
	21/06/00	7.2	2.2	2.6	3.4	26/06/01	9.0	5.0	6.2	2.4
	06/07/00	6.7	4.4	4.0	3.8	11/07/01	11.4	4.6	4.0	5.0
	18/08/00	1.0	1.6	1.2	2.2	24/07/01	10.4	1.6	1.8	5.0
					06/08/01	9.6	2.2	2.6	2.8	
Medium	17/05/00	9.6	1.0	2.0	4.0	11/06/01	7.8	2.6	3.6	5.6
	30/05/00	12.4	2.6	6.4	4.4	26/06/01	10.6	3.6	3.8	4.2
	21/06/00	2.4	2.6	2.8	4.0	11/07/01	11.8	3.2	2.8	3.8
	06/07/00	5.2	5.6	6.4	5.0	24/07/01	12.0	1.2	2.4	4.4
					06/08/01	6.2	1.4	2.0	3.0	
High	17/05/00	6.2	2.0	1.4	2.4	11/06/01	6.2	1.0	2.8	5.0
	30/05/00	5.2	1.4	1.8	3.6	26/06/01	8.2	4.8	3.2	5.2
	21/06/00	7.2	1.6	2.0	2.2	11/07/01	8.4	1.4	2.2	3.2
	06/07/00	3.1	2.0	2.0	2.8	24/07/01	10.2	1.4	1.0	4.6
					06/08/01	4.0	1.0	2.0	3.6	
Date = 0.7 *					Date = 0.8 *					
Risk area = 0.6 *					Risk area = 0.6 *					
Storage treatment = 0.7 *					Storage treatment = 0.7 *					
Risk area x Date = 1.3 *					Risk area x Date = 1.4 n.s					
Date x Storage treatment = 1.5 *					Date x Storage treatment = 1.6 *					
Risk area x Storage treatment = 1.3 n.s					Risk area x Storage treatment = 1.3 *					
Risk area x Storage treatment x Date = 2.5 *					Risk area x Storage treatment x Date = 2.8 n.s					
LSD (P = 0.05); * = significant; n.s = not significant; n = 5					LSD (P = 0.05); * = significant; n.s = not significant; n = 5					

TABLE VII

Effects of harvest date on total phenolics content, total polyphenol oxidase (PPO) activity, and soluble PPO activity in 'Pinkerton' avocado fruit from the "high risk" area during the 2001 harvest season

Harvest date	*Total Phenolics content ($\mu\text{g g}^{-1}\text{FW}$)	*Total PPO activity ($\Delta\text{OD } 420 \text{ min}^{-1} \text{ mg}^{-1} \text{ protein}$)	*Soluble PPO activity ($\Delta\text{OD } 420 \text{ min}^{-1} \text{ mg}^{-1} \text{ protein}$)
11/06/01	13.41 a	0.001224 b	0.000585 a
26/06/01	13.18 a	0.001234 b	0.000679 a
24/07/01	17.00 b	0.000982 ab	0.000705 a
06/08/01	17.71 b	0.000684 a	0.000637 a
LSD _(0.05)	2.03	0.000421	0.000534
Significance	$P < 0.001$	$P = 0.05$	n.s.

*n = 10 (5 fruit, 2 replications). Means followed by different lower-case letters are significantly different

TABLE VIII

Effects of storage temperature on total phenolics content, total polyphenol oxidase (PPO) activity, and soluble PPO activity in 'Pinkerton' avocado fruit harvested on 06/08/01 from the "medium risk" and "high risk" areas

Storage temperature (30 d)	Total phenolics content ($\mu\text{g g}^{-1}\text{FW}$)		Total PPO activity ($\Delta\text{OD } 420 \text{ min}^{-1} \text{ mg}^{-1}$ protein)		Soluble PPO activity ($\Delta\text{OD } 420 \text{ min}^{-1} \text{ mg}^{-1}$ protein)	
	"Medium"	"High"	"Medium"	"High"	"Medium"	"High"
No storage	16.7	17.2	0.000610	0.000784	0.000425	0.000769
8°C	22.3	26.9	0.000857	0.000336	0.000151	0.000274
5.5°C	16.1	20.6	0.000459	0.000364	0.000279	0.000310
2°C	11.4	18.8	0.000891	0.000831	0.000410	0.000696
LSD _(0.05) Temp	3.0 *		0.000387 n.s		0.000282 *	
LSD _(0.05) Risk area	2.1 *		0.000273 n.s		0.000199 *	
LSD _(0.05) Temp x Area	4.3 n.s		0.000547 n.s		0.000399 n.s	

* = significant; n.s = not significant; n = 10 (5 fruit, 2 replications)

TABLE IX

Effects of fruit origin on total phenolics content, total polyphenol oxidase (PPO) activity, and soluble PPO activity in 'Pinkerton' avocado fruit harvested on 06/08/01 from the "low risk", "medium risk" and "high risk" areas

Fruit origin	Total phenolics content ($\mu\text{g g}^{-1}\text{FW}$)	Total PPO activity ($\Delta\text{OD } 420 \text{ min}^{-1} \text{ mg}^{-1} \text{ protein}$)	Soluble PPO activity ($\Delta\text{OD } 420 \text{ min}^{-1} \text{ mg}^{-1} \text{ protein}$)
Low risk	19.31 a	0.00026 a	0.00028 a
Medium risk	16.68 a	0.00061 a	0.00042 a
High risk	18.26 a	0.00070 a	0.00074 a
LSD _(0.05)	4.0	0.00063	0.00062
Significance	n.s	n.s	n.s

*n = 10 (5 fruit, 2 replications). Means followed by different lower-case letters are significantly different

CHAPTER 3

The role of fruit mineral composition on fruit firmness and mesocarp discolouration in 'Pinkerton' avocado (*Persea americana* Mill.)

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SUMMARY

The successful marketing and export of fruit depends, to a large degree, on the predictability and consistency of quality. The 'Pinkerton' avocado, however, is prone to a physiological disorder known as "mesocarp discolouration" during and after storage. Furthermore, fruit have been found to arrive at their European destination with variable firmness. Mesocarp discolouration was initially thought to be the result of chilling injury, however differences in quality were noted between fruit from different origins. Fruit were subjected to mineral analysis following a suspicion that pre-harvest factors played a role in fruit quality. Excessive nitrogen concentrations were found to have the most significant role in the severity of mesocarp discolouration. In addition, decreasing copper, manganese and boron concentrations during the season also appeared to contribute to the development of the disorder. The results of this study indicate that interactions between minerals could be more important in determining quality than evaluating individual elements.

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The 'Pinkerton' avocado cultivar was introduced into South Africa primarily on account of its high yielding characteristics (Kruger and Kritzinger, 1999). Unfortunately this green skin cultivar has proved to have certain shortcomings. It is characterised by an extended flowering period, making the determination of fruit maturity difficult, and at the time was considered to have limited storage potential in terms of export by ship. Fruit have been found to have variable firmness on arrival at their European destination and/or to be severely affected by a disorder known as "mesocarp discolouration" or "grey pulp". This disorder is characterised by a grey-brown discolouration of the pulp, which intensifies upon cutting the fruit and exposing the cut surfaces to the atmosphere. Initial studies associated the disorder with chilling injury (Chaplin *et al.*, 1982); however, discoloration was also found in unstored fruit and at non-chilling temperatures (Vakis, 1982).

While temperature no doubt plays an important role in the keeping-quality of fruit from a post-harvest point of view, the actual causes of many disorders are often initiated pre-harvest (Arpaia, 1994; Ferguson *et al.*, 1999). This is demonstrated by the fact that when fruit from different orchards are stored in the same chamber they exhibit marked differences in storage quality. In avocados, post-harvest quality differences have been related to season and site effects, as well as to climatic conditions (Rowell, 1988; Kruger and Kritzinger, 1999; Woolf *et al.*, 1999), rootstock choices (Marques, 2002) and fertiliser treatments (Ginsberg, 1985; Koen *et al.*, 1990; Milne, 1998). An understanding of how these factors interact and how they affect fruit quality should, therefore, permit management systems to be adjusted accordingly. Mineral nutrition has received some attention over the years, with regards to establishing leaf and soil norms to ensure optimum growth and yield (Embleton and Jones, 1964; Köhne *et al.*, 1990; Koen and Du Plessis, 1991); however, it is becoming clear that these minerals important for tree growth are not necessarily also important for fruit quality (Arpaia *et al.*, 1996). Furthermore, in a study by Thorp *et al.* (1997), no correlations were found between fruit mineral concentrations and soil or leaf concentrations. To complicate matters further, Kremer-Köhne *et al.* (1993) found that minerals important for the prevention of one disorder are not necessarily beneficial for another.

Thus, due to the complex nature of the situation with 'Pinkerton' avocado it is necessary to limit the variables by prioritising what disorders are more problematic in the cultivar and identifying macro-factors that can be adjusted. In 'Pinkerton', early softening and mesocarp discolouration are the greatest problems. Physiological disorders such as these are generally associated with increased membrane permeability, resulting in the leakage of phenols from the vacuole into the cytoplasm, with subsequent oxidation by polyphenol

oxidase and fruit browning (Cutting *et al.*, 1992). Membrane stability is therefore a vital component of disorder development, as are all factors affecting membrane stability. Mineral concentrations are known to play an important role in this regard (Taleisnik and Grunberg, 1994; Cakmak *et al.*, 1995) and can be managed to some extent.

Calcium is thought to play a primary function in membrane stability (Kremer-Köhne *et al.*, 1993). However, the application of calcium to 'Pinkerton' fruit has had inconsistent results in terms of reducing mesocarp discolouration (Penter *et al.*, 2001). Potassium and magnesium are potentially antagonistic to calcium and may markedly increase membrane permeability (Bangerth, 1979). Avocado fruit quality is also affected by other minerals such as nitrogen (Arpaia *et al.*, 1996), boron (Smith *et al.*, 1997), magnesium, potassium (Koen *et al.*, 1990; Witney *et al.*, 1990), and zinc (Vorster and Bezuidenhout, 1988).

In this study, avocado fruit were obtained from "low", "medium" and "high risk" areas of South Africa to determine whether a fruit's origins would reflect which minerals play the most important role in terms of fruit softening and mesocarp discolouration development in 'Pinkerton' avocados. Furthermore, it was hoped that fruit mineral concentrations would help determine how fruit would respond to storage at various temperatures.

MATERIALS AND METHODS

Plant material and treatments

Avocado fruit (*Persea americana* Mill. 'Pinkerton') were obtained from various production areas with varying mesocarp discolouration histories throughout the 2001 season. The three fruit origins were termed "low risk", "medium risk" and "high risk" according to their potential for development of the disorder. These risk classes were based on fruit quality studies conducted in previous seasons (Kruger *et al.*, 2000). The "medium" and "high risk" orchards were situated on fairly "rich" soils previously planted to banana and therefore had a high organic matter content (data not shown). The "low risk" area chosen was situated on reasonably sandy soils with a slightly cooler climate. All the orchards were situated in Mpumalanga Province, South Africa. Once harvested, fruit were subjected to washed and waxed (Citrashine Pty Ltd., Johannesburg, RSA; 1 t^{-1}), at the same packhouse, before being sent by courier to the University of KwaZulu Natal, Pietermaritzburg, South Africa). The fruit took up to 72 h to be delivered. Fruit were randomly divided into four treatments (Table I), each treatment consisting of five fruit, in total, of similar sizes (count 12 to 16). Before storage, the five fruit for each treatment were numbered, weighed and placed in cartons.

After storage the weight of each fruit was recorded again, the firmness determined and the severity of internal disorders was noted.

Mesocarp discoloration

Fruit were bisected longitudinally and immediately rated, visually, for mesocarp discoloration using a scale of 0 to 10, with 0 = no visible discoloration and 10 = 100% of cut surface area black.

Fruit firmness

Fruit firmness was determined using a hand-held firmness tester (Bareiss, Oberdischingen, Germany). Two readings [on a scale of 100 (hard) to 0 (soft)] were taken per fruit. Measurements were taken at the maximum circumference of the fruit, turning the fruit 180° after each measurement. The densimeter measures fruit firmness by means of a metal ball (diameter 5 mm) that is pressed onto the fruit.

Mineral analysis

On arrival mesocarp tissue samples from the distal ends of the fruit in treatment 1 (Table 1) were cut into small blocks (1 cm³), flash frozen in liquid nitrogen and placed on a freeze-drier for approx. 5 d. Once dry, individual samples were finely milled and analysed using atomic absorption spectrometry by Cedara Agricultural College, KwaZulu Natal, South Africa. The minerals levels measured included nitrogen, calcium, potassium, magnesium, manganese, boron, iron, zinc, phosphorus, copper and sodium.

Statistical analysis

Statistical analysis was carried out on the data using GenStat® (VSN International, Hemel Hempstead, UK). Where applicable means were compared using least significant differences (LSD) at $P = 0.05$. Firmness data were subjected to multiple linear regression. However, due to the fact that the discoloration rating given to each fruit was a qualitative, non-normally distributed variable, the same regression could not be used to analyse these data. Since a large percentage of the fruit were found to have very little severe discoloration, the rating scale (0/10) was converted into a binomial variable (i.e., 0 = no discoloration; 1 = any discoloration) allowing use of logistic regression to analyse the data. This form of regression uses chi-square (χ^2) probabilities and works with deviance and not variance.

RESULTS AND DISCUSSION

Mesocarp discolouration and fruit firmness

The overall discolouration ratings showed a tendency for decreased discolouration as storage temperature was decreased, irrespective of fruit origin. This was best illustrated by the fruit from the “high risk” area (Figure 1). Fruit stored at lower storage temperatures were found to remain harder during storage (Figure 2). Storage at 2°C thus appeared to be optimum for overall fruit quality, and appeared to support the theory that mesocarp discolouration is affected by membrane breakdown. Furthermore, there was a tendency throughout the study for fruit that ripened more quickly to display a more intense mesocarp discoloration.

Risk areas

Fruit origin was found to have a significant ($P < 0.001$) effect on fruit firmness (Figure 3) and disorder development (Figure 4). The fruit from each area developing the severity of disorder associated with the respective risk levels, thus further emphasising the role of pre-harvest factors in determining fruit quality.

Effect of sampling date

Fruit quality was seen to deteriorate significantly ($P < 0.001$) as the season progressed in all the risk areas (Figure 4), with the “high risk” area showing the most dramatic increase in disorder severity at the same stage.

Mineral concentration

Significant ($P < 0.001$) differences in mineral concentrations were found between fruit of various origins and harvest dates. Comparisons between various mineral concentrations and severities of fruit disorder (Figure 4) from various origins appeared to show some relationships. For example, nitrogen, iron, magnesium and zinc concentrations (Table II) were found to increase with disorder development as one moved from a “low” to a “high risk” area. Furthermore, copper and manganese (Table II) appeared to decrease as the season progressed, which was accompanied by an increase in disorder severity. It appeared, therefore, that these minerals could have a role in disorder development, and similarly in fruit firmness. Analysis of the firmness data revealed that nitrogen, magnesium, manganese and iron were in fact the minerals that contributed most to fruit firmness (Table III), and together

with treatment and fruit origin accounted for 55.6% of the variance. The (calcium + magnesium)/potassium and nitrogen/calcium ratios were also considered, but did not appear to have a significant role.

A stepwise regression of discolouration revealed that nitrogen and manganese contributed the most to disorder development (Table IV). Storage temperature and fruit origin also contributed significantly to the severity of discolouration. As calcium, boron and some of the other elements were thought to contribute to discolouration in some way, and mineral interactions were known to confound the analysis (Table V), manganese and zinc were removed from the model (as these showed some significant interactions with some of the other elements) to see how this would affect the outcome. Results of the second analysis revealed that nitrogen, boron and calcium did, in fact, contribute significantly to discolouration development ($P < 0.003$). Because of these findings, each element was evaluated separately to negate interaction effects. Again, this third form of analysis rendered a new set of elements, of varying significance (Table VI). The information from the graphs and significance of each mineral (Table VI), with the known discolouration history of each fruit origin was thus combined to allow for some conclusions to be drawn.

High fruit nitrogen concentrations no doubt play a very significant role in disorder development ($P < 0.001$). To some extent this was expected due to the nature of the soil in the two "higher risk" orchards. High nitrogen contents in soils have previously been found to result in an increase in fruit nitrogen, with studies in 'Hass' avocados finding that this resulted in faster ripening and more internal disorders (Arpaia *et al.*, 1996). In addition, high flesh nitrogen concentrations have also been positively correlated with mesocarp discolouration in 'Fuerte' avocados (Koen *et al.*, 1990) and 'Pinkerton' (Kruger *et al.*, 2001). The results are not unique to avocados however, with superficial scald in apples (Emonger *et al.*, 1994) and translucence in pineapple (Soler, 1994; Paull and Reyes, 1996) also being related to high nitrogen concentrations. The high nitrogen content in the soil would also result in increased nitrogen content in the tree and subsequently more vegetative growth. Competition would thus arise between the fruit and vegetative growth for available reserves (Sippel *et al.*, 1993), with the vegetative growth being a stronger sink. The increased vegetativeness would also result in minerals, such as calcium, which move in the transpiration stream being directed to the actively transpiring and developing leaves, at the expense of the fruit (Shear and Faust, 1975). In the same way, carbohydrates would be directed to the new vegetative flush. Bower *et al.* (1990) reported that carbon fixation within the fruit would influence fruit growth and ripening. It has also been found that higher nitrogen concentrations in plant tissues may result

in thinner cell walls, explaining why fruit from the higher risk areas were softer after removal from storage (Figure 3). Snijder *et al.* (2002) recommended that fruit nitrogen concentrations be less than 1% (w/w) by March (southern hemisphere) to reduce mesocarp discolouration development and in this study nitrogen concentrations were above 1% (w/w) throughout the season in the “high risk” areas.

Copper is known to activate a group of oxidising enzymes such as polyphenol oxidase, monophenol oxidase, laccase and systems that oxidise ascorbic acid (Marschner, 1995). Zinc is often mutually coordinated with copper in the various oxidation-reduction reactions which lead to browning. Furthermore, copper is believed to be necessary for the normal metabolic activity of various plants, aiding chlorophyll formation and maintaining an adequate balance between nitrogen and reducing sugar content of plants (Lal and Subba Rao, 1954; Marschner, 1995). As nitrogen was seen to increase and copper to decrease (Table VI) during the season, maintaining an adequate supply of copper could prove vital to maintain fruit quality.

Manganese concentrations were seen to decrease gradually as the season progressed in the “low” and “high risk” areas (Table VI). Bezuidenhout and Vorster (1991) suspected that manganese could play a role in fruit quality, although the exact mechanism by which it did was uncertain. Zinc, copper, iron and manganese are important components of detoxifying enzymes, with some elements being directly involved in the photosynthetic electron transport chain. The photolysis of water is mediated by a manganese-containing enzyme attached to photosystem II (Marschner, 1995). Manganese also presumably acts as the binding site for the water molecules that are oxidised. Thus, the decrease in manganese concentration as the season progressed could upset the electron transport system. Furthermore, manganese is thought to play an important role in sugar formation and sugar metabolism (Lal and Subba Rao, 1954). Iron, a central molecule in cytochrome, plays a role in electron transfers between photosystems I and II (Marschner, 1995). Injections of iron into fruit have been found to induce symptoms similar to low temperature breakdown and superficial scald in apple (Wills *et al.*, 1989), which could in part explain the faster ripening in the “high risk” area. Furthermore, iron has been known to be interrelated with polyphenol oxidase, which is involved in flesh browning (Lal and Subba Rao, 1954). However, the decrease in iron concentration as the season progressed in the “low and high risk” areas were not accompanied by a decrease in disorder development as would be expected; perhaps explaining why iron did not contribute significantly to disorder development. Iron was

therefore thought to rather play a role in disorder development through its interactions with the other elements (Table II).

Boron has been found to be the most common nutrient deficiency in avocado trees (Whiley *et al.*, 1996). Marschner (1995) proposed primary roles for boron in cell wall structure and metabolism, plasma membrane integrity, phenol metabolism, and in diffusible auxin. While boron proved to be a significant element in the current study it was not clear from the data (Table VI) how it contributed. Fruit from the "low risk" area had the lowest boron concentrations but the best internal quality. It was, therefore, suspected that the interaction between boron and phosphorus (Table VI) could be important (Table II). Starch synthesis can be inhibited by high concentrations of phosphorus and this could be detrimental to energy generation. However, fruit mineral standards need to be established before this can be verified.

Zinc and copper are both an important component of superoxide dismutase (SOD). Under zinc deficiency the level of toxic oxygen species is high because of both depressed SOD activity and lower export rates of carbohydrates as a result of low sink activity (Marschner, 1995). This results in the peroxidation of membrane lipids and an increase in membrane permeability. While results from this study showed a significant ($P < 0.001$) decrease in zinc during the season in fruit from the "high risk" area, concentrations were generally lower in the "low risk" area.

Calcium has been associated with more physiological disorders than any other mineral (Bangerth, 1979; Wills *et al.*, 1989). Calcium is an integral component of cell membranes and is necessary for binding phospholipid molecules in membranes, which influences their selective permeability (Ferguson and Drobak, 1988). Calcium deficiencies in tissues are thought to cause membrane destabilisation, which in turn causes a breakdown in membrane permeability (Battey, 1990). In this study calcium levels were seen to fluctuate throughout the season and were not necessarily lower in the "high risk" areas (Table VI). However, together with the higher nitrogen levels in these areas this resulted in high nitrogen/calcium ratios, which have been associated with certain internal disorders in apple (Ferguson and Watkins, 1989) and pear (Curtis *et al.*, 1990) and could also be important in avocados.

Studying the effects of the various elements on post-harvest fruit quality has its challenges in that the effect of other pre-harvest factors are not always known and cannot be separated from the effect of mineral concentrations. Furthermore, these factors may differ between the respective growers. The extended flowering period of 'Pinkerton' also means

that often fruit of various maturities are sampled together at any one-harvest date. Furthermore, the interaction between the various elements makes it difficult to establish if some minerals are more important than others for overall fruit quality. The authors are, however, confident that the excessively high nitrogen concentrations played the primary role in mesocarp discolouration development. It is suspected that the excessive vegetative vigour out-competed the fruit for available reserves. This would have major effects on the storage capacity of the fruit as temperature affects the metabolic rate of the fruit and therefore carbohydrate consumption. A more comprehensive study over an extended period needs to be undertaken to assess the other elements. It may well be that different elements contribute to disorder development in different areas. In this study, where the mineral data of three fruit origins was pooled, it may well be that the significance of an element in a certain area could have been cancelled out by its insignificance in other area. In addition, research is urgently needed to establish fruit norms for the various elements. This will help to quantify what is meant by "excessive" and "deficient" so that results can be compared and interpreted using the available literature.

Further studies should also be done to consider the effect of pre-harvest conditions on mineral uptake into the fruit during fruit growth. An incorrect mineral concentration at a critical period of fruit growth could well be more damaging to fruit quality than concentrations after harvest (Lovatt, 1999). Furthermore, this study confirmed the importance of considering mineral interactions, rather than single elements, when assessing fruit quality. Unfortunately, there are few statistical packages that can accurately analyse this type of data. The confounding effect of these interactions on regression analysis could explain why low calcium concentrations are often found to play significant roles, however when applied to fruit, inconsistent results are obtained.

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REFERENCES

(See final reference section, pg's 129-155)

TABLE I

Treatments used to establish the role of temperature in disorder development

Treatment	Storage temperature (°C)	Storage period (d)
1	*(Not stored)	*(Not stored)
2	8	30
3	5.5	30
4	2	30

* Sampled on arrival

TABLE II

Mineral concentrations in fruit from different fruit origins throughout the 2001 season.

Harvest date	N %	Fe mg kg ⁻¹	Mg %	Zn mg kg ⁻¹	Cu mg kg ⁻¹	Mn mg kg ⁻¹	B mg kg ⁻¹	P %	Ca %
<i>Low risk</i>									
11/06/01	0.514	24.40	0.04	10.75	6.20	9.09	23.98	0.11	0.06
27/06/01	0.484	22.31	0.07	12.39	6.61	8.69	27.29	0.11	0.07
11/07/01	0.522	19.92	0.07	9.13	4.15	5.40	21.16	0.11	0.03
24/07/01	0.560	15.42	0.05	13.67	4.97	5.39	23.60	0.08	0.06
06/08/01	0.532	10.16	0.02	2.03	2.84	2.03	23.57	0.07	0.02
<i>Medium risk</i>									
11/06/01	1.006	28.4	0.08	15.73	4.36	6.54	60.58	0.21	0.02
27/06/01	1.076	28.91	0.14	16.76	7.54	7.54	46.06	0.20	0.04
11/07/01	1.140	23.47	0.09	10.07	2.52	3.77	27.24	0.13	0.04
24/07/01	1.036	25.14	0.11	15.52	3.37	8.39	35.23	0.21	0.06
06/08/01	1.242	40.91	0.12	15.87	3.76	8.76	55.09	0.27	0.04
<i>High risk</i>									
11/06/01	1.086	47.07	0.12	24.15	6.24	9.58	33.70	0.17	0.08
27/06/01	1.154	49.13	0.11	24.75	7.56	7.55	33.09	0.15	0.04
11/07/01	1.428	48.40	0.11	24.21	5.85	9.60	34.64	0.20	0.08
24/07/01	1.384	41.23	0.10	12.75	2.07	4.55	16.89	0.15	0.04
06/08/01	1.663	27.82	0.09	8.42	2.63	5.25	29.95	0.16	0.04
¹ LSD _(0.05)	0.239	18.01	0.04	6.58	2.39	3.50	12.65	0.05	0.04
n	75	75	75	75	75	75	75	75	75

¹LSD = Date x Fruit origin

TABLE III

Significance levels for minerals affecting fruit firmness

Mineral	Fruit firmness
	Significance level
Nitrogen (%)	< 0.001
Magnesium (%)	0.007
Manganese (mg kg ⁻¹)	0.030
Iron (mg kg ⁻¹)	0.107

TABLE IV

Significance levels for minerals affecting mesocarp discolouration

Mineral	Mesocarp discolouration
	Significance level
Nitrogen (%)	< 0.001
Manganese (mg kg ⁻¹)	< 0.001

TABLE V

Correlation matrix showing the interactions between various elements

	B	Ca	Cu	Fe	K	Mg	Mn	N	Na	P	Zn
B	1.00										
Ca	-0.28 ns	1.00									
Cu	0.20 *	0.39 ns	1.00								
Fe	0.29 ns	0.34 *	0.31 *	1.00							
K	0.48 *	0.05 ns	-0.26 ns	0.45 *	1.00						
Mg	0.49 *	0.24 *	0.23 *	0.73 **	0.51 **	1.00					
Mn	0.39 ns	0.65 **	0.66 **	0.57 **	0.27 *	0.52 **	1.00				
N	0.30 *	0.01 ns	-0.24 ns	0.68 **	0.65 **	0.68 **	0.11 ns	1.00			
Na	-0.14 ns	-0.54 ns	-0.20 ns	-0.27 ns	-0.05 ns	-0.18 ns	-0.49 ns	-0.12 ns	1.00		
P	0.80 **	-0.01 ns	0.03 ns	0.61 **	0.72 **	0.81 **	0.53 **	0.66 **	-0.26 ns	1.00	
Zn	0.39 *	0.50 **	0.61 **	0.84 **	0.31 *	0.71 **	0.73 **	0.41 *	-0.40 ns	0.53 **	1.00

ns = non-significant; * = $P = 0.05$; ** = $P < 0.001$

TABLE VI

Contribution of individual elements to mesocarp discolouration

Mineral (units)	Mesocarp discolouration	
	Deviance ratio	χ^2 (probability)
Nitrogen (%)	38.18	< 0.001
Copper (mg kg ⁻¹)	26.12	< 0.001
Manganese (mg kg ⁻¹)	12.34	< 0.001
Boron (mg kg ⁻¹)	7.93	0.005
Zinc (mg kg ⁻¹)	6.51	0.011
Nitrogen/Calcium ratio	4.51	0.034
Potassium (%)	4.09	0.043
Calcium (%)	3.01	0.083

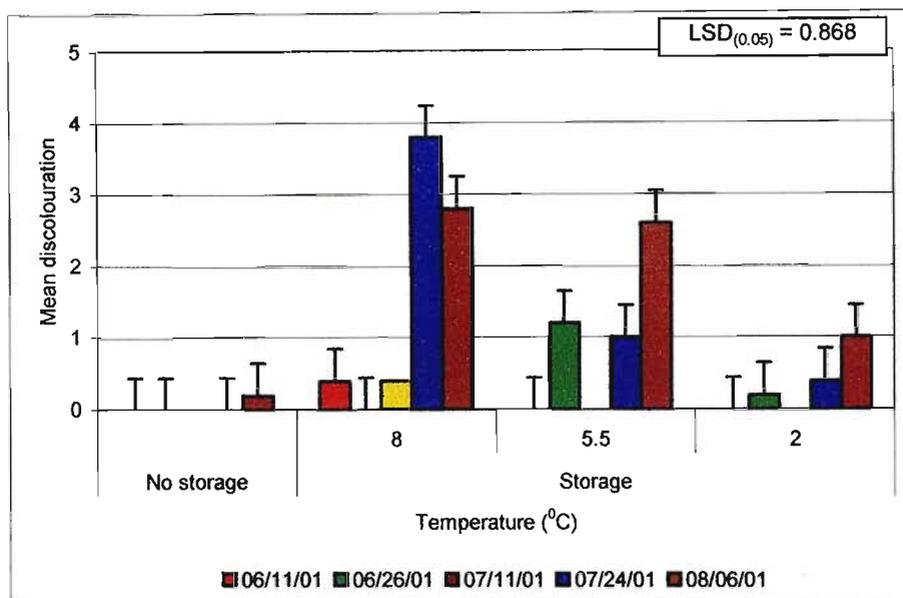


FIG. 1

Severity of mesocarp discoloration (range = 0 - 10), sampled immediately (no storage), or immediately after 30 d storage, as affected by storage temperature and harvest date in a "high risk" area. Values are means of five replications (\pm SE). LSD = Date x Temperature.

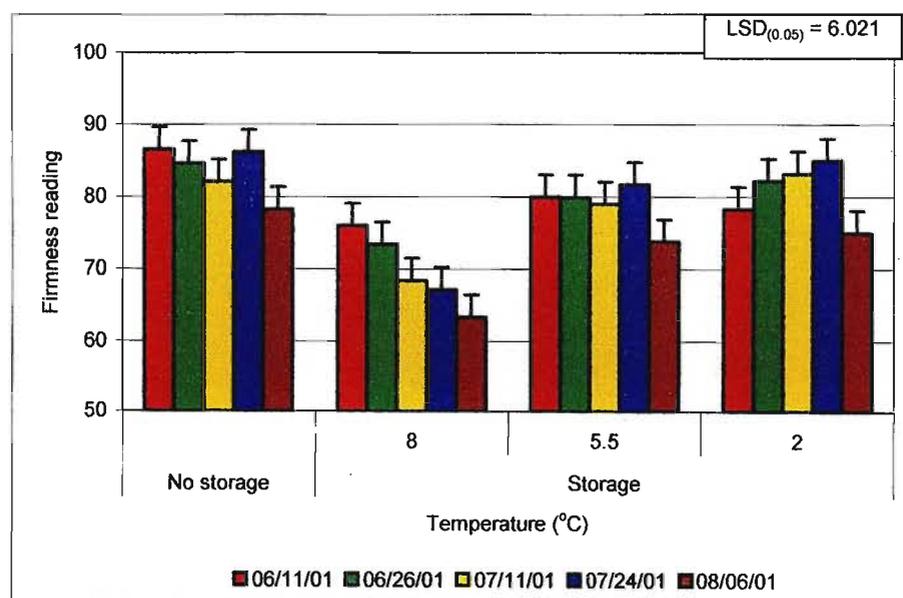


FIG. 2

Fruit firmness readings (0 = soft; 100 = hard) immediately upon arrival, or after removal from storage for 30 d at the temperatures shown. Fruit were from a "high risk" area. Values are means of five replications (\pm SE). LSD = Date x Temperature.

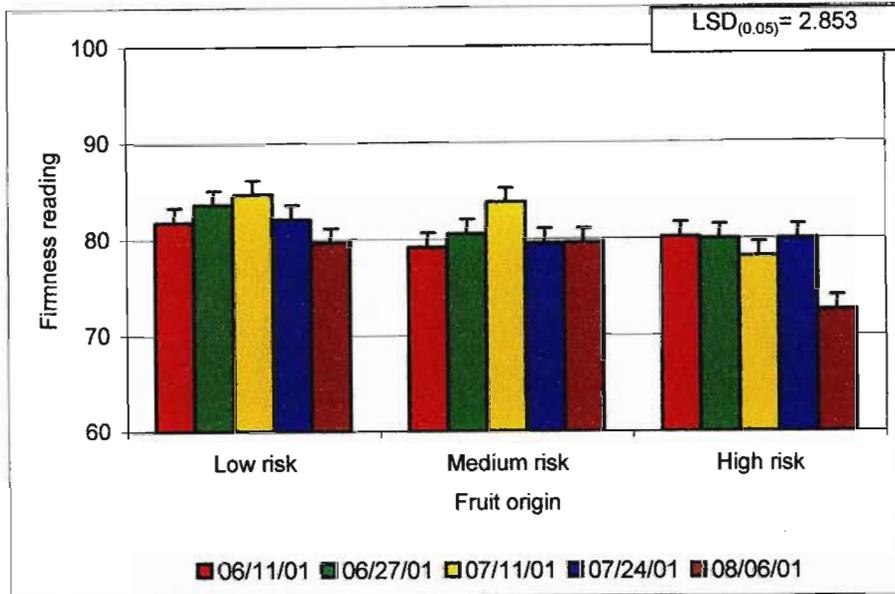


FIG. 3

Fruit firmness readings (0 = soft; 100 = hard) from various "risk areas" throughout the harvest season. Values are means of five replications (\pm SE). LSD = Date x Fruit origin.

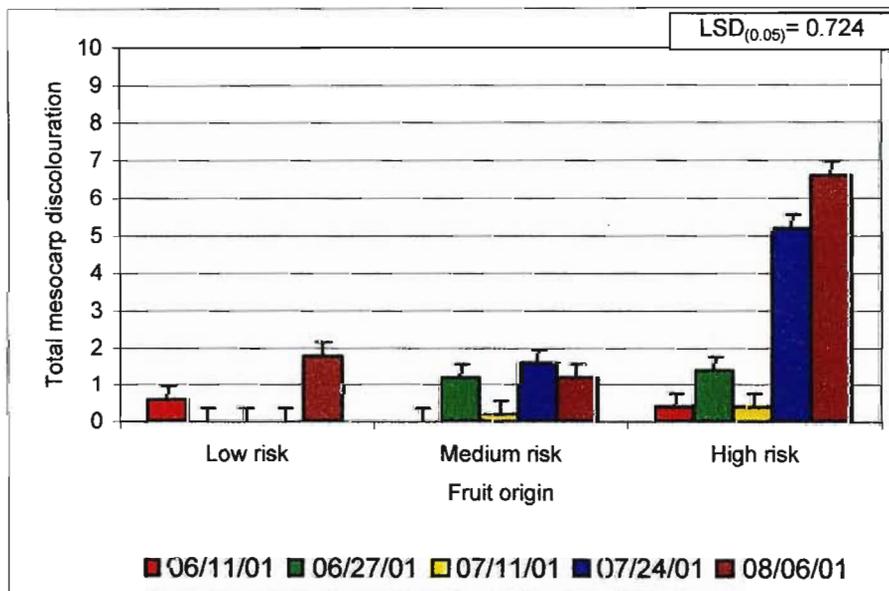


FIG. 4

Mesocarp discoloration ratings (range = 0 - 10) of fruit from different "risk areas" throughout the harvest season in 2001. Values are combined totals of all treatment means, consisting of five replications per treatment (\pm SE). LSD = Date x Fruit origin.

CHAPTER 4

The use of chlorophyll fluorescence for predicting internal fruit quality in 'Pinkerton' avocado (*Persea americana* Mill.) stored at different temperatures.

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SUMMARY

'Pinkerton' avocado fruit (*Persea americana* Mill.) are highly susceptible after storage to a physiological disorder known as mesocarp discolouration, which has threatened its export. Initial studies indicated that the disorder might be the result of chilling injury. In order to determine the affect of fruit origin fruit were obtained from different growing areas, of varying discolouration histories, throughout the 2000 harvest season and subjected to a 30-day storage period at either 8, 5.5 or 2°C. After storage half of the fruit from each treatment were allowed to ripen while the other half were sampled immediately for fruit quality and membrane integrity determinations (in the form of electrolyte leakage and chlorophyll fluorescence (Fv/Fm ratio)). Results were compared to control fruit, which were not stored. The Fv/Fm ratio was measured on both the mesocarp and exocarp tissue of the fruit, to determine if either or both of these readings could be correlated to poor internal fruit quality. Fruit origin and storage temperature were both found to have a significant effect on fruit quality, with fruit stored at 2°C having the best internal quality. Electrolyte leakage was also found to be the least at this temperature and was thus thought to give a good reflection of membrane integrity. The external Fv/Fm ratios, taken immediately after storage, were all well within the accepted range for a normal photosynthetic system in avocado skin, despite some external chilling injury being noticed at 2°C. Furthermore, no correlation between the external Fv/Fm ratios and internal fruit quality could be found. The internal Fv/Fm ratios were much lower than the external ratios, perhaps due to the lower chlorophyll a content. The internal ratios did not give a good indication of membrane integrity and results appeared to be in conflict with the electrolyte leakage and fruit quality results. It was, therefore, felt that chlorophyll fluorescence could not be used to predict poor internal fruit quality in 'Pinkerton' avocados.

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The Pinkerton avocado cultivar was introduced into South Africa in the 1980's and initially showed great potential as a high yielding greenskin cultivar. However, it was soon discovered that the cultivar did not ship well, having variable fruit quality on arrival at its destination. Fruit were found to exhibit an intense discolouration of the mesocarp and/or a certain percentage of fruit were found to be soft on arrival. Mesocarp discolouration was previously thought to be the result of chilling injury (Chaplin *et al.*, 1982), however symptoms were also noticed in unstored fruit (Vakis, 1982). While numerous studies have since been undertaken to find the cause of the disorder (Schutte, 1994; Sippel *et al.*, 1995; Zauberman and Jobin-Décor, 1995; Fuchs and Zauberman, 1998) a temporary solution was urgently sought in 'Pinkerton' as increasing consumer resistance to the cultivar threatened its export. It was thought that finding a way of predicting fruit quality, after storage, would allow for fruit to be sorted prior to shipping and/or resale thus minimising losses. Chlorophyll fluorescence offered a potential solution, being a non-invasive and non-destructive measurement (DeEll *et al.*, 1999) that could be performed fairly quickly. But, perhaps more importantly it was reported to detect cellular damage before the development of visible symptoms.

Chlorophyll fluorescence generally reflects the primary processes of photosynthesis that take place in the chloroplasts, such as light absorption, excitation energy transfer, and the photochemical reaction in photosystem II (PS II). When chloroplasts are damaged, consistent changes in fluorescence occur. In fruit the Fv/Fm ratio has been used as an indication of stress (Tijskens *et al.*, 1994; DeEll *et al.*, 1999). This ratio is generally determined in dark-adapted tissue, to relax non-photochemical quenching, and is used as an indicator of the quantum efficiency of PS II. Studies with cucumber found that the quantum yield of PS II decreased when fruit were stored at low temperatures in darkness, this treatment also led to the visual symptoms of chilling injury (Tijskens *et al.*, 1994). The primary process of chilling injury is thought to be membrane leakage caused by insufficient scavenging of radicals that form during or after the cold treatment (Hariyadi and Parkin, 1991). Membrane leakage is thought to be enhanced by lower temperatures, and to be well correlated with Fv/Fm (DeEll *et al.*, 1999). The cold storage appears to induce changes in the thylakoid membranes, resulting in a decreased exciton transfer efficiency of PS II, which seems to be temperature dependent.

In fruit the cell membrane system, and in particular the plasma membrane, make up an important aspect of ripening (Thompson, 1988). Maintaining membrane integrity during storage is therefore vital for sustaining the metabolic activity needed for controlled ripening and thus overall fruit quality. Storage temperature will play an important role in this respect

considering that the avocado is a highly climacteric and rapidly softening fruit. Storage at an incorrect temperature or any physiological stress occurring during storage has the potential of leading to a decrease in membrane integrity and thus the leakage of phenols from the vacuole of the cell into the cytoplasm, with subsequent oxidation by polyphenol oxidases (PPO) resulting in fruit blackening (Haslam, 1989). A decrease in membrane integrity has also been found to lead to increased electrolyte leakage (Thompson, 1988; Stanley, 1991). Chlorophyll fluorescence has already been used in avocados to reflect the effect of heat stress (Woolf and Laing, 1996). The study found a strong correlation between external browning and Fv/Fm in certain treatments. The Fv/Fm ratio should, therefore, be a fairly good indication of membrane integrity in the fruit at the time of measurement.

In this study the effect of storage temperature on fruit quality was evaluated using both chlorophyll fluorescence and electrolyte leakage as an indication of membrane integrity.

MATERIALS AND METHODS

Plant material and treatments

Avocado fruit (*Persea americana* Mill. 'Pinkerton') were obtained throughout the 2000 season from various production areas with varying mesocarp discolouration histories. The three fruit origins were termed "low risk", "medium risk" and "high risk" according to their potential for development of the disorder. These risk classes were based on fruit quality studies conducted in previous seasons (Kruger *et al.*, 2000). The "medium" and "high risk" orchards were situated on fairly "rich" soils previously planted to banana and therefore had high organic matter contents. The "low risk" area chosen was situated on reasonably sandy soils with a slightly cooler climate. All the orchards were situated in Mpumalanga Province, South Africa. Once harvested, fruit were washed and waxed (Citrashine Pty Ltd., Johannesburg, RSA; 1 l tonne⁻¹), at the same packhouse, before being sent by courier to the University of KwaZulu Natal, Pietermaritzburg, South Africa. The fruit took up to 72 h to be delivered. On arrival, fruit of similar sizes (count 12 to 16) from each origin were divided into the respective storage treatments, with each fruit being numbered, to maintain its individuality, and weighed. Ten fruit from each consignment acted as controls, with five fruit being sampled immediately on arrival, while five fruit were allowed to ripen at 20°C. The remaining fruit were then placed into storage at 2°C, 5.5°C or 8°C for 30 days, with 10 fruit per storage temperature. After storage five fruit from each temperature treatment were weighed and sampled immediately, while five were allowed to ripen. Evaluations of fruit quality, electrolyte leakage, chlorophyll fluorescence, and internal blackening were made

before and after storage as well as after attaining “eating ripeness”. The days taken to reach “eating ripeness” were also recorded.

Internal disorders

Fruit were bisected longitudinally and immediately rated visually for mesocarp discolouration using a scale of 0 to 10, where 0 = no discolouration and 10 = 100% of cut surface area black. In addition fruit were given a score of 0 = no discolouration, 1 = discolouration present.

Chilling injury

Fruit were given a score of 0 = no external injury, 1 = external chilling injury present.

Fruit firmness

Fruit firmness was determined using a hand-held firmness tester (Bareiss, Oberdischingen, Germany). Two readings (on a scale of 100 (hard) to 0 (soft)) were taken per fruit per sampling date. Measurements were taken at the maximum circumference of the intact fruit, turning the fruit 180° after each measurement. The firmness tester measured fruit firmness by means of a metal ball (5 mm diameter) that was pressed onto the fruit. “Eating ripe” was considered to be at a reading of 50 – 55 units.

Electrolyte leakage

The leakage of electrolytes from mesocarp tissue was determined by measuring the electrical conductance of cell effusates using a modified technique of Venkatarayappa *et al.* (1984). A mesocarp plug (1 cm diameter) was taken from the cut-half of each fruit at the distal end, halfway between the seed and the exocarp. Three discs of 2 mm thickness were cut from this plug and rinsed three times in distilled water before being placed in a single boiling tube containing 25 ml distilled water. The tubes were then placed on a shaker for 3 h and the electrical conductivity (EC) measured (Initial EC) using a multi-range conductivity meter (HI 9033, Hanna Instruments, Johannesburg, RSA). The tubes were then placed in a boiling water bath for 20 min; removed and allowed to cool. The EC of each tube was again recorded (Final EC) and the percentage leakage determined as $[(\text{Initial EC}/\text{Final EC}) \times 100/1]$.

Chlorophyll fluorescence

A slice of avocado tissue (exocarp and mesocarp respectively) was taken from the distal end of each fruit. Each slice was dark adapted for 5 minutes with leafclips and the F_v/F_m ratio determined (DeEll *et al.*, 1999) after illumination (1s light bombardment at 100% light intensity) using a Plant Efficiency Analyzer (PEA) (Hansatech, Norfolk, UK).

Statistical Analysis

Analysis of variance was carried out on data using GenStat® (VSN International, Hemel Hempstead, UK), and means were compared using least significant differences (LSD's) at $P = 0.05$.

RESULTS AND DISCUSSION

Internal disorders

The internal fruit quality during 2000 was generally acceptable, compared to reports of the previous season, and very little severe mesocarp discolouration was observed (ratings < 5). Differences in fruit quality were, however, seen between the various fruit origins (Figure 1). Storage temperature also had a significant affect on mesocarp discolouration ($P = 0.05$), with the 5.5°C and 8°C treatments rendering fruit with more severe discolouration (higher ratings), although in certain cases (*viz.* "medium risk") more fruit displayed discolouration in the 2°C treatment (higher scores) (Figure 1). There were thus some doubts as to whether mesocarp discolouration was the result of storage temperatures being too low.

Electrolyte leakage

The electrolyte leakage readings in all treatments where fruit that were sampled when "eating ripeness" had been attained, irrespective of storage temperature, were generally between 85 to 100% for all the growers. This was to be expected, as membrane integrity is known to decrease as fruit ripen and senescence occurs (Thompson, 1988). This could also, in part, be the reason why electrolyte leakage was higher in the 5.5°C and 8°C treatments sampled immediately after storage for fruit from all the risk areas (Figure 2). Fruit from these treatments were softer after removal from storage and took fewer days to ripen than fruit stored at 2°C (Table I).

Chlorophyll fluorescence

External Fv/Fm

Storage was found to have a significant ($P < 0.001$) effect on external Fv/Fm ratios, with all fruit receiving storage having lower Fv/Fm ratios than unstored fruit. Generally the lower storage temperatures gave the poorer Fv/Fm values, with values decreasing further after ripening (Figure 3). This could be due to the development of external chilling injury (pitting), especially in the 2°C treatment, although pitting was also noticed in the other treatments (Figure 4). Nonetheless, the mean external Fv/Fm ratio taken immediately after all the storage treatments was only slightly less than 0.8, which is considered to represent a normal functioning photosynthetic system in green avocado skin (Smillie, 1992) and healthy leaves (Adams *et al.*, 1990). The 30-day readings did not, therefore, appear to reflect possible chilling injury development. Furthermore, the lower Fv/Fm ratios found in the 2°C treatment could well be explained by the fact that these fruit took longer to ripen (Table I) and fluorescence is known to decrease with ripening (DeEll *et al.*, 1999). It would, therefore, appear that avocado fruit can be subjected to temperatures below 5.5°C, which is considered the standard shipping temperature (Bester, 1982), without too much damage to the photosynthetic apparatus. The observed chilling injury normally found at these low temperatures could thus be due to other factors or stresses occurring during storage, such as changes in membrane permeability (Vorster *et al.*, 1987).

Avocado fruit are known to change in their sensitivity to storage temperatures as the season progresses (Swarts, 1982). It was thus not unexpected that harvest date appeared to affect the external Fv/Fm, although it was only significant for fruit from the “high risk” and “low risk” areas ($P < 0.001$). This could however, be due to the differences in chilling injury severity often observed between fruit origins (Nelson *et al.*, 2002). Nonetheless, the Fv/Fm ratios were expected to be somewhat higher later in the season, when fruit are less sensitive to low temperatures, but this was not always apparent. Furthermore, no relationship between the internal fruit quality and the external Fv/Fm ratio was found, the ratios’ importance thus only extending skin deep.

Internal Fv/Fm

Treatment and harvest date had a highly significant ($P < 0.001$) effect on the internal Fv/Fm ratios for all three risk areas. Ratios were generally found to be much lower in mesocarp tissue than exocarp tissue. A preliminary study, evaluating chlorophyll content of the various tissues, revealed that there was much less chlorophyll (especially chlorophyll *a*) in

the mesocarp tissue than in that of the exocarp (data not shown), and this was thought to contribute to the lower ratios. Woolf and Laing (1996) also associated the Fv/Fm ratio with chlorophyll content in avocado exocarp tissue. Furthermore, a visual comparison between fruit from different risk areas showed that fruit from certain orchards had a much darker green mesocarp than others, with fruit from the "low risk" area having the lightest mesocarp. Preharvest factors thus appeared to play a significant role in the evaluation of Fv/Fm ratios, as discussed in a review by DeEll *et al.* (1999). While this meant that ratios could not be directly compared between risk areas, the treatments could nonetheless be compared to the control (unstored fruit) in each area (Figure 5). The internal Fv/Fm ratio indicated that membrane integrity was better in the 8°C and 5.5°C treatments than at 2°C. This data appeared to be in conflict with the electrolyte leakage results, which indicated poorer membrane integrity and indeed worse discolouration in these treatments. However, this could again be due to the fact that the quantum yield of PS II is thought to be dependent on temperature and time (Tijskens *et al.*, 1994; DeEll *et al.*, 1999) and that fruit stored at 2°C generally took longer to ripen. The increase in the Fv/Fm later in the season perhaps again reflected the decreased sensitivity to low temperatures later in the season, as discussed previously.

In conclusion, the authors have to agree with Woolf and Laing (1996) that the health of the photosynthetic apparatus does not appear to necessarily reflect the overall health of avocado skin or mesocarp, and that the functionality of the photosynthetic apparatus may be irrelevant to the quality of avocados, notwithstanding the fact that polyphenol oxidase, the enzyme related to the browning reaction, is located in chloroplasts.

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REFERENCES

(See final reference section, pg's 129-155)

TABLE I

Number of days taken to reach "eating ripeness" (firmness 50 - 55) after storage at different temperatures for 30 d in 'Pinkerton' avocado fruit obtained from different fruit origins ("low risk", "medium risk" and "high risk"), throughout the 2000 harvest season

Risk area	Harvest date	No storage	Storage temperature (°C)		
			8	5.5	2
Low	30/05/00	16.4	6.2	6.0	9.0
	20/06/00	15.8	6.4	4.4	9.6
	06/07/00	20.2	11.8	9.2	14.0
	16/08/00	15.2	4.8	4.4	8.2
	21/08/00	11.6	3.0	5.0	8.0
LSD _(0.05) = 1.0 (Treatment); n = 5					
Medium	18/04/00	16.6	7.6	10.4	12.0
	04/05/00	18.2	7.8	8.8	12.2
	17/05/00	14.4	5.8	4.6	9.6
	30/05/00	16.8	6.8	9.4	11.0
	20/06/00	15.0	5.4	7.4	10.6
	06/07/00	19.8	10.8	12.4	13.6
LSD _(0.05) = 1.2 (Treatment); n = 5					
High	17/05/00	16.2	5.2	6.6	13.0
	30/05/00	14.4	5.6	5.2	8.0
	20/06/00	12.4	3.0	4.4	8.6
	06/07/00	16.1	8.4	7.2	9.6
LSD _(0.05) = 1.0 (Treatment); n = 5					

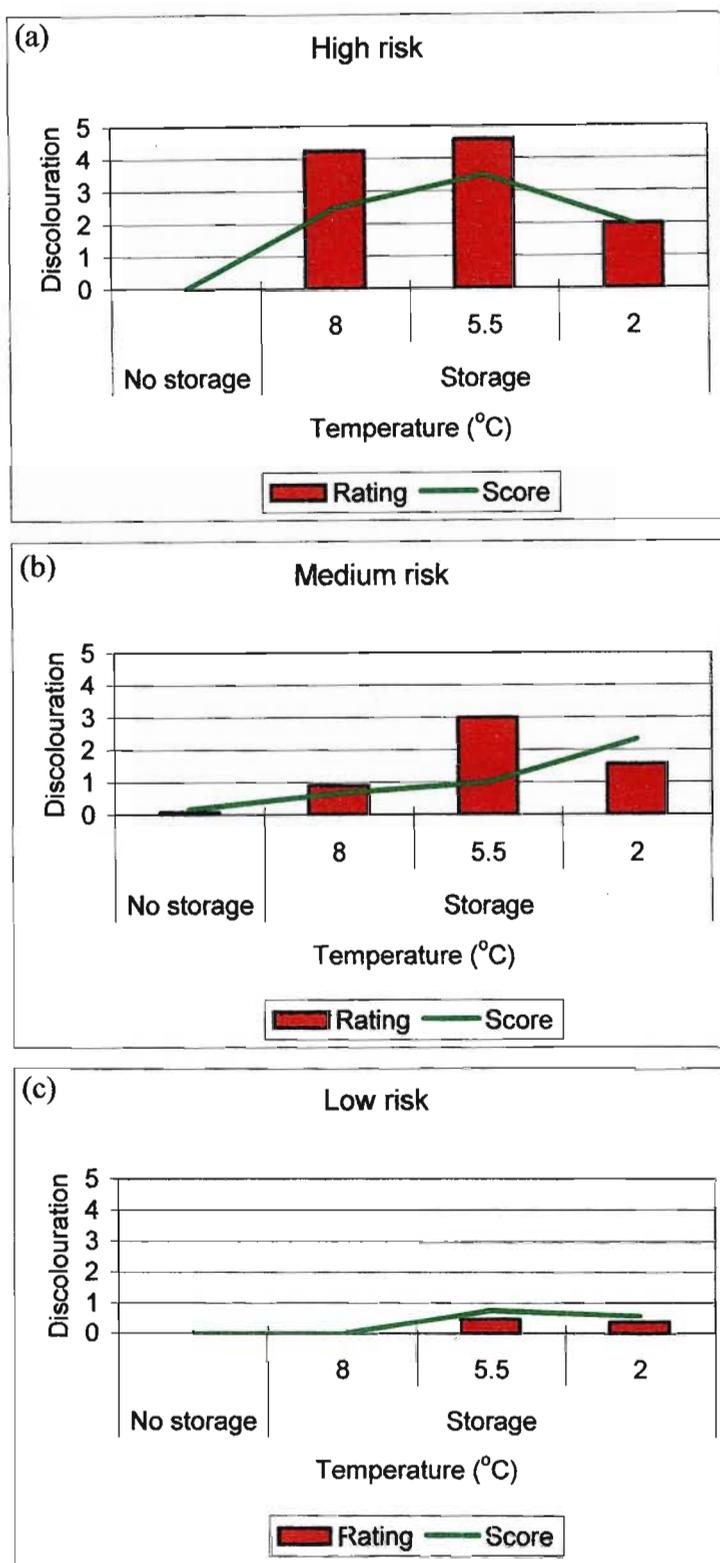


Fig. 1

Mean pooled discolouration ratings (0 = no discolouration; 10 = 100% of cut surface area black) (all dates) and mean score (no. of fruit affected) of 'Pinkerton' avocado fruit from different risk areas (a) high risk, (b) medium risk and (c) low risk, harvested throughout the 2000 season and stored at the temperatures shown.

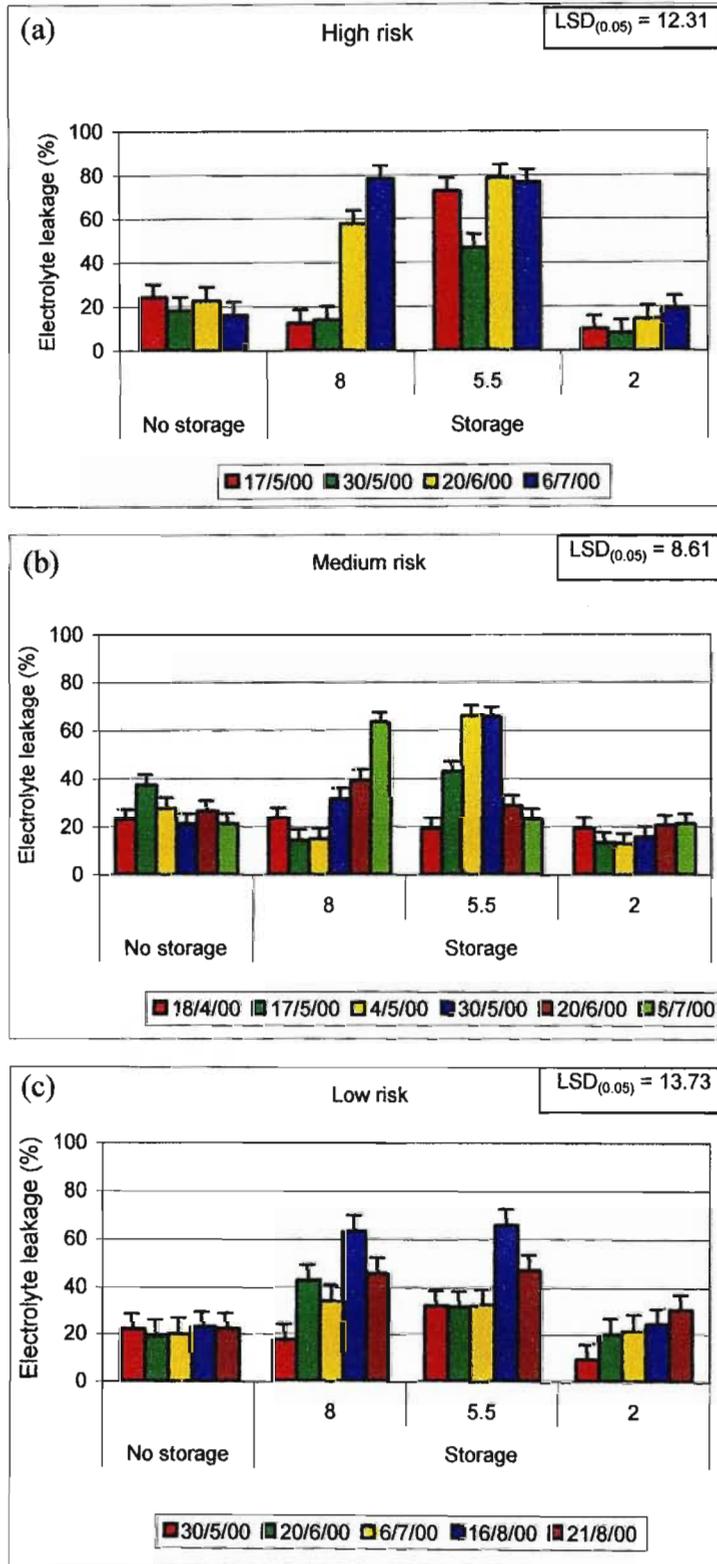


Fig. 2

Mean electrolyte leakage of 'Pinkerton' fruit from the different risk areas a) high risk, (b) medium risk and (c) low risk, throughout the 2000 season. Fruit were sampled immediately upon arrival, or after 30 d storage at the temperatures shown ($^{\circ}\text{C}$). $LSD = \text{Temperature} \times \text{Date}$; $n = 5$.

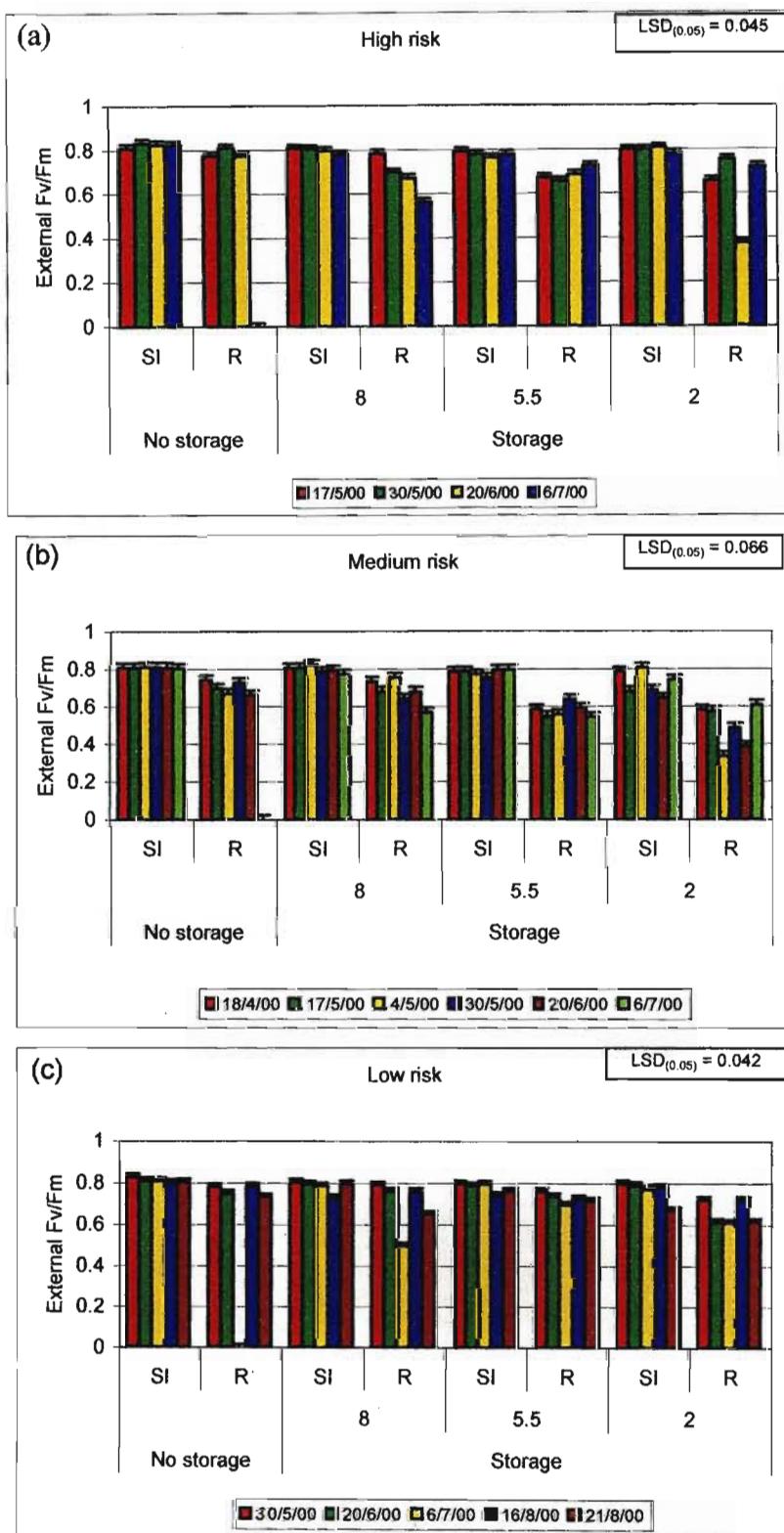


Fig. 3

Mean external Fv/Fm ratios of 'Pinkerton' fruit from the different risk areas a) high risk, (b) medium risk and (c) low risk, throughout the 2000 harvest season. Fruit were sampled without storage, or after 30 d storage at the temperatures shown (°C). SI = sampled immediately; R = allowed to ripen. LSD = Temperature; n= 5.

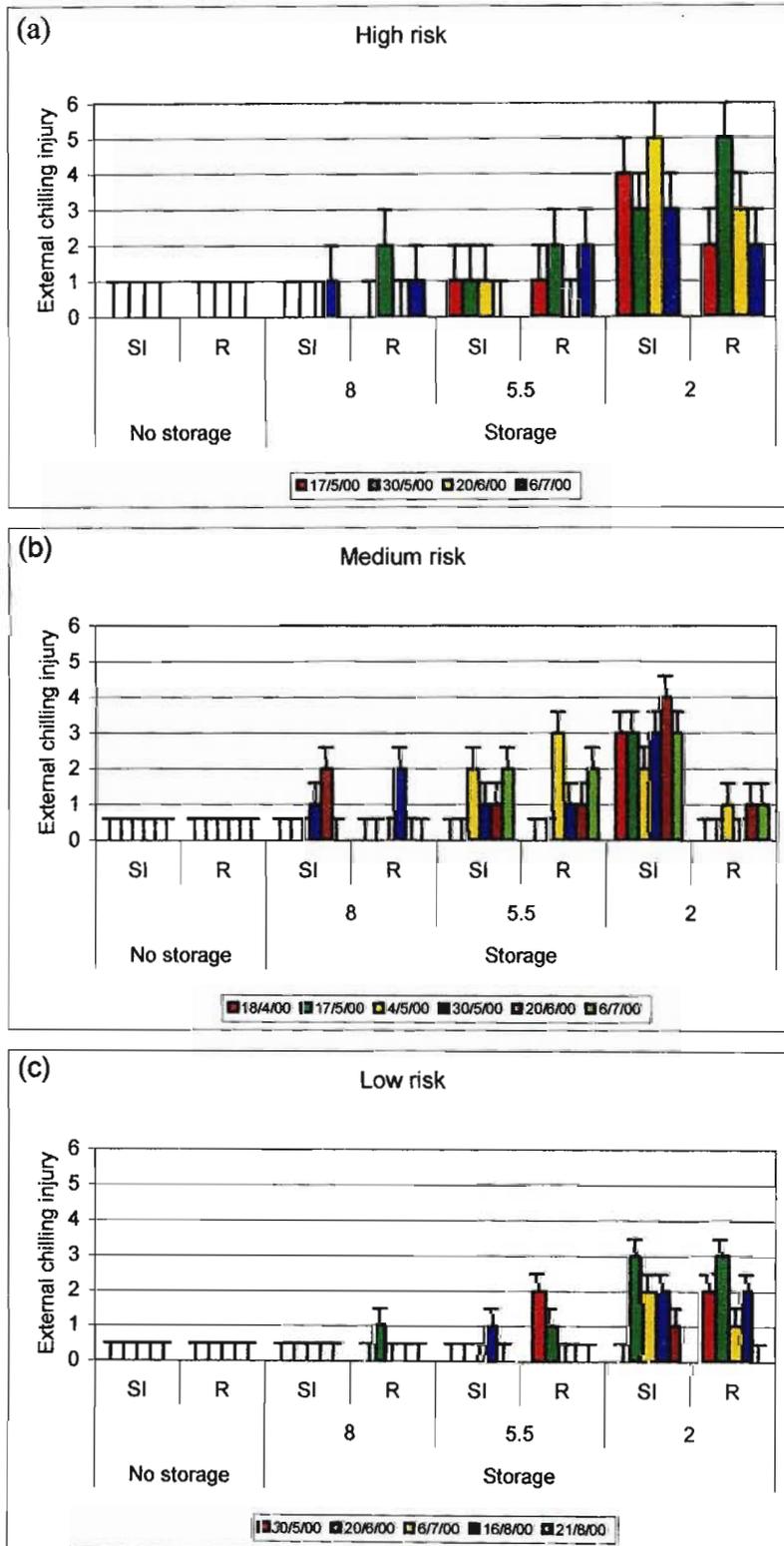


Fig. 4

Mean external chilling injury scores of 'Pinkerton' fruit from the different risk areas a) high risk, (b) medium risk and (c) low risk, throughout the 2000 harvest season. Fruit were sampled without storage, or after 30 d storage at the temperatures shown (°C). SI = sampled immediately; R = allowed to ripen. n = 5.

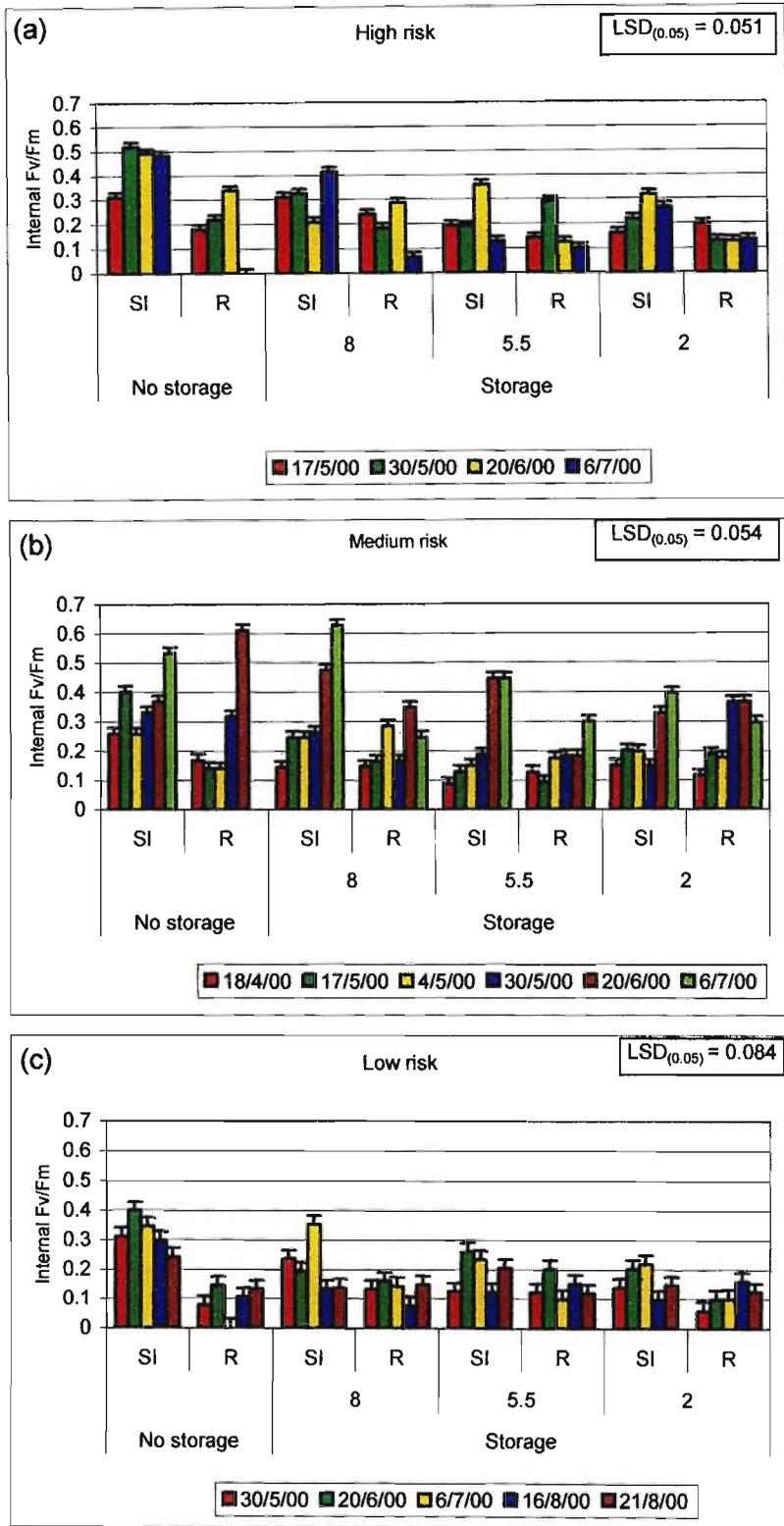


Fig. 5

Mean internal Fv/Fm ratios of 'Pinkerton' fruit from the different risk areas a) high risk, (b) medium risk and (c) low risk, throughout the 2000 harvest season. Fruit were sampled without storage, or after 30 d storage at the temperatures shown (°C). SI = sampled immediately; R = allowed to ripen. LSD = Temperature; n = 5.

CHAPTER 5

Postharvest treatments used to reduce external chilling injury in 'Pinkerton' avocado (*Persea americana* Mill.)

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SUMMARY

The results of previous research (Chapter 2) found that the poor internal quality of 'Pinkerton' avocados, which threatened the export of this cultivar, could be improved by storing fruit at 2°C. This posed a challenge to the industry as 'Pinkerton' avocado fruit are found to be sensitive to storage temperatures below 5.5°C, especially for extended periods (30 d), and fruit often develop external chilling injury. Thus, in order to market 'Pinkerton' fruit of an overall high quality a solution to the problem of external chilling injury development was needed, especially as South Africa is also constantly trying to enter new markets. Furthermore, export to some countries might require fruit to be subjected to a period of cold disinfestations at temperatures lower than 2°C in order to meet quarantine standards. In this study, it was hoped that techniques could be found to precondition fruit to low temperature storage. Preconditioning treatments consisted of fruit that were kept at either 10°C, 15°C or 20°C for 1 d or 2 d before being placed into storage for 30 d at 2°C or 5.5°C. All preconditioning treatments were compared to fruit that were placed directly into storage. The effect of fruit packaging on weight loss and chilling injury was also investigated using unwaxed fruit, commercially waxed and unwaxed fruit individually sealed in micro-perforated polypropylene bags with an anti-mist coating on the inside (polybags). While chilling injury was more severe in fruit stored at 2°C, keeping fruit at 10°C for 2 d significantly reduced the chilling injury severity, compared to control fruit placed directly into storage. The role of weight loss in chilling injury development was not always clear. Chilling injury was more severe in unconditioned fruit and fruit preconditioned for 1 d, however, fruit preconditioned at 15°C or 20°C prior to storage were found to lose the most weight, especially when

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preconditioned for 2 d. Fruit stored at 5.5°C also lost significantly more weight than fruit stored at 2°C. Unwaxed fruit lost the most weight during the preconditioning treatments and storage and polybag fruit the least, with chilling injury being more severe in waxed and then unwaxed fruit. In this study proline accumulation appeared to reflect the level of stress experienced by the fruit. Proline concentrations were the lowest in the polybag treatments, which lost the least weight during preconditioning and storage. However, while the waxed fruit did not lose the most weight during the preconditioning treatments, proline levels were higher in these fruit. As external chilling injury was also more severe in waxed fruit it is suspected that the waxed fruit may have been exposed to an additional stress, namely suffocation caused by the clogging of lenticels. The success of low temperature conditioning in reducing chilling injury may thus not be related directly to weight loss but rather to biochemical and physiological modifications induced by the treatments.

The desire to reach distant markets with avocado fruit often means that fruit are subjected to a storage period of up to 30 days. In order to control the ripening of these climacteric fruit and to ensure optimal fruit quality on arrival, these fruit are subjected to low temperatures during storage in an attempt to slow down all biological processes. Unfortunately, 'Pinkerton' fruit are susceptible to the development of certain physiological disorders during this storage period. While previous research (Chapter 2) found that the development of the disorder most threatening to the export of this cultivar (mesocarp discolouration) could be reduced by storing fruit at 2°C, thus below the industry standard of 5.5°C, the potential for damage to the fruits' exocarp was increased. Thus a solution was needed that would ensure both good internal and external quality. The ability to store fruit at very low temperatures would also increase the potential for South African fruit to be exported to new markets as some countries require cold disinfestation treatments in order to minimise the risk of insect pests entering their country. Fortunately, certain postharvest techniques have been found to alleviate low temperature injury in various chilling-sensitive commodities; these include preconditioning, heat treatments, intermittent warming, controlled atmosphere storage, waxing, film packaging, genetic modification, and applications of certain chemicals and plant growth regulators (Morris, 1982; Wang, 2001). This paper will concentrate on the effect of low temperature conditioning, waxing and fruit packaging on external chilling injury.

Low temperature conditioning involves holding cold-sensitive tissue at temperatures slightly above those at which injury occurs to induce tolerance to these normally damaging temperatures and thus delay the development of injury symptoms. This technique has been successful in reducing chilling injury in grapefruit (Hatton and Cubbedge, 1983; Chalutz *et al.*, 1985), tomato seedlings (Wheaton and Morris, 1967), tomato fruit (Saltveit, 1991), papaya (Chen and Paull, 1986), zucchini squash (Wang, 1994), and more recently in avocados (Woolf *et al.*, 2003; Hofman *et al.*, 2003). Adaptation to lower temperatures is thought to be the result of various biochemical and physiological modifications induced by the conditioning treatment.

Chilling injury can also be prevented in many crops by reducing moisture loss from tissues (Ben-Yehoshua *et al.*, 1983a; Wang, 1990), with conditions of high relative humidity being thought to inhibit the collapse of the epidermal and underlying cells. High relative humidity, in the atmosphere around commodities, can generally be achieved by waxing fruit or by using film packaging. Waxing and film packaging are also thought to increase the carbon dioxide (CO₂) and decrease the oxygen (O₂) concentrations of the internal atmosphere during storage (Durand *et al.*, 1984). These factors are believed to contribute to

the reduction in chilling injury in many crops (Wang, 1993). In avocados the use of either waxing (Lunt *et al.*, 1981) or film packaging (Eksteen and Truter, 1985; Wang, 1993; Bower and Jackson, 2003) has been successful in reducing chilling injury, although, Bower and Magwaza (2004) found that polypropylene packaging in 'Fuerte' avocados was more effective in reducing external chilling injury than waxing.

The amino acid proline is thought to be a non-specific indicator of stress as it accumulates in plants after they have been exposed to different stresses (Aspinall and Paleg, 1981). The exact mechanism whereby proline accumulates under stress, and the precise role of proline accumulation has not been unequivocally determined to date. Proline accumulation is argued by some researchers to be advantageous to a plant as far as stress tolerance is concerned (Singh *et al.*, 1973). For example, grapefruit, which have accumulated relatively high concentrations of carbohydrates and proline, have been found to be less likely to develop chilling injury caused by low, non-freezing temperatures (Purvis, 1981; Purvis and Grierson, 1982). Conversely, some researchers argue that proline accumulation is simply an indication of the damage suffered by the plant during stress conditions (Hanson *et al.*, 1979).

Objectives of this study were (a) to identify postharvest treatments that would lead to the successful storage, in terms of overall fruit quality, of 'Pinkerton' avocados, with special emphasis on the reduction of external chilling injury; (b) to identify the degree and kind of stress (moisture vs temperature) placed on the fruit by the various postharvest treatments; and (c) to elucidate the role of proline concentrations in avocado stress physiology.

MATERIALS AND METHODS

Plant material and treatments

'Pinkerton' avocado fruit (*Persea americana* Mill.) were obtained from a grower near Wartburg in KwaZulu Natal (29°27'S, 30°40'E) on 03/08/04 and 23/08/04. One third of the fruit were commercially waxed at the packhouse (Canuaba Tropical, Sasol Waxes, RSA; 0.71 £ tonne⁻¹ fruit), one third were left unwaxed, and the last third were left unwaxed and individually heat-sealed in 30 µm thick polypropylene bags with 9 µm perforations and an anti-mist coating on the interior (Polylam Packaging, Johannesburg, RSA) on arrival at the University of KwaZulu Natal (6-8 h after harvest). Prior to waxing, fruit were placed in a fungicide dip (Sporekill, Hygrotech, Pietermaritzburg, RSA; 0.25 £ 100 £⁻¹ water). On arrival fruit were divided into the respective treatments, labelled, weighed and visually assessed for any external blemishes, with ten individual fruit replications per treatment (5 fruit being sampled immediately after a treatment and 5 allowed to ripen). Treatments consisted of fruit

preconditioned at 10, 15 or 20°C for 1 or 2 d before being placed in storage at either 2°C or 5.5°C. Fruit in the control treatments were not preconditioned in any way and were placed either directly into storage at 2°C or 5.5°C, sampled immediately or left to ripen at 20°C. The maturity of the fruit, as determined by moisture content, was determined on arrival. The polybags were removed once the fruit were removed from storage, to allow for ripening. After each treatment stage (*viz.* preconditioning, storage or ripening) the fruit were weighed, fruit firmness was determined and fruit were visually assessed for any signs of external chilling injury or anthracnose (*Colletotrichum gloeosporioides* Penz.). After cold storage 5 fruit per treatment were removed for destructive analysis to see if any mesocarp discolouration was present, while the remaining fruit were allowed to reach “eating ripeness”. The number of days taken to reach “eating ripeness” was recorded for all treatments. On sampling the exocarp of each fruit was cut into small pieces (1 cm²) and flash frozen in liquid nitrogen before being placed in a freezer until further analysis could be conducted.

Maturity

The maturity of each consignment was ascertained on arrival by determining the moisture content (Kruger *et al.*, 1995) of a sample of mesocarp tissue (20 g). The tissue was cut into small pieces (1 cm³) and immersed in liquid nitrogen. Once frozen, the samples were placed on a freeze drier for 5 d. This was determined to be sufficient time to remove moisture and attain constant weight.

External chilling injury

Chilling injury was assessed by giving the external black discolouration (pitting) a visual rating using a scale of 0 to 10, with 0 = no visible discolouration and 10 = 100% of surface area black.

Anthracnose and mesocarp discolouration

The presence of anthracnose was given a score of 0 = no infection and 1 = some infection. For mesocarp discolouration ratings fruit were bisected longitudinally and immediately rated using a visual scale of 0 to 10, with 0 = no visible discolouration and 10 = 100% of cut surface area black.

Fruit firmness

“Eating ripeness” was determined using a hand-held firmness tester (Bareiss, Oberdischingen, Germany). Two readings (on a scale of 100 (hard) to 0 (soft)) were taken per fruit. Measurements were taken at the maximum circumference of the fruit, turning the fruit 180° after each measurement. The fruit firmness tester measures firmness by means of a metal ball (diameter 5 mm) that is pressed onto the fruit. “Eating ripe” was considered to be at a reading of 50 – 55 units.

Proline analysis

Avocado exocarp tissue was analysed for proline concentration using a modified method of Bates *et al.* (1973). For each fruit sample (analysed in triplicate) 1 g frozen avocado exocarp was cut into very small pieces before being homogenised in 10 ml 3% sulphosalicylic acid using an Ultra-turrax T25 (Janke and Jackson, Staufen, Germany). Samples were then filtered through Whatman® No. 1 filter paper and the supernatant/filtrate collected. Two ml of the filtrate was then combined with 2 ml acid-ninhydrin and 2 ml glacial acetic acid. The samples were incubated for 1 h in a boiling waterbath, and the reaction terminated on ice. Four ml toluene was added to the reaction mixture and vortexed for 15 s. Time was allowed for the toluene to separate from the aqueous phase, before the toluene phase was extracted. The absorbance of the samples was determined at 520 nm using a Beckman Coulter DU 800 spectrophotometer (Fullerton, California, USA). The proline concentration was determined using a standard curve, using L-proline (Sigma) as standard. The μmole proline per g of fresh weight sample was determined using the formula $[(\mu\text{g proline/ml} \cdot \text{ml toluene})/115.13 \mu\text{g}/\mu\text{mole}]/[\text{g of sample}/5]$ (Bates *et al.*, 1973). All the fruit that were sampled came from treatments that were terminated immediately after storage at 2°C and consisted of 5 single fruit replications per treatment.

Statistical Analysis

Data was subjected to analysis of variance (ANOVA) using the GenStat® statistical package (VSN International Ltd, Hemel Hempstead, UK). Least significant difference (LSD) was used to separate treatment means. Due to the existence of many significant interactions (between factors) all the results are displayed in tables indicating the various interactions and their significance. Proline data were subjected to multiple linear regressions.

RESULTS

Maturity

The mean moisture content of fruit harvested on 03/08/04 was 76.8% and those harvested on 23/08/04 was 75.5%.

Preconditioning weight loss

The unwaxed and waxed fruit lost significantly ($P < 0.001$) more weight, during the preconditioning treatments, than the fruit individually sealed in polybags, irrespective of temperature, length of conditioning or harvest date (Table I). Keeping the fruit at higher temperatures (*viz.* 15°C or 20°C) and for greater periods (2 d) resulted in increased weight loss. Preconditioning the fruit at 10°C and/or placing the fruit in polybags was thus the most successful in terms of reducing weight loss prior to storage.

Storage weight loss

For both harvest dates the unwaxed fruit lost the most weight during storage followed by the waxed fruit (Table II). Fruit stored at 5.5°C lost significantly ($P < 0.001$) more weight during storage than fruit stored at 2°C. The relative humidity in the 2°C container was 80-85% while that in 5.5°C was at around 75-80%. As the polybags were only removed from the fruit after storage, the difference between storage and preconditioning weight loss could not be determined. Nevertheless, polybag fruit still lost less than 1% of their original fruit weight by the time they were removed from storage. This was attributed to the presence of some free water in the bags after storage, and to readings of a 100% relative humidity within the bags. Small differences in weight loss were seen between the preconditioning treatments and a significant interaction was found between fruit packaging, preconditioning temperature and storage temperature on the weight loss during storage. During the 30 d storage period the control fruit and fruit preconditioned at 10°C lost more weight than fruit from other treatments (Table II).

Ripening weight loss

Fruit originally sealed in polybags, lost the most weight during the ripening period compared to the other fruit packaging treatments (Table III). The unconditioned/control fruit and fruit preconditioned for 1 d, irrespective of temperature, lost significantly ($P < 0.001$) more weight than fruit receiving 2 d preconditioning. Furthermore, fruit stored at 2°C lost significantly ($P < 0.001$) more weight during ripening than fruit stored at 5.5°C (Table III).

Total weight loss

For both harvest dates, the total weight loss of fruit sampled immediately after storage revealed that fruit packaging and storage temperature had a significant ($P < 0.001$) effect on weight loss (Table IV) with unwaxed fruit losing considerably more weight than polybag fruit, and fruit stored at 2°C losing less weight than those stored at 5.5°C. Prior to ripening, weight loss was also significantly affected ($P < 0.001$) by the length of preconditioning, with weight loss being greater in the 2 d treatments. More weight was lost during storage (Table II) than during the actual preconditioning treatments (Table I). After attaining "eating ripeness" the differences in weight loss, between the fruit packaging and preconditioning treatments, while being significant ($P < 0.001$) were less dramatic (Table V). The final weight loss figures were significantly affected ($P = 0.05$) by interactions between all the postharvest treatment factors.

Days to ripening

Fruit stored at 2°C, regardless of fruit packaging or harvest date, took significantly ($P < 0.001$) longer to ripen than fruit stored at 5.5°C (Table VI). Fruit packaging also significantly ($P < 0.001$) affected the number of days taken to ripen, with the waxed fruit, stored at 2°C, taking the longest to ripen, for both harvest dates, followed by fruit originally sealed in polybags (Table VI). However, in the 5.5°C storage treatments the fruit sealed in polybags took the longest to ripen after the first harvest date (03/08/04), although the waxed fruit appeared to take longer after the second harvest date (23/08/04) in some treatments. Harvest date was found to significantly affect ($P < 0.001$) on days taken to ripen with fruit harvested on 23/08/04 taking less time to ripen than fruit harvested on 03/08/04. The preconditioning treatments were also found to significantly affect the days taken to ripening (Table VI). In summary, however, the days taken to reach "eating ripeness" were significantly affected by a number of high order interactions between the postharvest treatments (Table VI). Nevertheless, it appeared that weight loss played a significant role in ripening time. The unwaxed fruit consistently lost the most weight during preconditioning and storage and also ripened the fastest, and similarly the fruit stored at 5.5°C lost more weight and subsequently took less time to ripen.

Fruit firmness

Fruit firmness, immediately after storage, was affected by significant ($P < 0.001$) interactions between the various treatment factors (Table VII). Nevertheless, unwaxed fruit

appeared the least firm and polybag fruit the most firm. After storage at 5.5°C all fruit (except the polybag fruit) were less firm than those stored at 2°C.

External chilling injury

During both harvest dates, regardless of treatment, the external chilling injury severity immediately after storage (Table VIII) or after ripening (Table IX) was never found to exceed 4, out of a possible rating of 10. Nevertheless, the severity of external chilling injury was found to be higher in fruit stored at 2°C than at 5.5°C. Preconditioning treatments significantly affected chilling injury severity, with the lowest ratings being found in the 2 d preconditioning at 10°C treatments, whether fruit were stored at 2°C or 5.5°C. For both harvest dates the waxed fruit appeared to be more severely affected by chilling injury and pitting was often observed around the lenticels of the fruit (Figure 1). Storing fruit in polybags significantly reduced chilling injury, however this was negated to a certain extent by a higher incidence of fungal infections.

Mesocarp discolouration

Very little mesocarp discolouration was observed in this study, and ratings out of 10 never exceeded 3, with an average below 1 (Table X). Mesocarp discolouration ratings were significantly ($P = 0.05$) affected by interactions between the various treatment factors (Table X). Fruit packaging had a significant affect ($P < 0.001$) on mesocarp discolouration, with waxed fruit showing the highest incidence of discolouration.

Anthraco

Anthraco scores were affected by significant interactions between treatment factors (Table XI). A higher incidence of anthracose infection was found at 2°C than in fruit stored at 5.5°C. The waxed and polybag fruit were more severely affected than the unwaxed fruit. However, the fungal lesions in the polybag fruit did not initially resemble the "typical" anthracose symptoms. Immediately after storage (i.e. while fruit were hard) the exocarp of fruit sealed in polybags had large dull black areas, with smooth boundaries, and these were generally concentrated on the lower half of the fruit (Figure 2). As the fruit ripened these areas on the exocarp started to collapse, resembling chilling injury/pitting (Figure 3). Removal of the exocarp immediately after storage showed that the mesocarp tissue beneath the "infected" exocarp was not always affected (Figure 4). As gases, such as carbon dioxide, were found to pass readily through the perforations in the bag, and a possible plasticiser

effect was ruled out, we feel confident that these black areas were in fact the result of a fungal infection. This is also supported by microscopic studies, which revealed the presence of fungal hyphae on the exocarp of the fruit. Furthermore, the typical symptoms of a fungal infection (Figure 5) started to develop as the fruit ripened and the affected areas collapsed, with the infection extending into the mesocarp tissue beneath these areas. Thus, for the sake of simplification we have scored this fungal infection under anthracnose.

Proline analysis

Proline analysis of fruit exocarp, sampled immediately after storage at 2°C, revealed that preconditioning, harvest date, fruit packaging and interactions between these factors had significant effects ($P < 0.001$) on proline concentrations (Table XII). Proline concentrations were significantly higher ($P < 0.001$) in fruit harvested on 23/08/04. During both harvest dates waxed fruit, receiving no preconditioning treatments or 1 d preconditioning, had the highest proline concentrations and fruit sealed in polybags the lowest. However, when fruit were subjected to preconditioning for 2 d the waxed fruit had the lowest proline concentrations and the unwaxed fruit, for the most part, had the highest. Overall, the 2 d preconditioning treatment at 10°C rendered fruit with the lowest proline concentrations (Table XII). Regression analysis revealed that chilling injury and preconditioning weight loss contributed the most to proline concentrations ($P < 0.001$), followed by total weight loss (immediately after storage) ($P = 0.05$). However, together these factors only accounted for 21% of the variance. When the analysis was divided into fruit packaging treatments the significance of chilling injury, preconditioning and total weight loss remained significant for the unwaxed and waxed fruit, but only the presence of chilling injury was found to contribute to proline concentrations in the polybag fruit. The addition of preconditioning time, harvest date and fruit packaging treatment to the analysis accounted for 40% of the variance.

DISCUSSION

Both the preconditioning treatments and the fruit packaging treatments had a significant effect on the weight loss and external chilling injury severity of 'Pinkerton' avocado fruit. In terms of weight loss, the 2 d storage delay, with fruit held at either 15°C or 20°C resulted in the greater weight loss. This was to be expected, as was the greater weight loss of unwaxed fruit, and fruit stored at 5.5°C compared to 2°C. The lower relative humidity in the 5.5°C container was thought to create a greater water vapour pressure deficit between the fruit and air within this container, resulting in greater weight loss. Minimising weight loss prior

to storage, and during storage, is thought to be crucial to sustaining membrane integrity and thus the optimal functioning of cells (Wang, 1993). Maintaining a low water vapour pressure deficit during storage is therefore crucial in minimising weight loss during this time, as supported by the insignificant weight loss of fruit sealed in polybags during storage. Throughout the study the chilling injury severity was the lowest in fruit sealed in polybags during preconditioning and storage.

However, care should be taken when storing fruit at a relative humidity close to 100%, as this condition is favourable to the spread and growth of pathogens. The accumulation of free water in the polybags could have been limited to a certain extent by modifying the technique used to apply the polybags; for example, keeping the bags and fruit in the cold rooms for a set time period to equilibrate before applying the bags, and/or by placing an absorptive material at the bottom of the bag. Ensuring timely fungicide applications both during the season and after harvest can also decrease the presence of pathogenic fungi on the fruit. In this study, the unwaxed (and therefore polybag) fruit were not put through the normal packhouse treatment and did not, therefore, receive a fungicide dip prior to storage. The results do, however, indicate that this would not have solved the problem completely as the waxed fruit were also affected by anthracnose infections. The higher incidence of anthracnose infections in fruit stored at 2°C was thought to be related to the increased number of days taken to reach "eating ripeness" after storage at 2°C (Table VI), as found by Eksteen and Truter (1985).

Weight loss after storage appeared to have no effect on chilling injury severity as the polybag fruit lost significantly more weight during ripening (Table III), and at a greater rate, as reflected by the similar number of days, to waxed fruit, taken to reach "eating ripeness" (Table VI) while still developing very little chilling injury. Furthermore, no significant differences were found in chilling injury ratings between fruit sampled immediately after storage (Table VIII) and fruit allowed to ripen (Table IX). The higher weight loss during ripening, of the fruit originally sealed in polybags, also appeared to indicate that 'Pinkerton' avocado fruit need to lose a certain amount of weight in order to attain "eating ripeness".

Chilling injury could not be attributed solely to weight loss, as the waxed fruit in this study were the most significantly affected by chilling injury despite the fact that the unwaxed fruit lost more weight prior to ripening. The higher incidence of chilling injury in the waxed fruit could have been caused by either the thickness or type of the wax not being optimal for very low temperature storage (i.e., below the 5.5°C standard) (Johnston and Banks, 1998). In fact, Bower *et al.* (2003) found that the type of fruit packaging used in avocados significantly

affected the incidence of external chilling damage. Furthermore, some studies have indicated that waxing can affect the gaseous exchange of fruit either through the incorrect thickness of the wax or by the clogging of stomatal pores (Ben-Yehoshua *et al.*, 1983b). The method of wax application can also be detrimental to fruit quality. In avocados lenticels may become damaged if the brushes used in the application of the wax are too hard. This could account for chilling injury symptoms often being more prevalent around the lenticels of the fruit (Figure 1). The high proline levels in the waxed fruit, which were placed either directly into storage or preconditioned for 1 d prior to storage at 2°C, seem to suggest that these fruit were experiencing an additional stress other to that inflicted by weight loss. The unwaxed fruit consistently lost the most weight during preconditioning and storage and would thus be expected to exhibit the highest proline concentrations, which was not always the case (Table XII).

The low chilling injury ratings of the polybag treatments, accompanied by low proline concentrations, appear to support the theory that proline concentrations may well reflect the extent of damage caused by a stress (Hanson *et al.*, 1979) rather than be used as a predictor of stress tolerance. Thus, the low stress levels experienced by the polybag fruit prior to storage may well have enabled the fruit to better withstand the low-temperature storage (2°C). The degree of hydration in plant membranes is known to affect membrane fluidity. In general, it is found that when the cell water percentage falls below a certain level, membranes lose the ability to maintain homeostatic viscosity and this in turn may affect the thermodynamic stability of the membrane (Nilsen and Orcutt, 1996). However, the role of weight loss in chilling injury development was not always clear in this study, and it is possible that the reduced chilling injury might well have been the result of other biochemical and physiological modifications induced by the conditioning treatments. These changes could include increases in the degree of unsaturation of fatty acids in the membranes, in response to temperature conditioning, which would in turn affect membrane fluidity and permeability. Preconditioning treatments have also been found to affect the sugar content of plant tissues (Purvis, 1990). Bower and Jackson (2003) found that carbon dioxide evolution rates were lower in fruit sealed in polybags during storage, than in unwaxed and waxed fruit. Over time this was suspected to result in a decrease in the respiratory requirement for carbohydrates during storage thus possibly leading to a more controlled rate of energy consumption, which would in turn enable the fruit to tolerate the stress induced by low temperature storage.

CONCLUSION

The high level of interactions between the various postharvest treatments, fruit packaging treatments and harvest dates clearly illustrate that avocados are living organisms and as such cannot be handled uniformly throughout the season. In fact, postharvest care starts as soon as the fruit are harvested. An understanding of how postharvest conditions affect fruit quality, in terms of external chilling injury, will therefore enable the manipulation of these factors in order to ensure optimal fruit quality, after storage at 2°C, and possibly allow for fruit to be entered into new markets where a period of cold disinfestation is required to meet quarantine standards. Low temperature preconditioning treatments show great potential in allowing fruit to be stored at very low temperatures while maintaining high fruit quality, thus further studies should try to elucidate how preconditioning treatments acclimatise avocado fruit to these conditions. This would possibly enable the manipulation, or at least management, of these factors preharvest; for example, determining the effect of the fatty acid saturation of membranes on chilling development. Preharvest temperatures are thought to influence the degree of lipid saturation and might help in identifying which growing areas are more suitable to low storage temperatures of 2°C. In fact, storage temperatures lower than 2°C might need to be attained in order to guarantee pest eradication and thus additional work needs to be done on establishing what this temperature is. The effect of waxing on chilling development also needs further investigation as the formulation and thickness of the wax application may be easier to manipulate in the short term. Furthermore, the method of application, in the packhouse, may need to be slightly modified in accordance with the type of wax used. The use of micro-perforated polypropylene pallet wraps during storage also needs to be considered as this may prove to be more practical in terms of dealing with large fruit numbers.

REFERENCES

(See final reference section, pg's 129-155)

TABLE I

Weight loss of unwaxed, waxed and fruit sealed in micro-perforated polypropylene bags, harvested on 03/08/04 or 23/08/04, during the respective preconditioning treatments

Preconditioning time (d)	Preconditioning temperature (°C)	Weight loss (%)					
		03/08/04			23/08/04		
		Unwaxed	Waxed	Polybag	Unwaxed	Waxed	Polybag
1	10	0.46	0.33	0.08	0.31	0.21	0.09
	15	0.99	0.76	0.12	1.26	0.81	0.08
	20	1.22	0.97	0.13	1.12	0.87	0.07
2	10	0.64	0.52	0.12	0.60	0.40	0.14
	15	2.07	1.83	0.20	2.08	1.71	0.22
	20	2.59	1.92	0.28	2.01	1.49	0.21

Date = 0.04**

Packaging = 0.05**

Preconditioning time = 0.04**

Preconditioning temperature = 0.05**

Packaging x Date = 0.05*

Packaging x Preconditioning time = 0.07**

Date x Preconditioning time = 0.06**

Date x Preconditioning temperature = 0.07**

Packaging x Preconditioning temperature = 0.09**

Preconditioning temperature x Preconditioning time = 0.07**

Packaging x Date x Preconditioning temperature = 0.12**

Packaging x Date x Preconditioning time = 0.07*

Packaging x Preconditioning temperature x Preconditioning time = 0.12**

Date x Preconditioning temperature x Preconditioning time = 0.10**

Packaging x Date x Preconditioning temperature x Preconditioning time = 0.13*

* = significant (LSD_{0.05}); ** = significant (LSD_{0.001}); n = 10

TABLE II

Weight loss of unwaxed, waxed and fruit sealed in micro-perforated polypropylene bags, harvested on 03/08/04 or 23/08/04, during storage at 2°C or 5.5°C (30 d) and after exposure to the various preconditioning treatments

Precon. time (d)	Precon. temperature (°C)	Storage temperature (°C)	Weight loss (%)					
			03/08/04			23/08/04		
			Unwaxed	Waxed	Polybag	Unwaxed	Waxed	Polybag
0	none	2	5.0	3.6	0.5	4.6	3.3	0.4
		5.5	7.6	5.9	0.4	7.1	5.9	0.3
1	10	2	4.7	3.6	0.4	4.3	3.1	0.4
			4.1	3.7	0.5	4.3	3.0	0.5
			4.6	3.3	1.0	4.0	3.1	0.4
	15	5.5	7.7	5.6	0.4	6.9	5.2	0.4
			7.1	5.6	0.5	6.5	5.0	0.5
			7.0	5.2	0.6	6.6	5.3	0.5
2	10	2	5.3	3.3	0.4	4.9	3.2	0.4
			4.4	3.3	0.8	4.0	3.4	0.5
			4.1	2.9	0.7	4.2	3.2	0.6
	15	5.5	7.6	5.7	0.8	7.2	5.5	0.4
			6.8	5.2	0.6	6.9	4.3	0.5
			6.6	5.0	0.8	6.6	4.3	0.6

Date = 0.1**

Packaging = 0.1**

Storage temperature = 0.1**

Preconditioning temperature = 0.2**

Packaging x Preconditioning time = 0.2**

Packaging x Storage temperature = 0.2**

Preconditioning temperature x Storage temperature = 0.2**

Preconditioning temperature x Preconditioning time = 0.2*

Preconditioning time x Storage temperature = 0.1*

Packaging x Preconditioning temperature x Storage temperature = 0.3*

* = significant (LSD_{0.05}); ** = significant (LSD_{0.001}); n = 10

TABLE III

Weight loss of unwaxed, waxed and fruit sealed in micro-perforated polypropylene bags, harvested on 03/08/04 or 23/08/04, during ripening (after preconditioning and storage at 2°C or 5.5°C (30 d))

Precon. time (d)	Precon. temperature (°C)	Storage temperature (°C)	Weight loss (%)					
			03/08/04			23/08/04		
			Unwaxed	Waxed	Polybag	Unwaxed	Waxed	Polybag
0	none	2	8.7	10.2	9.5	8.6	8.0	9.0
		5.5	5.4	4.8	7.5	6.9	6.7	7.7
1	10	2	7.3	8.8	8.4	7.0	7.3	9.9
			7.2	7.9	9.8	9.6	7.8	10.2
			8.2	8.8	11.3	8.2	7.7	9.0
	10	5.5	5.1	4.9	8.6	5.6	4.6	6.8
			5.4	6.1	9.0	5.5	4.7	7.9
			4.9	4.2	9.8	6.0	5.8	8.0
2	10	2	5.7	6.3	8.5	7.2	6.2	8.0
			6.6	9.2	9.3	5.4	6.6	8.9
			6.7	7.6	9.0	7.5	6.8	9.2
	10	5.5	4.5	4.7	6.7	5.0	5.3	7.7
			4.3	4.7	7.3	4.7	4.0	6.8
			4.7	5.1	6.4	5.1	4.8	8.0

Packaging = 0.3**

Storage temperature = 0.3**

Preconditioning time = 0.3**

Preconditioning temperature = 0.4**

Packaging x Date = 0.6**

Packaging x Storage temperature = 0.5**

Date x Storage temperature = 0.4**

Preconditioning temperature x Storage temperature = 0.4*

Date x Preconditioning temperature x Preconditioning time = 0.7*

Packaging x Date x Storage temperature = 0.5*

Date x Preconditioning temperature x Storage temperature = 0.6*

Date x Packaging x Preconditioning temperature x Preconditioning time = 1.3*

Packaging x Preconditioning temperature x Preconditioning time x Storage temperature = 1.3*

Date x Preconditioning temperature x Preconditioning time x Storage temperature = 1.0*

Packaging x Date x Precon temperature x Precon time x Storage temperature = 1.8*

* = significant ($LSD_{0.05}$); ** = significant ($LSD_{0.001}$); n = 5

TABLE IV

Total weight loss of unwaxed, waxed and fruit sealed in micro-perforated polypropylene bags, harvested on 03/08/04 or 23/08/04, after exposure to various preconditioning treatments, and storage at 2°C or 5.5°C (30 d)

Precon. time (d)	Precon. temperature (°C)	Storage temperature (°C)	Weight loss (%)					
			03/08/04			23/08/04		
			Unwaxed	Waxed	Polybag	Unwaxed	Waxed	Polybag
0	none	2	5.3	3.4	0.5	4.5	3.1	0.4
		5.5	7.7	5.7	0.4	7.2	4.7	0.4
1	10	2	5.5	4.1	0.4	4.5	3.5	0.4
			5.0	5.0	0.5	5.8	3.9	0.5
			5.7	3.9	1.4	5.5	3.9	0.5
	15	5.5	7.6	6.1	0.5	7.1	6.0	0.4
			8.5	7.0	0.5	7.9	6.9	0.5
			7.4	6.6	0.6	7.2	6.4	0.5
			20	7.4	6.6	0.6	7.2	6.4
2	10	2	6.1	4.4	0.4	5.7	4.1	0.4
			6.9	4.5	0.7	6.1	5.0	0.6
			6.4	4.9	0.7	5.9	4.9	0.6
	15	5.5	8.3	5.8	0.6	8.4	6.4	0.5
			9.6	6.3	0.6	9.0	5.8	0.5
			9.1	6.5	0.8	8.1	5.6	0.6

Date = 0.2**

Packaging = 0.2**

Storage temperature = 0.2**

Preconditioning time = 0.2**

Preconditioning temperature = 0.2**

Packaging x Preconditioning temperature = 0.4**

Packaging x Preconditioning time = 0.3**

Packaging x Storage temperature = 0.3**

Preconditioning temperature x Storage temperature = 0.2*

Packaging x Preconditioning time x Storage temperature = 0.4*

* = significant ($LSD_{0.05}$); ** = significant ($LSD_{0.001}$); n = 5

TABLE V

Total weight loss of unwaxed, waxed and fruit sealed in micro-perforated polypropylene bags, harvested on 03/08/04 or 23/08/04, after preconditioning, storage at 2°C or 5.5°C (30d) and ripening

Precon. time (d)	Precon. temperature (°C)	Storage temperature (°C)	Weight loss (%)					
			03/08/04			23/08/04		
			Unwaxed	Waxed	Polybag	Unwaxed	Waxed	Polybag
0	none	2	13.4	14.0	9.9	13.3	11.5	9.3
		5.5	13.0	11.0	8.0	13.8	13.9	8.1
1	10	2	12.3	12.8	8.8	11.8	10.7	10.2
			12.5	12.2	10.3	15.2	11.5	10.7
			13.7	13.3	12.0	13.1	11.7	9.4
	15	5.5	13.8	11.1	9.0	13.6	10.1	7.2
			14.7	12.1	9.4	13.6	9.5	8.3
			13.7	10.0	10.5	14.4	11.9	8.4
2	10	2	12.2	10.2	8.9	12.7	9.9	8.4
			13.3	15.1	10.1	11.2	11.9	9.4
			13.6	12.5	9.8	14.0	11.7	9.8
	15	5.5	13.0	11.9	7.6	12.7	11.0	8.1
			13.0	12.1	7.9	14.2	9.7	7.3
			13.1	12.6	7.3	14.2	11.0	8.5

Date = 0.3*

Packaging = 0.4**

Storage temperature = 0.4**

Preconditioning temperature = 0.5**

Packaging x Date = 0.5*

Packaging x Storage temperature = 0.6**

Packaging x Preconditioning time = 0.6*

Date x Storage temperature = 0.4*

Date x Preconditioning temperature x Storage temperature = 0.8*

Date x Preconditioning temperature x Preconditioning time x Storage temperature = 1.4*

Packaging x Preconditioning temperature x Preconditioning time x Storage temperature = 1.7*

* = significant ($LSD_{0.05}$); ** = significant ($LSD_{0.001}$); n = 5

TABLE VI

Days taken to reach "eating ripeness" of unwaxed, waxed and fruit sealed in micro-perforated polypropylene bags, harvested on 03/08/04 or 23/08/04, after preconditioning and storage at 2°C or 5.5°C (30 d)

Precon. time (d)	Precon. temp (°C)	Storage temp (°C)	Days taken to ripen					
			03/08/04			23/08/04		
			Unwaxed	Waxed	Polybag	Unwaxed	Waxed	Polybag
0	none	2	11.0	13.6	10.2	7.6	10.8	9.0
		5.5	5.0	5.2	6.8	6.2	7.0	6.4
1	10	2	8.2	12.4	9.6	6.4	10.0	9.0
			8.4	11.4	10.0	9.8	10.8	9.2
			9.2	12.4	12.4	7.8	10.8	8.8
	15	5.5	4.8	6.4	9.0	5.0	6.0	6.0
			5.2	9.6	11.0	5.0	7.4	6.8
			4.6	6.6	9.4	5.0	7.0	6.2
2	10	2	6.0	9.8	8.8	7.4	8.8	7.6
			7.2	10.0	9.8	6.0	8.6	9.6
			7.2	11.8	9.2	6.6	9.8	8.8
	15	5.5	4.4	6.0	7.6	4.8	7.0	7.4
			4.6	6.6	7.4	4.0	5.8	7.2
			5.4	7.4	6.6	4.4	7.0	6.6

Date = 0.3**

Packaging = 0.4**

Storage temperature = 0.3**

Preconditioning time = 0.4**

Preconditioning temperature = 0.4*

Date x Storage temperature = 0.5**

Packaging x Preconditioning time = 0.5*

Packaging x Storage temperature = 0.6**

Packaging x Preconditioning temperature = 0.5*

Preconditioning temperature x Storage temperature = 0.7**

Preconditioning temperature x Preconditioning time = 0.6*

Date x Preconditioning temperature x Storage temperature = 1.0**

Packaging x Date x Storage temperature = 0.6*

Packaging x Date x Preconditioning temperature x Preconditioning time = 1.6*

Date x Preconditioning temperature x Preconditioning time x Storage temperature = 1.3*

* = significant (LSD_{0.05}); ** = significant (LSD_{0.001}); n = 5

TABLE VII

Fruit firmness of preconditioned unwaxed, waxed and fruit sealed in micro-perforated polypropylene bags, harvested on 03/08/04 or 23/08/04, immediately after storage at 2°C or 5.5°C (30 d)

Precon. Time (d)	Precon. Temp (°C)	Storage temp (°C)	Firmness					
			03/08/04			23/08/04		
			Unwaxed	Waxed	Polybag	Unwaxed	Waxed	Polybag
0	none	2	85.7	86.9	88.9	83.4	84.7	88.9
		5.5	79.5	82.9	90.0	78.3	80.7	85.4
1	10	2	85.7	87.1	88.8	83.2	85.5	88.8
			84.7	85.1	87.9	83.2	85.3	86.4
			87.2	86.4	89.6	83.2	86.3	86.3
	15	5.5	79.1	84.9	88.8	81.4	82.8	85.9
			80.7	83.1	86.1	78.8	81.7	85.5
			80.8	84.9	87.2	78.6	81.5	86.0
2	10	2	83.2	84.6	87.7	85.1	84.5	86.2
			82.6	83.7	87.9	85.7	84.8	86.5
			82.6	86.3	86.9	82.8	85.3	87.9
	15	5.5	82.4	83.7	88.3	79.6	84.2	87.7
			80.8	83.0	87.9	78.6	82.9	87.9
			81.3	81.9	87.3	78.8	82.9	87.4

Date = 0.4**

Packaging = 0.5**

Storage temperature = 0.4**

Preconditioning temperature = 0.4*

Date x Preconditioning temperature = 0.6*

Date x Preconditioning time = 0.7**

Date x Storage temperature = 0.4*

Packaging x Storage temperature = 0.7**

Packaging x Preconditioning temperature = 0.9**

Preconditioning temperature x Storage temperature = 0.8**

Preconditioning time x Storage temperature = 0.7**

Packaging x Date x Preconditioning time x Storage temperature = 1.6**

Packaging x Date x Precon temperature x Precon time x Storage temperature = 2.5*

* = significant (LSD_{0.05}); ** = significant (LSD_{0.001}); n = 10

TABLE VIII

Chilling injury of preconditioned unwaxed, waxed and fruit sealed in micro-perforated polypropylene bags, harvested on 03/08/04 or 23/08/04, rated immediately after storage at 2°C or 5.5°C (30 d)

Precon. time (d)	Precon. temp (°C)	Storage temp (°C)	External chilling injury rating (0..10) [‡]					
			03/08/04			23/08/04		
			Unwaxed	Waxed	Polybag	Unwaxed	Waxed	Polybag
0	none	2	1.2	1.6	0.4	1.0	2.6	0.2
		5.5	0.2	0.2	0	1.0	2.2	0.2
1	10	2	0.8	0.6	0	0.4	0.4	0.2
		15	0	0.6	0	1.6	2.6	0.2
		20	1.8	2.8	0.4	1.6	2.6	0.2
	5.5	10	0.8	0	0	0	0.4	0
		15	0	0.6	0	0	0	0
		20	0.8	1.6	0	0.2	0.2	0
2	10	2	0	0	0	0.2	0.4	0.2
		15	0.6	0.4	0	0	0.4	0
		20	1.4	3.2	0	1.8	0.8	0.2
	5.5	10	0.4	0	0	0	0	0
		15	0	0	0	0	0	0
		20	0	0	0	0	0.6	0

Date = 0.1*

Packaging = 0.2**

Storage temperature = 0.2**

Preconditioning time = 0.2**

Preconditioning temperature = 0.2**

Date x Preconditioning temperature = 0.3*

Packaging x Storage temperature = 0.3**

Packaging x Preconditioning temperature = 0.4**

Preconditioning time x Storage temperature = 0.3**

Date x Preconditioning temperature x Preconditioning time = 0.4*

Date x Preconditioning temperature x Storage temperature = 0.4*

Date x Preconditioning time x Storage temperature = 0.3*

Packaging x Date x Preconditioning temperature = 0.4*

Date x Preconditioning temperature x Preconditioning time x Storage temperature = 0.6*

Packaging x Date x Precon temperature x Precon time x Storage temperature = 1.1*

* = significant (LSD_{0.05}); ** = significant (LSD_{0.001}); n = 5[‡] 0 = no injury, 10 = 100% surface area of fruit affected

TABLE IX

Chilling injury severity of preconditioned unwaxed, waxed and fruit sealed in micro-perforated polypropylene bags, harvested on 03/08/04 or 23/08/04, which were stored at 2°C or 5.5°C (30 d) and rated after ripening

Precon. Time (d)	Precon. Temp (°C)	Storage temp (°C)	External chilling injury rating (0..10) [‡]					
			03/08/04			23/08/04		
			Unwaxed	Waxed	Polybag	Unwaxed	Waxed	Polybag
0	none	2	1.4	1.4	0.2	1.4	3.8	0.2
		5.5	0.2	1.0	0	0.2	0.6	0.4
1	10	2	0	0.2	0	0.8	1.4	0.4
			1.2	0.4	0	1.2	2.0	0.4
			1.6	2.2	0	2.0	2.2	0.8
	15	5.5	0	0.2	0	0	0.2	0
			0.2	0.2	0	0.2	0	0
			0	0.6	0	0	2.0	0.2
2	10	2	0	0	0	0	0.2	0
			0	0	0	0	0.2	0
			0.6	0.8	0.2	1.2	1.0	0.4
	15	5.5	0	0	0	0	0.4	0
			0	0	0	0.2	0	0
			0.4	0	0	0.6	0.4	0

Date = 0.1**

Packaging = 0.2**

Storage temperature = 0.1**

Preconditioning time = 0.2**

Preconditioning temperature = 0.2**

Date x Storage temperature = 0.2**

Packaging x Date = 0.3**

Packaging x Preconditioning time = 0.2*

Packaging x Storage temperature = 0.3**

Packaging x Preconditioning temperature = 0.4**

Preconditioning temperature x Storage temperature = 0.3**

Preconditioning time x Storage temperature = 0.3**

Packaging x Date x Storage temperature = 0.3*

Packaging x Date x Preconditioning temperature x Storage temperature = 0.6*

* = significant (LSD_{0.05}); ** = significant (LSD_{0.001}); n = 5

[‡] 0 = no injury, 10 = 100% surface area of fruit affected

TABLE X

Mesocarp discolouration ratings of unwaxed, waxed and fruit sealed in micro-perforated polypropylene bags, harvested on 03/08/04 or 23/08/04, after preconditioning, storage at either 2°C or 5.5°C (30 d), and ripening

Precon. Time (d)	Precon. Temp (°C)	Storage temp (°C)	Mesocarp discolouration (0..10) [‡]					
			03/08/04			23/08/04		
			Unwaxed	Waxed	Polybag	Unwaxed	Waxed	Polybag
0	none	2	0.8	0.6	0.6	0.2	0.4	0.2
		5.5	0	2.8	0	0	0	0.2
1	10	2	0.2	0.8	0.2	0.2	0.8	0
			0.4	0.4	0.6	0.6	0.6	0.2
			0.2	0.6	0.2	0.6	0.8	0
	5.5	10	0	1.0	0.4	0	0.2	0
		15	0	0.2	0	0	0	0
		20	0	0.4	0.4	0.4	1.4	0.2
2	10	2	0.2	0.4	0.4	0	0	0
			0.2	0.2	0.6	0	0	0.6
			0	0.2	0	0.4	0.2	0.6
	5.5	10	1.4	0	0	0	0.6	0.2
		15	0.2	0	0	0.4	0.2	0.2
		20	1.4	0	0	0.8	0.4	0

Date = 0.1*

Packaging = 0.2**

Storage temperature = n.s.

Preconditioning time = n.s.

Preconditioning temperature = n.s.

Date x Preconditioning temperature = 0.3*

Packaging x Storage temperature = 0.2*

Packaging x Preconditioning time = 0.3*

Packaging x Date x Storage temperature = 0.3*

Packaging x Preconditioning time x Storage temperature = 0.4*

Packaging x Date x Preconditioning temperature x Storage temperature = 0.6*

Packaging x Date x Preconditioning time x Storage temperature = 0.4*

* = significant (LSD_{0.05}); ** = significant (LSD_{0.001}); n.s. = non-significant; n = 5

[‡] 0 = no discolouration, 10 = 100% of cut surface area black

TABLE XI

Anthracnose score of unwaxed, waxed and fruit sealed in polybags, harvested on 03/08/04 or 23/08/04, after preconditioning, storage at either 2°C or 5.5°C (30 d), and ripening

Precon. Time (d)	Precon. Temp (°C)	Storage temp (°C)	Anthracnose score (0/1) [‡]					
			03/08/04			23/08/04		
			Unwaxed	Waxed	Polybag	Unwaxed	Waxed	Polybag
0	none	2	0.2	0.6	0.6	0.2	0.6	0.4
		5.5	0	0.2	0.2	0	0.2	0
1	10	2	0	0.4	0.2	0.2	0.6	0
			0.4	0.2	0.6	0.4	0.6	0.2
			0	0.2	1.0	0.2	0.4	0.6
	5.5	10	0	0.2	0.2	0	0	0
		15	0	0.4	0.2	0	0	0
		20	0	0.2	0.2	0	0.4	0.2
2	10	2	0	0.2	0.2	0	0	0
			0	0.4	0.6	0	0	0.2
			0	0	0.2	0	0.2	0.4
	5.5	10	0.2	0	0	0	0.2	0
		15	0	0	0	0	0	0.2
		20	0.2	0	0	0	0.2	0

Date = n.s.

Packaging = 0.1**

Storage temperature = 0.1**

Preconditioning time = 0.1**

Preconditioning temperature = 0.1*

Packaging x Date = 0.1*

Packaging x Storage temperature = 0.1*

Packaging x Preconditioning temperature = 0.1*

Preconditioning temperature x Storage temperature = 0.1*

Preconditioning time x Storage temperature = 0.1*

Packaging x Preconditioning time x Preconditioning temperature = 0.2*

Date x Preconditioning temperature x Preconditioning time x Storage temperature = 0.3*

* = significant (LSD_{0.05}); ** = significant (LSD_{0.001}); n.s. = non-significant; n = 5

[‡] 0 = no pathogen detected; 1 = pathogen detected

TABLE XII

Proline concentration of unwaxed, waxed and fruit sealed in polybags, harvested on 03/08/04 or 23/08/04, after preconditioning, and storage at either 2°C or 5.5°C (30 d)

Preconditioning time (d)	Preconditioning temperature (°C)	Proline concentration (μ mole proline/ g fresh weight)					
		03/08/04			23/08/04		
		Unwaxed	Waxed	Polybag	Unwaxed	Waxed	Polybag
0	none	0.019	0.025	0.016	0.039	0.059	0.028
1	10	0.018	0.037	0.012	0.034	0.033	0.027
	15	0.020	0.022	0.017	0.044	0.056	0.025
	20	0.027	0.023	0.022	0.039	0.047	0.026
2	10	0.017	0.008	0.009	0.021	0.024	0.026
	15	0.021	0.014	0.014	0.025	0.021	0.027
	20	0.024	0.017	0.019	0.041	0.026	0.028

Date = 0.003**

Packaging = 0.004**

Preconditioning time = 0.004**

Preconditioning temperature = 0.004**

Packaging x Date = 0.004*

Packaging x Preconditioning time = 0.007**

Date x Preconditioning temperature = 0.005*

Date x Preconditioning time x Preconditioning temperature = 0.008*

Packaging x Date x Preconditioning time x Preconditioning temperature = 0.014*

* = significant ($LSD_{0.05}$); ** = significant ($LSD_{0.001}$); n = 5



Figure 1. Photo illustrating chilling injury damage/pitting around the lenticels of a waxed 'Pinkerton' fruit after cold storage at 2°C.



Figure 2. Photo illustrating the symptoms of the unidentified fungal infection in unwaxed fruit preconditioned and stored in micro-perforated polypropylene bags.



Figure 3. Photo illustrating the typical symptoms of external chilling injury/pitting in 'Pinkerton' avocado fruit (unwaxed).



Figure 4. Photo illustrating the symptoms of the unidentified fungal infection on the exocarp tissue, and immediately underneath.



Figure 5. Photo illustrating the typical symptoms of an anthracnose infection in ripening avocado fruit.

GENERAL DISCUSSION AND CONCLUSIONS

Initially the aim of this research was to find ways of reducing the development of mesocarp discolouration in 'Pinkerton' avocado, while at the same time trying to identify factors that increase the potential for disorder development, so that the future of the cultivar could be ensured. As mesocarp discolouration was not a new or unique disorder to avocado fruit, the study started out by testing various hypotheses made during past studies. One such hypothesis was that mesocarp discolouration was the result of "chilling injury".

Throughout the study there was unequivocal evidence to suggest that postharvest storage temperature does, in fact, have a significant effect on the severity of mesocarp discolouration development in 'Pinkerton' avocado fruit. However, contrary to common beliefs the disorder was not found to be the result of too low, but rather too high a storage temperature. Storage at 2°C proved to be highly beneficial in maintaining internal fruit quality. Fruit remained hard during the 4-week storage period, which is a highly desirable trait for export consideration. Membrane integrity was also retained at this temperature. It appears that storage at 2°C slows down the metabolic activity of the fruit, possibly enough to prevent imbalances in metabolism, and subsequently the accumulation of toxic intermediates. Fruit stored at intermediate temperatures have often been found to develop more "chilling disorders" than fruit stored at higher or lower temperatures (Ulrich, 1958). In the case of 'Pinkerton' it would appear that temperatures between 5.5°C and 8°C fall in the "intermediate" range. The improved membrane integrity, found in fruit stored at 2°C, becomes even more important when considering the fact that the total phenolic content of fruit, while lower at 2°C, was found to increase during the harvest season, thereby increasing the potential for mesocarp discolouration later in the season. Furthermore, fruit from historically poor quality/ "high risk" areas had higher PPO activities to start with and would therefore benefit greatly from the improved membrane integrity found in fruit stored at 2°C.

Nevertheless, while postharvest storage conditions may be beneficial in reducing the severity of mesocarp discolouration identifying and treating the cause of the disorder is the preferred mode of action. The differences in mesocarp discolouration severity between fruit origins and between seasons indicate that preharvest conditions play a significant role. This presents a more complex interaction of variables, and furthermore these may differ between areas. However, determining the mineral status of fruit appears to be a valid starting point. From the

results of the study excessive nitrogen concentrations (>1%) at harvest proved to be detrimental to fruit quality. This is supported by the work of Snijder *et al.* (2002). The high nitrogen content of the fruit implied that the soil and tree might contain very high nitrogen levels and subsequently excessive tree vigour may have been a problem. Vegetative/shoot growth is known to be a stronger sink for minerals, such as calcium (Witney *et al.*, 1990), as well as other metabolites (carbohydrates) and water. Bower and Van Lelyveld (1985) previously found that preharvest water stress could have an adverse effect on PPO activity and consequently fruit quality. Thus irrigation schedules would have to be adapted to the vegetative state of the tree. The carbohydrate status of the fruit would also be important to postharvest fruit quality as the fruit depends on this for maintaining the energy supply during respiration and ripening, with membrane integrity also being affected. While 'Pinkerton' is not severely affected by an alternate bearing pattern there is circumstantial evidence to suggest that prevalence of the disorder is. Furthermore, the soluble C7 sugar, mannoheptulose, and a related C7 sugar alcohol, perseitol, have been found to play a role in fruit ripening. These sugars display a distinct seasonal fluctuation in response to vegetative growth flushes in 'Hass' (Liu *et al.*, 1999). Further studies could, therefore, investigate how excessive vigour and crop load affects the carbohydrate status of 'Pinkerton' fruit, especially as this cultivar has an extended flowering period.

The condition of the tree will also affect the fruit mineral content and thus fruit from different areas will be expected to differ. The mineral status of a tree is affected by many factors (which may interact), such as light, temperature, water, and plant growth regulators. The relationship between calcium and auxin may be a good example of this, with auxin concentrations being high in metabolically active organs, such as developing shoots, and thus being stronger sinks for calcium. Calcium is also thought to move in the transpiration stream of the plant and the new vegetative flush would have a high transpiration rate. In this study, however, calcium was not found to contribute significantly to disorder development as previous studies have indicated (Chaplin and Scott, 1980; Cutting *et al.*, 1992). Furthermore, while the ratio of nitrogen:calcium was found to play a significant role, many of the other elements were found to contribute more significantly. However, it is difficult to eliminate the roles of certain elements altogether, as it may be that their concentrations play a significant role at certain periods of fruit growth and not necessarily during postharvest storage. Furthermore, the carbohydrate status of a fruit may play a deciding role in whether a certain element is deficient or not. There are, thus, dynamic sets of interactions that can affect the

tree at any one time and this will require intelligent and adaptive management practices throughout the changing conditions of the season.

In summary, the factors contributing to mesocarp discolouration development are complex. However, there do appear to be some factors, which can be managed to a certain extent, such as preharvest water stress and mineral status. Studies are currently being undertaken in South Africa to identify critical periods at which certain mineral concentrations play a role. These studies include regularly sampling fruit for mineral content, from about two weeks post petal fall until harvest, and should help with establishing benchmark concentrations so that management practices can be adapted. In the meantime ways of managing nitrogen need to be found to reduce vigour and improve source:sink relationships. Management practices might include choosing more suitable rootstocks for the prevailing conditions. Rootstocks have been known to affect mineral uptake of trees (Marques, 2002) as well as tree vigour (Wolstenholme, 2003). Unfortunately the resistance of the rootstock to *Phytophthora cinnamomi* will also have to be considered under South African conditions. Applying suitable mulches to soils that contain high nitrogen concentrations may also be beneficial in decreasing the available soil nitrogen (Wolstenholme, 2004). Mulches with a high carbon:nitrogen ratio (>30) would result in micro-organisms using the soil nitrogen to break down the carbon/mulch thus withdrawing excess nitrogen from the soil.

Unfortunately the chlorophyll fluorescence technique used in this study did not prove to be useful in predicting internal fruit quality. However, improvements in this field are being made daily and the equipment used to determine chlorophyll fluorescence is getting more advanced, thus this technology may become useful in the future.

In order to finalise a postharvest protocol suitable for commercial application, when handling 'Pinkerton' fruit, certain factors had to be addressed. This included studying the effects of a 3 d storage delay, experienced during 2000 and 2001, on fruit quality, as well as the affect of 2°C storage on external chilling injury development. Past studies conducted by other researchers (Kruger *et al.*, 2000) using fruit from the same risk areas were unsuccessful at storing fruit at temperatures below 5.5°C from both an internal and external point of view. It was suspected that fruit used in the other studies were placed into storage a lot sooner than fruit in this study as fruit were received on the same day as the fruit were harvested. The optimal procedure after harvest is thought to entail getting fruit into the packhouse as soon as

possible, with cooling following shortly thereafter. This technique was thought to reduce fruit moisture loss, which has been correlated to fruit quality (Bower *et al.*, 1989), and to reduce the rate of metabolism of the stored fruit in order to prolong its postharvest life. While many fruit types benefit from rapid cooling after harvest, some fruit are more resistant to “chilling injury” if held at temperatures slightly above those at which injury normally occurs before storage.

Studies, conducted in 2004, appeared to support the fact that a storage delay could be beneficial in terms of reducing both mesocarp discolouration and external chilling injury in ‘Pinkerton’ avocados. However, the temperature at which the fruit are held prior to storage plays a significant role. In this study holding fruit at 10°C for 2 d consistently rendered fruit with the best internal and external quality regardless of how fruit were packaged. Low temperature conditioning has also been successful in reducing external chilling injury in ‘Hass’ avocados (Woolf *et al.*, 2003; Hofman *et al.*, 2003). It appears, therefore, that this form of preconditioning shows considerable potential in terms of being able to store fruit at very low temperatures without damage. The ability of fruit to undergo a cold disinfection treatment therefore becomes an attainable goal, in terms of meeting quarantine standards while maintaining high fruit quality, and opens up the possibility of exporting fruit to new lucrative markets.

The role of fruit packaging in external chilling injury development still needs further investigation. Previous research had indicated that decreasing moisture loss (as determined by weight loss) during storage could possibly overcome the development of external chilling injury (Bower and Jackson, 2003). Fruit sealed in micro-perforated polypropylene bags (polybags) lost the least weight during preconditioning and storage, developed the least external chilling injury and consistently had the lowest proline concentrations. Unwaxed fruit, while losing the most weight during preconditioning and storage did not always display the most external chilling injury, nor were proline concentrations consistently higher in these fruit. If we accept the theory that proline concentrations reflect the level of stress within a plant tissue then it is safe to assume that the waxed fruit were exposed to a stress other than that inflicted by moisture loss. Waxing can affect the gaseous exchange of fruit and further studies might include observing the fruit exocarp under a scanning electron microscope to see whether lenticels do become clogged and whether these areas become the primary sites for membrane collapse and “pitting”. Measuring the carbon dioxide or ethylene production of fruit

subjected to different packaging treatments, and different wax thicknesses may also prove to be enlightening. However, the interaction between temperature and the various packaging treatments will also have to be taken into account, as this may account for the differences found between studies using similar materials.

Kosiyachinda and Young (1976) showed that fruit susceptibility to disorders was affected by the stage of climacteric development. Fruit were found to be more susceptible to chilling disorders as they approached the climacteric peak. In practice, Zauberman and Jobin-Décor (1995) found that the storage temperature of Australian 'Hass' could be reduced to 2°C for preclimacteric fruit. Increasing maturity as the season progresses would thus be expected to reduce the time taken to reach the climacteric peak and thus care would have to be taken not to delay storage for too long later in the season. Packaging treatments that will result in some kind of modified atmosphere around the fruit, either by reducing weight loss or by affecting gaseous exchange, will possibly affect the period of preconditioning needed to acclimatise fruit to low temperature storage. As weight loss is affected by harvest date studies will have to be undertaken over the whole harvest season to determine the best handling procedure. Furthermore, as preharvest conditions, such as temperature, are known to affect the sensitivity of fruit to subsequent postharvest chilling, these studies will also have to be conducted with fruit from different origins.

In terms of more advanced studies the determination of lipid saturation in 'Pinkerton' fruit from different origins may also help in predicting how fruit will respond to various storage temperatures. It is reported that as temperature increases the degree of lipid unsaturation is reduced, while as temperature decreases, unsaturation increases. Insertion and removal of sterols from membranes is suspected to be part of the mechanism by which membranes adjust to temperature changes (Nilsen and Orcutt, 1996). As membrane permeability increases a change in ionic leakage occurs, as well as a possible increase in the activation energy of membrane-bound enzyme systems. Ultimately this can lead to imbalances in metabolism, which eventually lead to cell death. Determining the physiological and biochemical makeup of avocado fruit may, thus, enable horticulturalists to predict how fruit will respond to postharvest chilling.

FINAL RECOMMENDATIONS

Better ways than presently used to manipulate tree cropping in 'Pinkerton' may be essential. The influence of water stress, the role of minerals and perhaps of special note, carbohydrate relationships, need to be investigated further. It is necessary to ensure that during fruit development membrane systems, sufficiently robust to cope with pre- and postharvest stress, are laid down. The fruit must also contain sufficient carbohydrates to sustain the rapid energy requirement of the large fruit, which 'Pinkerton' normally has, without resorting to potentially destructive alternative energy forms. Coupled to this, a new postharvest strategy including low temperature storage (2-4°C) imposed after suitable acclimation, plus packaging to eliminate external chilling injury, is likely to ensure that the poor quality image of 'Pinkerton' is changed.

While further work is still needed, as outlined in the previous section, the relative roles of numerous factors relating to the 'Pinkerton' problem have been elucidated, and even without complete knowledge of the complex interaction of systems causing the problems, it is believed that a protocol for an acceptable risk in exporting the cultivar has been achieved. The technique may also improve the quality of the less risky cultivars. In South Africa the 'Hass' cultivar makes up a very large portion (49%) of the export crop and, unlike the greenskin cultivars, fruit quality in 'Hass' is not hindered as much by the development of external chilling injury, as these symptoms become masked as the fruit ripen and the exocarp darkens. However, if the risk of external chilling can be decreased in this cultivar, then the potential for secondary fungal infections also decreases and fruit of a better quality can be expected thus potentially enhancing returns. Furthermore, fruit of consistent and predictable quality will be more competitive in the market.

REFERENCES

- ABRAHAMSON, J.L., BAKER, L.G., STEPHENSON, J.T. and WOOD, J.M. (1983). Proline dehydrogenase from *Escherichia coli* K12. Properties of the membrane-associated enzyme. *European Journal of Biochemistry*, **134**, 77-82.
- ADAMS, W.W., DEMMIG-ADAMS, B., WINTER, K. and SCHREIBER, U. (1990). The ratio of variable to maximum chlorophyll fluorescence from photosystem II, measure in leaves at ambient temperature and at 77K, as an indicator of the photon yield of photosynthesis. *Planta*, **180**, 166-174.
- ADATO, I. and GAZIT, S. (1974). Water-deficit stress, ethylene production, and ripening in avocado fruits. *Plant Physiology*, **53**, 45-46.
- AHMAD, I. and HELLEBUST, J.A. (1988). The relationship between inorganic nitrogen metabolism and proline accumulation in osmoregulatory responses of two euryhaline microalgae. *Plant Physiology*, **88**, 348-354.
- ALIA, A., SARADHI, P.P. and MOHANTY, P. (1991). Proline enhances primary photochemical activities in isolated thylakoid membranes of *Brassica juncea* by arresting photo inhibitory damage. *Biochemical and Biophysical Research Communications*, **193**, 54-58.
- AMERANTE, C. and BANKS, N.H. (2001). Postharvest physiology and quality of coated fruits and vegetables. *Horticultural Reviews*, **26**, 161-238.
- ARPAIA, M.L. (1994). Preharvest factors influencing post-harvest quality of tropical and subtropical fruit. *HortScience*, **29**, 982-985.
- ARPAIA, M.L., MEYER, J.L., WITNEY, G.W., BENDER, G.S., STOTTLEMYER, D.S. and ROBINSON, P.R. (1996). The Cashin Creek nitrogen fertilizer trial – what did we learn? *California Avocado Society Yearbook*, **80**, 85-98.
- ASPINALL, D. and PALEG, L.G. (1981). Proline accumulation: physiological aspects. In: *The Physiology and Biochemistry of Drought Resistance in Plants* (Paley, L.G. and Aspinall, D., Eds). Academic Press, Sydney, Australia. 205-241.
- ATKINSON, D.E. (1977). *Cellular Energy Metabolism and its Regulation*. Academic Press, New York, NY, USA. 293 pp.
- BAIN, J.M. and MERCER, F.V. (1964). Organization resistance and the respiratory climacteric. *Australian Journal of Biological Science*, **17**, 78-85.
- BANGERTH, F. (1979). Calcium related physiological disorders of plants. *Annual Review of Phytopathology*, **17**, 97-122.

- BATES, L.S., WALDREN, R.P. and TEARE, I.D. (1973). Rapid determination of free proline for water-stress studies. *Plant and Soil*, **39**, 205-207.
- BATTEY, N.H. (1990). Calcium deficiency disorders of fruits and vegetables. *Postharvest News and Information*, **1**, 23-27.
- BENNETT, A.B., SMITH, G.M. and NICHOLS, B.G. (1987). Regulation of climacteric respiration in ripening avocado fruit. *Plant Physiology*, **83**, 973-976.
- BEN-YEHOSHUA, S. (1964). Respiration and ripening of discs of avocado fruit. *Physiologia Plantarum*, **17**, 71-80.
- BEN-YEHOSHUA, S. (1985). Individual seal-packaging of fruit and vegetables in plastic film – A new postharvest technique. *HortScience*, **20**, 32-37.
- BEN-YEHOSHUA, S., BURG, S.P. and YOUNG, R. (1983b). Resistance of citrus fruit to C₂H₄, O₂, CO₂ and H₂O mass transport. *Proceedings of the Growth Regulator Society*. East Lansing, Michigan, USA. (Cited by Ben-Yehoshua, 1985).
- BEN-YEHOSHUA, S., KOBILER, I. and SHAPIRO, B. (1981). Effects of cooling versus seal-packaging with high-density polyethylene on keeping qualities of various citrus cultivars. *Journal of the American Society for Horticultural Science*, **106**, 536-540.
- BEN-YEHOSHUA, S., SHAPIRO, B. and MORAN, R. (1987). Individual seal packaging enables the use of curing at high temperatures to reduce decay and heal injury of citrus fruits. *HortScience*, **22**, 777-783.
- BEN-YEHOSHUA, S., SHAPIRO, B., EVEN-CHEN, Z. and LURIE, S. (1983a). Mode of action of plastic film extending life of lemon and bell pepper fruit by alleviation of water stress. *Plant Physiology*, **73**, 87-93.
- BERGH, B. and ELLSTRAND, N. (1986). Taxonomy of avocado. *California Avocado Society Yearbook*, **70**, 127-133.
- BERRY, J. and BJÖRKMAN, O. (1980). Photosynthetic response and adaptation to temperature in higher plants. *Annual Review of Plant Physiology*, **31**, 491-543.
- BESTER, J.M. (1982). Avocados and temperature control. *South African Avocado Growers' Association Yearbook*, **5**, 14-15.
- BEZUIDENHOUT, J.J. (1983). Die voorkoms van mesokarpverkleuring by 'Fuerte' avokados op die Rungis Mark gedurende 1982. *South African Avocado Growers' Association Yearbook*, **6**, 24-27.
- BEZUIDENHOUT, J.J. and KUSCHKE, E. (1982). Die avocado ondersoek by Rungis, Frankryk, gedurende, 1981. *South African Avocado Growers' Association Yearbook*, **5**, 18-24.

- BEZUIDENHOUT, J. J. and VORSTER, L. L. (1991). Verband van vrugkwaliteit met klimaat- en grondfaktore. *South African Avocado Growers' Association Yearbook*, **14**, 40-41.
- BIALE, J.B. (1941). The climacteric rise in respiration rate of the 'Fuerte' avocado fruit. *Proceedings of the American Society for Horticultural Science*, **39**, 137-142.
- BIALE, J.B. and YOUNG, R.E. (1971). The avocado pear. In: *The Biochemistry of Fruit and Their Products, Vol 2* (Hulme, A.C., Ed). Academic Press, New York, NY, USA. 1-63.
- BLACKMAN, F.F. and PARIJA, P. (1928). Analytical studies in plant respiration. I: The respiration of a population of senescent ripening apples. *Proceedings of the Royal Society B. Biological Science*, **103**, 412-418.
- BLANKE, M.M. (1991). Respiration of apple and avocado fruits. *Postharvest News and Information*, **2**, 429-436.
- BLANKE, M.M. and NOTTON, B.A. (1991). Kinetics and physiological significance of photosynthetic phosphoenolpyruvate carboxylase in avocado fruit. *Journal of Plant Physiology*, **137**, 553-558.
- BLUM, A. and EBERCON, A. (1976). Genotypic responses in sorghum to drought stress. III. Free proline accumulation and drought resistance. *Crop Science*, **16**, 361-367.
- BOSS, P.K., GARDNER, R.C., JANSSEN, B.J. and ROSS, G.S. (1995). An apple polyphenol oxidase cDNA is up-regulated in wounded tissues. *Plant Molecular Biology*, **27**, 429-433.
- BOWER, J.P. (1984). Effect of fruit water stress and irrigation regime in the ripening of stored avocado fruit, cv Fuerte. *South African Avocado Growers' Association Yearbook*, **7**, 55-56.
- BOWER, J.P. (1987). The calcium accumulation pattern in avocado fruit as influenced by long-term irrigation regime. *South African Avocado Growers' Association Yearbook*, **10**, 97-99.
- BOWER, J.P. (1988). Pre- and postharvest measures for long-term storage of avocados. *South African Avocado Growers' Association Yearbook*, **11**, 68-72.
- BOWER, J.P. and CUTTING, J.G.M. (1987). Some factors affecting post-harvest quality in avocado fruit. *South African Avocado Growers' Association Yearbook*, **10**, 143-146.
- BOWER, J.P. and CUTTING, J.G.M. (1988). Avocado fruit development and ripening physiology. *Horticultural Reviews*, **10**, 229-271.
- BOWER, J.P. and JACKSON, J. (2003). Effect of fruit coating and packaging on external and internal quality. *South African Avocado Growers' Association Yearbook*, **26**, 15-19.

- BOWER, J.P. and MAGWAZA, L.S. (2004). Effect of coatings and packaging on external and internal quality with emphasis on "cold injury". *South African Avocado Growers' Association Yearbook*, **27**, 51-55.
- BOWER, J.P. and VAN LELYVELD, L.J. (1985). The effect of stress history and container ventilation on avocado fruit polyphenol oxidase activity. *Journal of Horticultural Science*, **60**, 545-547.
- BOWER, J.P., CUTTING, J.G.M. and VAN LELYVELD, L.J. (1986). Long term irrigation regime as influencing avocado abscisic acid content and quality. *South African Avocado Growers' Association Yearbook*, **9**, 43-45.
- BOWER, J.P., CUTTING, J.G.M. and WOLSTENHOLME, B.N. (1989). Effect of pre- and post-harvest water stress on the potential for fruit quality defects in avocado (*Persea americana* Mill.). *South African Journal of Plant and Soil*, **6**, 219-222.
- BOWER, J.P., DENNISON, M.T. and FOWLER, K. (2003). Avocado and mango cold storage damage as related to water loss control. *Acta Horticulturae*, **628**, 401-406.
- BOWER, J. P., CUTTING, J. G. M., LOVATT, C. J. and BLANKE, M. M. (1990). Interaction of plant growth regulator and carbohydrate in flowering and fruit set. *Acta Horticulturae*, **275**, 425-433.
- BOWLER, C., VAN MONTAGU, M. and INZE, D. (1992). Superoxide dismutase and stress tolerance. *Annual Review of Plant Physiology and Molecular Biology*, **43**, 83-116.
- BRADFORD, M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, **72**, 248-254.
- BRAMLAGE, W.J. (1982). Chilling injury of crops of temperate origin. *HortScience*, **17**, 165-168.
- BRUINSMA, J. (1981). Hormonal regulation of senescence, ageing, fading and ripening. *Acta Horticulturae*, **138**, 141-163.
- BRUNE, W. and VAN LELYVELD, L.J. (1982). Biochemical comparison of leaves of five avocado (*Persea americana* Mill.) cultivars and its possible association with susceptibility to *Phytophthora cinnamomi* rootrot. *Phytopathologische Zeitschrift*, **104**, 243-254.
- BURG, S.P. (1968). Ethylene, plant senescence and abscission. *Plant Physiology*, **43**, 1503-1511.

- BURNS, J.K. and PRESSEY, R. (1987). Ca^{2+} in cell walls of ripening tomato and peach. *Journal of the American Society for Horticultural Science*, **112**, 783-787.
- CAKMAK, I., KURZ, H. and MARSCHNER, H. (1995). Short-term effects of boron, germanium and high light intensity on membrane permeability in boron deficient leaves of sunflower. *Physiologia Plantarum*, **95**, 11-18.
- CHALUTZ, E., WAKS, J. and SCHIFFMANN-NADEL, M. (1985). Reducing susceptibility of grapefruit to chilling injury during cold treatment. *HortScience*, **20**, 226-228.
- CHAPLIN, G.R. and SCOTT, K.J. (1980). Association of calcium in chilling injury susceptibility of stored avocados. *HortScience*, **15**, 514-515.
- CHAPLIN, G.R., WILLS, R.B.H. and GRAHAM, D. (1982). Objective measurement of chilling injury in the mesocarp of stored avocados. *HortScience*, **17**, 238-239.
- CHAPLIN, G.R., WILLS, R.B.H. and GRAHAM, D. (1983). Induction of chilling injury in stored avocados with exogenous ethylene. *HortScience* **18**, 952-953.
- CHAREST, C. and CHON, T.P. (1990). Cold acclimation of wheat (*Triticum aestivum*): properties of enzymes involved in proline metabolism. *Physiologia Plantarum*, **80**, 159-168.
- CHATTPAR, H.S., MATTOO, A.K. and MODI, V.V. (1971). Biochemical studies on chilling injury in mangoes. *Phytochemistry*, **10**, 1007-1009.
- CHEN, N.M. and PAULL, R.E. (1986). Development and prevention of chilling injury in papaya fruit. *Journal of the American Society for Horticultural Science*, **111**, 639-643.
- CHIANG, H-H and DANDEKAR, A.M. (1995). Regulation of proline accumulation in *Arabidopsis thaliana* (L.) Heynh. During development and in response to desiccation. *Plant, Cell and Environment*, **18**, 1280-1290.
- CHRISTIANSEN, M.N. and ASHWORTH, E.N. (1978). Prevention of chilling injury to seedling cotton with anti-transpirants. *Crop Science*, **18**, 907-908.
- CHU, T.M., ASPINALL, D. and PALEG, L.G. (1974). Stress metabolism. VI. Temperature stress and the accumulation of proline in barley and radish. *Australian Journal of Plant Physiology*, **1**, 87-97.
- CHU, T.M., JUSAITIS, M., ASPINALL, D. and PALEG, L.G. (1978). Accumulation of free proline at low temperatures. *Physiologia Plantarum*, **43**, 254-260.
- CHVAPIL, M. (1973). New aspects in the biological role of zinc: a stabilizer of macromolecules and biological membranes. *Life Sciences*, **13**, 1041-1049.
- CONWAY, W.S., SAMS, C.E., MCGUIRE, R.G. and KELMAN, A. (1992). Calcium treatments of apples and potatoes to reduce postharvest decay. *Plant Disease*, **76**, 329-334.

- COUEY, H.M. (1982). Chilling injury of crops of tropical and sub-tropical origin. *HortScience*, **17**, 162-165.
- CRISOSTO, C.H., JOHNSON, R.S., DEJONG, T. and DAY, K.R. (1997). Orchard factors affecting postharvest stone fruit quality. *HortScience*, **32**, 820-823.
- CROWLEY, D.E., SMITH, W., FABER, B. and MANTHEY, J.A. (1996). Zinc fertilization of avocado trees. *HortScience*, **116**, 969-974.
- CURTIS, D., RIGUETTI, T.L., MIELKE, E. and FACTEAU, T. (1990). Postharvest mineral analysis of corkspotted and extra fancy 'Anjou' pears. *Journal of the American Society for Horticultural Science*, **116**, 969-974.
- CUTTING, J.G.M. and BOWER, J.P. (1990). Spring vegetative flush removal: the effect on yield, size, fruit mineral composition and quality. *South African Avocado Growers' Association Yearbook*, **13**, 33-34.
- CUTTING, J.G.M. and VORSTER, L.L. (1991). Differences in fruit ripening and quality from trees with different yields. *South African Avocado Growers' Association Yearbook*, **14**, 56.
- CUTTING, J.G.M. and WOLSTENHOLME, B.N. (1992). Maturity and water loss effects on avocado (*Persea americana* Mill.) postharvest physiology in cool environments. *Journal of Horticultural Science*, **67**, 569-575.
- CUTTING, J.G.M., BOWER, J.P. and WOLSTENHOLME, B.N. (1986). Stress, delayed harvest and fruit quality in 'Fuerte' avocado fruit. *South African Avocado Growers' Association Yearbook*, **9**, 39-42.
- CUTTING, J.G.M., BOWER, J.P. and WOLSTENHOLME, B.N. (1988). Effect of harvest date and applied ABA on polyphenol oxidase levels in avocado (*Persea americana* Mill.) fruit. *Journal of Horticultural Science*, **63**, 509-515.
- CUTTING, J.G.M., BOWER, J.P. and WOLSTENHOLME, B.N. (1989). Effect of abscisic acid on abscisic acid metabolism, PPO activity, phenolics and quality in ripening avocado (*Persea americana* Mill.) fruit. *South African Avocado Growers' Association Yearbook*, **12**, 53-55.
- CUTTING, J.G.M., WOLSTENHOLME, B.N. and HARDY, J. (1992). Increasing relative maturity alters the base mineral composition and phenolic concentration of avocado fruit. *Journal of Horticultural Science*, **67**, 761-768.
- DALLMAN, T.F., EAKS, I.L., THOMSON, W.W. and NOTHNAGEL, E.A. (1988). Postharvest density changes in membranes from ripening avocado fruits. *Journal of the American Society for Horticultural Science*, **113**, 729-733.

- DAVIES, D.D. (1973). Control of and by pH. *Symposia of the Society for Experimental Biology*, **27**, 513-529.
- DEELL, J.R., VAN KOOTEN, O., PRANGE, R.K. and MURR, D.P. (1999). Applications of chlorophyll fluorescence techniques in postharvest physiology. *Horticultural Reviews*, **23**, 69-107.
- DEMARTY, M., MORVAN, C. and THELLIER, M. (1984). Calcium and the cell wall. *Plant, Cell and Environment*, **7**, 441-448.
- DE RONDE, J.A. (2000). *Proline Biosynthesis in Transgenic Soybean Plants*. PhD Thesis. University of Natal, Pietermaritzburg, South Africa. 203 pp.
- DONKIN, D.J. (1995). *Some Aspects of Cold Storage of 'Fuerte' Avocados (Persea americana Mill.) grown in the Natal midlands*. M.Sc Thesis. University of Natal, Pietermaritzburg, South Africa. 108 pp.
- DRAPER, S.R. (1972). Amino acid changes associated with low temperature treatment of *Lolium perenne*. *Phytochemistry*, **11**, 639-641.
- DRY, I.B. and ROBINSON, S.P. (1994). Molecular cloning and characterisation of grape berry polyphenol oxidase. *Plant Molecular Biology*, **26**, 495-502.
- DU PLESSIS, S.F. and KOEN, T.J. (1992). Relationship between mineral nutrition and postharvest fruit disorders of 'Fuerte' avocados. In: *Proceedings of the World Avocado Congress II* (Lovatt, C.J., Ed). California, USA, 395-402.
- DURAND, B.J., ORCAN, L., YANKO, U., ZAUBERMAN, G. and FUCHS, Y. (1984). Effects of waxing on moisture loss and ripening of 'Fuerte' avocado fruit. *HortScience*, **19**, 421-422.
- EAKS, I.L. (1976). Ripening, chilling injury and respiration response of 'Hass' and 'Fuerte' avocado fruits at 20°C following chilling. *Journal of the American Society for Horticultural Science*, **101**, 538-540.
- EAKS, I.L. (1983). Effects of chilling on respiration and ethylene production of 'Hass' avocado fruit at 20°C. *HortScience*, **18**, 235-237.
- EAKS, I.L. (1985). Effect of calcium on ripening, respiratory rate, ethylene production, and quality of avocado fruit. *Journal of the American Society for Horticultural Science*, **110**, 145-148.
- EAKS, I.L. and MORRIS, E.L. (1957). Deterioration of cucumbers at chilling and non-chilling temperatures. *Proceedings of the American Society for Horticultural Science*, **69**, 388.
- EKSTEEN, G.J. and TRUTER, A.B. (1985). Effects of controlled and modified atmosphere storage on quality of eating ripe avocados. *South African Avocado Growers' Association Yearbook*, **8**, 78-80.

- EKSTEEN, G.J., BEZUIDENHOUT, J., KEEVY, C., REAY, N. and ROBINSON, R. (1998). The effect of intransit and storage conditions on the quality of avocados – 1997 season. *South African Avocado Growers' Association Yearbook*, **21**, 100-108.
- EMBLETON, T. W. and JONES, W. W. (1964). Avocado nutrition in California. *Proceedings of the Florida State Horticultural Society*, **77**, 401-405.
- EMONGER, V.E., MURR, D.P. and LOUGHEED, E.C. (1994). Preharvest factors that predispose apples to superficial scald. *Postharvest Biology and Technology*, **4**, 289-300.
- ENGELBRECHT, A.H.P. (1987). Localisation of polyphenol oxidase activity in avocado fruit. *South African Avocado Growers' Association Yearbook*, **10**, 140-143.
- FARAGHER, J.D., MAYAK, S. and WATCHEL, E.J. (1986). Changes in physical properties of cell membranes and their role in senescence of rose flower petals. *Acta Horticulturae*, **181**, 371-375.
- FAUST, M. and SHEAR, C.B. (1968). Corking disorders of apples: a physiological and biochemical review. *Botanical Reviews*, **34**, 441-469.
- FERGUSON, I.B. (1984). Calcium in plant senescence and fruit ripening. *Plant, Cell and Environment*, **7**, 477-489.
- FERGUSON, I.B. and DROBAK, B.K. (1988). Calcium and the regulation of plant growth and senescence. *HortScience*, **23**, 262-266.
- FERGUSON, I.B. and WATKINS, C.B. (1989). Bitter pit in apple fruit. *Horticultural Reviews*, **11**, 289-355.
- FERGUSON, I., VOLZ, R. and WOOLF, A. (1999). Preharvest factors affecting physiological disorders of fruit. *Postharvest Biology and Technology*, **15**, 255-262.
- FLORES, A., GRAU, A., LAURICH, F. and DÖRFFLING, K. (1988). Effect of new terpenoid analogues of abscisic acid on chilling and freezing resistance. *Journal of Plant Physiology*, **132**, 362-369.
- FLOYD, R.A. and NAGY, Z.S. (1984). Formation of long-lived hydroxyl free radical adducts of proline and hydroxy proline in a Fenton reaction. *Biochimica et Biophysica Acta*, **790**, 94-97.
- FORNEY, C.F. and LIPTON, W.J. (1990). Influence of controlled atmospheres and packaging on chilling sensitivity. In: *Chilling Injury of Horticultural Crops* (Wang, C.Y., Ed). CRC Press, Boca Raton, FL., USA. 257-267.
- FRENKEL, C. (1987). Factors regulating the respiratory upsurge in developing storage tissues. In: *The Physiology and Biochemistry of Plant Respiration* (Palmer, J.M., Ed). Cambridge University Press, Cambridge, UK, 33-46.

- FUCHS, Y. and ZAUBERMAN, G. (1998). Effect of postharvest treatments and storage conditions on avocado fruit ripening and quality. In: *Proceedings of the World Avocado Congress III*, Israel. 323-330.
- FUCHS, Y., ZAUBERMAN, G. and AKERMAN, M. (1986). Polygalacturonase, cellulase and softening, as related to ethylene production in avocado fruit stored at chilling temperature. *Acta Horticulturae*, **179**, 801-804.
- GARTLAN, J.S., MCKEY, D.B., WATERMAN, P.G., MBI, C.N. and STRUHSAKER, T.T. (1980). A comparative study of the phytochemistry of two African rainforests. *Biochemical Systematics and Ecology*, **8**, 401-422.
- GINSBERG, L. (1985). Post harvest physiological problems of avocados. *South African Avocado Growers' Association Yearbook*, **8**, 8-11.
- GLENN, G. and POOVAIAH, B. (1990). Calcium-mediated postharvest changes in texture and cell wall structure and composition in 'Golden Delicious' apples. *Journal of the American Society for Horticultural Science*, **115**, 962-968.
- GOLAN, A. and SADOVSKI, A. (1977). Evaluation of browning potential in avocado mesocarp. *Journal of Food Science*, **25**, 853-855.
- GOLAN, A., KAHN, V. and SADOVSKI, A.Y. (1977). Relationship between polyphenols and browning in avocado mesocarp. Comparison between the 'Fuerte' and 'Lerman' cultivars. *Journal of Agricultural and Food Chemistry*, **25**, 1253-1260.
- GONZALEZ-MELER, M.A., RIBAS-CARBA, M., GILES, L. and SIEDOW, J.N. (1999). The effect of growth and measurement temperature on activity of the alternative respiratory pathway. *Plant Physiology*, **120**, 765-777.
- GRAHAM, D. and PATTERSON, B.D. (1982). Responses of plants to low, non-freezing temperatures: proteins, metabolism, and acclimation. *Annual Review of Plant Physiology*, **33**, 247-372.
- GUILLEN, P., DOMENECH, A., LARRIGAUDIÈRE, C. and VENDRELL, M. (1998). Ethylene-induced rise of Abscisic acid levels and ACC oxidase activity in immature melons. *Journal of Horticultural Science and Biotechnology*, **73**, 313-316.
- GUINN, G. (1971). Changes in sugars, starch, RNA, protein, and lipid-soluble phosphate in leaves of cotton plants at low temperatures. *Crop Science*, **11**, 262-265.
- HANSON, A.D., NELSEN, C.E., PEDERSEN, A.R. and EVERSON, E.H. (1979). Capacity for proline accumulation during water stress in barley and its implications in breeding for drought resistance. *Crop Science*, **19**, 489-493.

- HARE, P.D. (1995). *Molecular characterisation of the gene encoding Δ^1 -pyrroline-5-carboxylate reductase isolated from Arabidopsis thaliana (L.) Heynh.* M.Sc Thesis, University of Natal, Pietermaritzburg, South Africa. 342 pp.
- HARE, P.D. (1998). *A regulatory role for proline metabolism in Arabidopsis thaliana (L.) Heynh.* PhD Thesis. University of Natal, Pietermaritzburg, South Africa. 366 pp.
- HARE, P.D. and CRESS, W.A. (1997). Metabolic implication of stress-induced proline accumulation in plants. *Plant Growth Regulation*, **21**, 79-102.
- HARIYADI, P. and PARKIN, K.L. (1991). Chilling-induced oxidative stress in cucumber fruits. *Postharvest Biology and Technology*, **1**, 33-45.
- HASLAM, E. (1989). *Plant Polyphenols: Vegetable Tannins Revisited.* Cambridge University Press, Cambridge, UK. 230 pp.
- HATTON, T.T. (1990). Reduction of chilling injury with temperature manipulation. In: *Chilling Injury of Horticultural Crops* (Wang, C.Y., Ed). CRC Press, Boca Raton, FL, USA. 269-280.
- HATTON, T.T. and CUBBEDGE, R.H. (1982). Conditioning Florida grapefruit to reduce chilling injury during low temperature storage. *Journal of the American Society for Horticultural Science*, **107**, 57-60.
- HATTON, T.T. and CUBBEDGE, R.H. (1983). Preferred temperature for prestorage conditioning of 'Marsh' grapefruit to prevent chilling injury at low temperatures. *HortScience*, **18**, 721-722.
- HEINO, P., SANDMAN, G., LONG, V., NORDIN, K. and PALVA, E.T. (1990). Abscisic acid deficiency prevents development of freezing tolerance in *Arabidopsis thaliana* (L.) Heynh. *Theoretical and Applied Genetics*, **79**, 801-806.
- HIMELRICK, D.G. and MCDUFFIE, R.F. (1983). The calcium cycle: uptake and distribution in apple trees. *HortScience*, **18**, 147-151.
- HIRON, R.W.P. and WRIGHT, S.T.C. (1973). The role of endogenous abscisic acid in the response of plants to stress. *Journal of Experimental Botany*, **24**, 769-781.
- HOFMAN, P.J., STUBBINGS, B.A., ADKINS, M.F., CORCORAN, R.J., WHITE, A. and WOOLF, A.B. (2003). Low temperature conditioning before cold disinfestations improves 'Hass' avocado fruit quality. *Postharvest Biology and Technology*, **28**, 123-133.
- HOFMAN, P.J., VUTHAPANICH, S., KLIEBER, A., WHILEY, A.W. and SIMONS, D.H. (2002). Tree yield and fruit minerals concentrations influence 'Hass' avocado fruit quality. *Scientia Horticulturae*, **92**, 113-123.

- HOUCK, L.G., JENNER, J.F. and BIANCHI, J. (1990). Holding lemon fruit at 5°C or 15°C before cold treatment reduces chilling injury. *HortScience*, **25**, 1174.
- HU, H. and BROWN, P.H. (1994). Localization of boron in cell walls of squash and tobacco and its association with pectin. *Plant Physiology*, **105**, 681-689.
- HUANG, L. and ROMANI, R.J. (1991). Metabolically driven self-restoration of energy-linked functions by avocado mitochondria. *Plant Physiology*, **95**, 1096-1105.
- HULME, A.C., SMITH, W.H. and WOOLTORTON, L.S.C. (1964). Biochemical changes with the development of low-temperature breakdown in apple. *Journal of the Science of Food and Agriculture*, **15**, 303.
- HUNT, M.D., EANNETTA, N.T. HAIFENG, Y., NEWMAN, S.M. and STEFFENS, J.C. (1993). cDNA cloning and expression of potato polyphenol oxidase. *Plant Molecular Biology*, **21**, 59-68.
- ISMAIL, M.A. and GRIERSON, W. (1977). Seasonal susceptibility of grapefruit to chilling injury as modified by certain growth regulators. *HortScience*, **12**, 118-120.
- JOHNSTON, J.W. and BANKS, N.H. (1998). Selection of a surface coating and optimization of its concentration for use on 'Hass' avocado (*Persea americana* Mill.) fruit. *New Zealand Journal of Crop and Horticultural Science*, **26**, 143-151.
- JONES, J.J. (1942). Respiration and chemical changes of papaya fruit in relation to temperature. *Plant Physiology*, **15**, 711-715.
- JONES, B., PECH, J.C., BOUZAYEN, M., LELIÈVRE, J.M., GUIZ, M., ROMOJARO, F. and LATCHÈ, A. (2001). Ethylene and developmentally-regulated processes in ripening climacteric fruit. *Acta Horticulturae*, **553**, 133-138.
- KADMAN, A. and COHEN, A. (1977). Experiments with zinc applications to avocado trees. *California Avocado Society Yearbook*, **61**, 81-85.
- KAHN, V. (1975). Polyphenol oxidase activity and browning of three avocado varieties. *Journal of the Science of Food and Agriculture*, **26**, 1319-1324.
- KAHN, V. (1977a). Latency properties of polyphenol oxidase in two avocado cultivars differing in their rate of browning. *Journal of the Science of Food and Agriculture*, **28**, 233-239.
- KAHN, V. (1977b). Some biochemical properties of polyphenoloxidase from two avocado varieties differing in their browning rates. *Journal of Food Science*, **42**, 38-43.
- KAISER, C. and WOLSTENHOLME, B.N. (1994). Aspects of delayed harvest of 'Hass' avocado (*Persea americana* Mill.) fruit in a cool subtropical climate. I. Fruit lipid and fatty acid accumulation. *Journal of Horticultural Science*, **69**, 437-445.

- KAWADA, K. WHEATON, T.A., PURVIS, A.C. and GRIERSON, W. (1979). Levels of growth regulators and reducing sugars of 'Marsh' grapefruit peel as related to seasonal resistance to chilling injury. *HortScience*, **14**, 446.
- KAYS, S.J. (1991). Postharvest handling of perishable plant products. Van Nostrand-Reinhold, New York, NY, USA. 532 pp.
- KIKUTA, Y. and ERICKSON, L.C. (1968). Seasonal changes of avocado lipids during fruit development and storage. *Californian Avocado Society Year book*, **52**, 102-108.
- KINGSTON, C.M. (1991). Maturity indices for apple and pear. *Horticultural Reviews*, **13**, 407-432.
- KIRKBY, E.A. and PILBEAM, D.J. (1984). Calcium as a plant nutrient: Review. In: *Special issue: Calcium* (Smith, H. Ed). *Plant, Cell and Environment*, **7**, 397-406.
- KNOWLES, N.R. and KNOWLES, L.O. (1989). Correlations between electrolyte leakage and degree of saturation of polar lipids from aged potato (*Solanum tuberosum* L.) tuber tissue. *Annals of Botany*, **65**, 217-223.
- KOEN, T.J. and DU PLESSIS, S.F. (1991). Optimal leaf analysis norms for avocado (cv. Fuerte). In: *Proceedings of the World Avocado Congress II "The Shape of Things to Come"* (Lovatt, C.J., Holthe, P.A. and Arpaia, M.L., Eds). Orange, CA, USA, Vol. 1, 289-299.
- KOEN, T.J., DU PLESSIS, S.F. and TERBLANCHE, J.H. (1990). Nutritional factors involved in physiological postharvest fruit disorders of avocados (cv 'Fuerte'). *Acta Horticulturae*, **275**, 543-550.
- KÖHNE, J.S., KOEN, T.J., PARTRIDGE, C.J., WESTCOTT, D.B., ABERCROMBIE, R.A., BOTHA, J. and FARRELL, D. (1990). Fertilization guidelines for high yields and good fruit quality in avocado. *South African Avocado Growers' Association Yearbook*, **13**, 8-10.
- KÖHNE, J.S., KREMER-KÖHNE, S. and SCHUTTE, T. (1992). Recent developments in avocado production research. Unpublished Report of Merensky Technological Services, Tzaneen, South Africa. 12 pp.
- KOSIYACHINDA, S. and YOUNG, R.E. (1976). Chilling sensitivity of avocado fruit at different stages of the respiratory climacteric. *Journal of the American Society for Horticultural Science*, **101**, 665-667.
- KRAMER, P.J. (1983). *Water Relations of Plants*. Academic Press, New York, NY, USA. 474 pp.

- KREMER-KÖHNE, S., KÖHNE, J.S. and SCHUTTE, J.M. (1993). Effect of potassium, magnesium and nitrogen soil applications on 'Fuerte' avocado fruit quality. *South African Avocado Growers' Association Yearbook*, **16**, 33-36.
- KRITZINGER, M. and KRUGER, F.J. (1997). Preliminary results on the evaluation of hot water heatshock treatments of South African avocados. *South African Avocado Growers' Association Yearbook*, **20**, 1-5.
- KRITZINGER, M., KRUGER, F.J. and BEZUIDENHOUT. (1998). Further evaluation of hot water/air heatshock temperature of South African avocados. *South African Avocado Growers' Association Yearbook*, **21**, 93-96.
- KRUGER, F. J. and KRITZINGER, M. (1999). 'Pinkerton' grey pulp problem addressed. *Neltropika*, **306**, 5-6.
- KRUGER, F.J., KRITZINGER, M. and MALUMANE, R. (2000). Recommendations for controlling the postharvest problems of the Pinkerton cultivar. *South African Avocado Growers' Association Yearbook*, **23**, 8-14.
- KRUGER, F.J., STASSEN, P.J.C. and SNIJDER, B. (1995). A preliminary study on variation in the maturity parameters of avocados from the Kiepersol/Hazyview area. *South African Avocado Growers' Association Yearbook*, **18**, 67-73.
- KRUGER, F. J., DU PLESSIS, M. H., KRITZINGER, M., MALUMANE, R., PENTER, M., SNIJDER, B. and CLAASSENS, N. (2001). Updating 'Pinkerton' export parameters and evaluation of new and upgraded avocado postharvest applications. *South African Avocado Growers' Association Yearbook*, **24**, 49-51.
- KRUGER, F.J., SNIJDER, B., MATHUMBU, J.M., LEMMER, D. and MALUMANE, R. (2004). Establishing appropriate maturity and fruit mineral content norms for the main avocado export cultivars. *South African Avocado Growers' Association Yearbook*, **27**, 10-16.
- KRUGER, F.J., TAIT, L., KRITZINGER, M., BEZUIDENHOUT, M. and CLAASSENS, V. (1999). Postharvest browning in South African subtropical export fruits. *Acta Horticulturae*, **485**, 225-229.
- LAL, K. N. and SUBBA RAO, M. S. (1954). *Micro-Element Nutrition of Plants*. Banaras Hindu University Press, India. 246 pp.
- LARCHER, W. (1995). *Physiological plant ecology*. Springer-Verlag, Berlin, Germany. 506 pp.
- LAX, A.R., VAUGHN, K.C. and TEMPLETON, G.E. (1984). Nuclear inheritance of polyphenol oxidase in *Nicotiana*. *Journal of Heredity*, **75**, 285-287.

- LEE, S.K. and YOUNG, R.E. (1984). Temperature sensitivity of avocado fruit in relation C₂H₄ treatment. *Journal of the American Society for Horticultural Science*, **109**, 689-692.
- LEGGETT-BAILEY, J. (1962). *Techniques in Protein Chemistry*. Elsevier Publishing Company, London, U.K. 310 pp.
- LEVITT, J. (1972). *Response of Plants to Environmental Stress*. Academic Press, New York, NY, USA. 497 pp.
- LEVITT, J. (1980). *Responses of plants to environmental stresses, Vol 1. Chilling, freezing, and high temperature stresses*. Academic Press, New York, NY, USA. 497 pp.
- LEVY, Y. (1980). Field determination of free proline accumulation and water-stress in lemon trees. *HortScience*, **15**, 302-303.
- LINK, H. (1980). Effects of nitrogen supply on some components of fruit quality in apples. In: *Mineral Nutrition of Fruit Trees* (Atkinson, D., Jackson, J.E., Sharples, R.O. and Waller, W.M., Eds). Butterworths, London, UK. 285 pp.
- LIU, X., ROBINSON, R.W., MADORE, M.A., WITNEY, G.W. and ARPAIA, M.L. (1999). 'Hass' avocado carbohydrate fluctuations. I. Growth and phenology. *Journal of the American Society for Horticultural Science*, **124**, 671-675.
- LIU, X., SIEVERT, J., ARPAIA, M.L. and MADORE, A. (2002). Postulated physiological roles of the seven-carbon sugars, mannoheptulose, and perseitol in avocado. *Journal of the American Society for Horticultural Science*, **127**, 108-114.
- LOVATT, C.J. (1999). Timing citrus and avocado foliar nutrient applications to increase fruit set and size. *HortTechnology*, **9**, 607-612.
- LOVATT, C.J. (2001). Properly timed soil-applied nitrogen fertilizer increases yield and fruit size of 'Hass' avocado. *Journal of the American Society for Horticultural Science*, **126**, 555-559.
- LOWMAN, M.D. and Box, J.D. (1983). Variation in leaf toughness and phenolic content among five species of Australian rain forest trees. *Australian Journal of Ecology*, **8**, 17-25.
- LOWRY, O.H., ROSEBROUGH, N.J., FARR, A.L. and RANDALL, R.J. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry*, **193**, 265-275.
- LUNT, R.E., SMITH, H. and DARVAS, M.M. (1981). A comparison between waxing and cellophane wrapping of avocados for export. *South African Avocado Growers' Association Yearbook*, **4**, 57-62.
- LURIE, S. and BEN-ARIE, R. (1983). Microsomal membrane changes during the ripening of apple fruit. *Plant Physiology*, **73**, 636-638.

- LURIE, S., SONEGO, L. and BEN-ARIE, R. (1987). Permeability, microviscosity and chemical changes in the plasma membrane during storage of apple fruit. *Scientia Horticulturae*, **32**, 73-83.
- LYONS, J.M. (1973). Chilling injury in plants. *Annual Review of Plant Physiology*, **24**, 445-466.
- LYONS, J.M. and RAISON, J.K. (1970). Oxidative activity of mitochondria isolated from plant tissues sensitive and resistant to chilling injury. *Plant Physiology*, **45**, 386-389.
- MADAN, S., NAINAWATEE, H.S., JAIN, R.K. and CHOWDHURY, J.B. (1995). Proline and proline metabolising enzymes in *in vitro*-selected NaCl-tolerant *Brassica juncea* L. under salt stress. *Annals of Botany*, **76**, 51-57.
- MAPSON, L.W., SWAIN, T. and TOMALIN, A.W. (1963). Influence of variety, cultural conditions and temperature of storage on enzymic browning of potato tubers. *Journal of the Science of Food and Agriculture*, **14**, 673-684.
- MARKHART, A.H. III. (1986). Chilling injury: A review of possible causes. *HortScience*, **21**, 1329-1333.
- MARSCHNER, H. (1995). *Mineral Nutrition of Higher Plants*. Academic Press, San Diego, CA, USA. 889 pp.
- MARQUES, J. R. (2002). *'Hass' Avocado Fruit Quality: The Role of Fruit Minerals and Rootstocks*. PhD Thesis, University of Queensland, Australia. 191 pp.
- MATOH, T. (1997). Boron in plant cell walls. In: *Boron in Soils and Plants: Reviews* (Dell, B., Brown, P.H. and Bell, R.W., Eds). Kluwer Academic Publishers, Dordrecht, The Netherlands, 59-70.
- MATSUYAMA, N. and DIMOND, A.E. (1973). Effect of nitrogenous fertilizer on biochemical processes that could affect lesion size of rice blast. *Phytopathology*, **63**, 1202-1203.
- MAYER, A.M. and HAREL, E. (1979). Polyphenol oxidases in plants. *Phytochemistry*, **18**, 193-214.
- MAYER, A.M. and HAREL, E. (1991). Phenoloxidases and their significance in fruits and vegetables. In: *Food Enzymology* (Fox, P.F., Ed). Elsevier Publishing Company, London, UK. 371-398.
- MCKERSIE, B.D., SENARATNA, T., WALKER, M.A., KENDALL, E.J. and HETHERINGTON, P.R. (1988). Deterioration of membranes during aging in plants: evidence for free radical mediation. In: *Senescence and Aging in Plants* (Nooden, L.S. and Leopold, A.C., Eds). Academic Press, San Diego, California, USA, 441-464.

- MEIR, S., AKERMAN, M., FUCHS, Y. and ZAUBERMAN, G. (1995). Further studies on the controlled atmosphere storage of avocados. *Postharvest Biology and Technology*, **5**, 323-330.
- MEIR, S., PHILOSOPH-HADAS, S., ZAUBERMAN, G., FUCHS, Y., AKERMAN, M. and AHARONI, N. (1991). Increased formation of fluorescent lipid-peroxidation products in avocado peels precede other signs of ripening. *Journal of the American Society for Horticultural Science*, **116**, 823-826.
- MILLER, E.V. and HEILMAN, A.S. (1952). Ascorbic acid and physiological breakdown in the fruits of the pineapple. *Science*, **116**, 505
- MILNE, D.L. (1998). Avocado quality assurance: Who? Where? When? How? *South African Avocado Growers' Association Yearbook*, **21**, 39-47.
- MONTOYA, M.M., DE LA PLAZA, J.L. and LOPEZ-RODRIGUEZ, V. (1994). Electrical conductivity of avocado fruit during cold storage and ripening. *Food Science and Technology, Lebensmittel-Wissenschaft, Technologie*, **27**, 34-38.
- MORRIS, L.L. (1982). Chilling injury of horticultural crops: an overview. *HortScience*, **17**, 161-162.
- MORRIS, L.L. and PLATENIUS, H. (1938). Low temperature injury to certain vegetables. *Proceedings of the American Society for Horticultural Science*, **36**, 609-613.
- MUELLER, W.C. and BECHMAN, C.H. (1978). Ultrastructural localization of polyphenol oxidase and peroxidase in roots and hypocotyls of cotton seedlings. *Canadian Journal of Botany*, **56**, 1579-1587.
- MURATA, T. (1969). Physiological and biochemical studies of chilling injury in bananas. *Physiologia Plantarum*, **22**, 401-406.
- MURATA, N., ISHIZAKI-NISHIZAWA, O., HIGASHI, S., HAYASHI, H., TASAKA, Y. and NISHIDA, I. (1992). Genetically engineered alteration in the chilling sensitivity of plants. *Nature*, **356**, 710-713.
- NAYLOR, A.W. (1983). The many faceted problem of chilling injury. In: *Molecular and Physiological Aspects of Stress in Plants (Proceedings of the Southern Section of the American Society of Plant Physiology)* (Purvis, A., Ed). University of Tennessee, Knoxville, TN, USA. 55-74.
- NELSON, R.M., BEZUIDENHOUT, J.J. and DONKIN, D.J. (2001). An overview of the export market situation and fruit quality during the 2000 avocado season. *South African Avocado Growers' Association Yearbook*, **24**, 5-10.

- NELSON, R.M., BEZUIDENHOUT, J.J. and DONKIN, D.J. (2002). Factors influencing export fruit quality: 2001 season. *South African Avocado Growers' Association Yearbook*, **25**, 54-62.
- NILSEN, E.T. and ORCUTT, D.M. (1996). *The Physiology of Plants Under Stress. Abiotic Factors*. John Wiley and Sons Inc., New York, NY, USA. 689 pp.
- OUDIT, D.D. and SCOTT, K.J. (1973). Storage of 'Hass' avocados in polyethylene bags. *Tropical Agriculture (Trinidad)*, **50**, 241-243.
- PALMA, T., MARANGONI, A.G. and STANLEY, D.W. (1995). Environmental stresses affect tomato microsomal membrane function differently than natural ripening and senescence. *Postharvest Biology and Technology*, **6**, 257-273.
- PALMER, J.K. (1971). The Banana. In: *The Biochemistry of Fruits and Their Products, Vol 2* (Hulme, A.C., Ed). Academic Press, New York, NY, USA. 65-105.
- PALTA, J.P. (1990). Stress interactions at the cellular and membrane levels. *HortScience*, **25**, 1377-1381.
- PANTASTICO, E.B., MATTOO, A.K., MURATE, T and OGATA, K. (1975). Chilling injury. In: *Post-harvest physiology, handling and utilisation of tropical and subtropical fruits and vegetables* (Pantastico, E.B., Ed). AVI Publishing, Westport, Connecticut, USA. 339-362.
- PAULL, R.E. and REYES, M.E.Q. (1996). Preharvest weather conditions and pineapple fruit translucency. *Scientia Horticulturae*, **66**, 59-67.
- PEARSON, D. (1975). Seasonal English market variation in the composition of South African and Israeli avocados. *Journal of the Science of Food and Agriculture*, **26**, 207-213.
- PENTER, M.G., SNIJDER, B. and KRITZINGER, M. (2001). The use of calcium for fruit quality improvement in Pinkerton avocados. *South African Avocado Growers' Association Yearbook*, **24**, 25-28.
- PHANG, J.M. (1985). The regulatory functions of proline and pyrroline-5-carboxylic acid. *Current Topics in Cellular Regulation*, **25**, 91-132.
- PLANK, R. (1938). Contribution to the theory of cold injury to fruit. *Food Research*, **3**, 175-187. (Cited by Lyons, 1973)
- PLATT-ALOIA, K.A. and THOMSON, W.W. (1981). Ultrastructure of the mesocarp of mature avocado fruit and changes associated with ripening. *Annals of Botany*, **48**, 451-465.
- PLATT-ALOIA, K.A. and THOMSON, W.W. (1987). Freeze-fracture evidence for lateral phase separations in the plasmalemma of chilling-injured avocado fruit. *Protoplasma*, **136**, 71-80.

- PLATT-ALOIA, K.A. and THOMSON, W.W. (1992). Ultrastructure of avocados: ripening, chilling injury, and isolation of idioblast oil cells. In: *Proceedings of the World Avocado Congress II* (Lovatt, C.J., Ed). California, USA, 417-425.
- PRATT, H.K. and GOESCHL, J.D. (1969). Physiological roles of ethylene in plants. *Annual Review of Plant Physiology*, **20**, 541-584.
- PURVIS, A.C. (1980). Influence of canopy depth on susceptibility of 'Marsh' grapefruit to chilling injury. *HortScience*, **15**, 731-733.
- PURVIS, A.C. (1981). Free proline in peel of grapefruit and resistance to chilling injury during cold storage. *HortScience*, **16**, 160-161.
- PURVIS, A.C. (1990). Relation of chilling stress to carbohydrate composition. In: *Chilling Injury of Horticultural Crops* (Wang, C.Y., Ed). CRC Press, Boca Raton, FL, USA. 211-221.
- PURVIS, A.C. and GRIERSON, W. (1982). Accumulation of reducing sugar and resistance of grapefruit peel to chilling injury as related to winter temperatures. *Journal of the American Society for Horticultural Science*, **107**, 139-142.
- PURVIS, A.C., KAWADA, K. and GRIERSON, W. (1979). Relationship between midseason resistance to chilling injury and reducing sugar level in grapefruit peel. *HortScience*, **14**, 227-229.
- PURVIS, A.C. and YELENOSKY, G. (1983a). Sugar and proline accumulation in grapefruit and leaves during cold hardening of young trees. *Journal of the American Society for Horticultural Science*, **107**, 222-226.
- PURVIS, A.C. and YELENOSKY, G. (1983b). Translocation of carbohydrates and proline in young grapefruit trees at low temperatures. *Plant Physiology*, **73**, 877-880.
- QUINN, P.J. (1988). Effects of temperature on cell membranes. In: *Plants and Temperature* (Long, S.P. and Woodward, F.I., Eds). The Company of Biologists Limited, Cambridge, UK. 237-258.
- RAISON, J.K. (1973). Temperature-induced phase changes in membrane lipids and their influence on metabolic regulation. *Symposia of the Society for Experimental Biology*, **27**, 485.
- RAISON, J.K. (1980). Effect of low temperature on respiration. In: *The Biochemistry of Plants, Vol 2. Metabolism and Respiration* (Davies, D.D., Ed). Academic Press, New York, NY, USA. 613-626.
- RAISON, J.K. and ORR, F.R. (1990). Proposals for a better understanding of the molecular basis of chilling injury. In: *Chilling Injury of Horticultural Crops* (Wang, C.Y., Ed). CRC Press, Boca Raton, FL, USA. 145-164.

- RANADIVE, A.S. and HAARD, N.F. (1971). Changes in polyphenolics on ripening of selected pear varieties. *Journal of the Science of Food and Agriculture*, **22**, 86-89.
- RHODES, M.J.C. (1980). Respiration and senescence of plant organs. In: *The Biochemistry of Plants, Vol 2. Metabolism and Respiration* (Davies, D.D., Ed). Academic Press, New York, NY, USA. 419-462.
- RHODES, M.J.C. (1981). The maturation and ripening of fruits. In: *Senescence in Plants* (Thimann, K.V., Ed). CRC Press, Boca Raton, FL, USA. 157-205.
- RIKIN, A. and RICHMOND, A.E. (1976). Amelioration of chilling injuries in cucumber seedlings by abscisic acid. *Physiologia Plantarum*, **38**, 95-97.
- RIKIN, A., ATSMON, D. and GILER, C. (1979). Chilling injury in cotton: Prevention by abscisic acid. *Plant, Cell Physiology*, **20**, 1537-1546.
- RISSE, L.A. and CHUN, D. (1987). Influence of various conditioning times and temperatures and intermittent warming on chilling injury and decay of nonwrapped and film wrapped peppers. *Proceedings of the Florida State Horticultural Society*, **100**, 29-32.
- ROOSENS, N.H.C.J., THU, T.T., ISKANDAR, H.M. and JACOBS, M. (1998). Isolation of the ornithine- δ -aminotransferase cDNA and effect of salt stress on its expression in *Arabidopsis thaliana*. *Plant Physiology*, **117**, 263-271.
- ROWELL, A.W.G. (1988). Cold storage capacity of avocados from different geographic regions. *South African Avocado Growers' Association Yearbook*, **11**, 41-47.
- ROWELL, A.W.G. and DURAND, B.J. (1982). Avocado fruit quality studies in the Nelspruit area from 1977-1981. *South African Avocado Growers' Association Yearbook*, **5**, 28-29.
- SACHER, J.A. (1962). Relations between changes in membrane permeability and the climacteric in banana and avocado. *Nature, London*, **195**, 577-578.
- SACHER, J.A. (1976). Studies of permeability, RNA and protein turnover during ageing of fruit and leaf tissues. *Symposia of the Society for Experimental Biology*, **21**, 269-304.
- SALTVEIT, M.E. (1991). Prior temperature exposure affects subsequent chilling sensitivity. *Physiologia Plantarum*, **82**, 529-536.
- SALTVEIT, M.E. and MORRIS, L.L. (1990). Overview on chilling injury of horticultural crops. In: *Chilling Injury of Horticultural Crops* (Wang, C.Y., Ed). CRC Press, Boca Raton, FL, USA. 3-15.
- SÁNCHEZ, E., RUIZ, J.M. and ROMERO, L. (2002). Proline metabolism in response to nitrogen toxicity in fruit of french bean plants (*Phaseolus vulgaris* L. cv Strike). *Scientia Horticulturae*, **93**, 225-233.

- SARADHI, P.P., ARORA, S. and PRASAD, K.V.S.K. (1995). Proline accumulates in plants exposed to UV radiation and protects them against induced peroxidation. *Biochemica et Biophysica Research Communication*, **290**, 1-5.
- SCHOBERT, B. and TSCHESCHE, H. (1978). Unusual solution properties of proline and its interactions with proteins. *Biochemica et Biophysica Acta*, **541**, 270-277.
- SCHROEDER, C.A. (1953). Growth and development of the Fuerte avocado fruit. *Proceedings of the American Society for Horticultural Science*, **61**, 103-109.
- SCHUTTE, J.M. (1994). Die invloed van verskillende temperatuur regimes op die vrugkwaliteit van Pinkerton. *South African Avocado Growers' Association Yearbook*, **17**, 21.
- SEYMOUR, G.B. and TUCKER, G.A. (1993). Avocado. In: *Biochemistry of Fruit Ripening* (Seymour, G.B., Taylor, J.E. and Tucker, G.A., Eds). Chapman and Hall, London, UK. 53-81.
- SHARON, N. (1980). Carbohydrates. *Scientific American*, **243**, 90-116.
- SHARON, O. and KAHN, V. (1979). Browning potential, PPO, catalase and acid phosphatase activities during ripening of non-chilled and chilled avocado. *Journal of the Science of Food and Agriculture*, **30**, 634-638.
- SHARON-RABER, O. and KAHN, V. (1983). Avocado mesocarp; browning potential, carotenoid content, polyphenol oxidase, catalase and peroxidase activities: comparison between six avocado cultivars. *Journal of Food Science*, **48**, 1874-1875.
- SHEAR, C.B. (1975). Calcium-related disorders of fruits and vegetables. *HortScience*, **10**, 361-365.
- SHEAR, C.B. (1980). Interaction of nutrition and environment on mineral composition of fruits. In: *Mineral Nutrition of Trees* (Atkinson, D., Jackson, J.E., Sharples, R.O. and Waller, W.M., Eds). Butterworths, London-Boston. 41-50.
- SHEAR, C. B. and FAUST, M. (1975). Preharvest nutrition and postharvest physiology of apples. In: *Postharvest Biology and Handling of Fruits and Vegetables* (Haard, N. F. and Salunkhe, D. K., Eds). Avi Publishing Company, CT, USA. 35-42.
- SHELP, B. (1993). Physiology and biochemistry of boron in plants. In: *Boron and its Role in Crop Production* (Gupta, U.C., Ed). CRC Press, Boca Raton, FL, USA. 53-85.
- SHEWFELT, R.L. (1993). Stress physiology: a cellular approach to quality. In: *Postharvest Handling: A Systems Approach* (Shewfelt, R.L. and Prussia, S.E., Eds). Academic Press Inc., San Diego, CA, USA. 257-276.
- SHKOLNIK, M.Y. (1984). *Trace Elements in Plants*. Elsevier, New York, NY, USA. 463 pp.

- SIMON, E.W. (1978). The symptoms of calcium deficiency in plants. *New Phytologist*, **80**, 1-15.
- SINGH, T.N., PALEG, L.G. and ASPINALL, D. (1973). Stress metabolism. I. Nitrogen metabolism and growth in the barley plant during water stress. *Australian Journal of Biological Science*, **26**, 45-56.
- SIPPEL, A.D., HOLMES, M.A., CLAASSENS, N.J.F. and CLAASSENS, V. (1993). Analysis of 'Pinkerton' avocado fruit growth. *South African Avocado Growers' Association Yearbook*, **16**, 90-95.
- SIPPEL, A.D., HOLMES, M.A. and CLAASSENS, N.J.F. (1995). Manipulation of fruit set period of the Pinkerton avocado and its effect on fruit and tree characteristics. *South African Avocado Growers' Association Yearbook*, **18**, 77-79.
- SIPPEL, A.D., HOLMES, M.A., CLAASSENS, N.J.F. and CLAASSENS, V. (1994). Avocado fruit maturity (cv. Pinkerton) as affected by fruitset period. *South African Avocado Growers' Association Yearbook*, **17**, 91-94.
- SMILLIE, R.M. (1992). Calvin cycle activity in fruit and the effect of heat stress. *Scientia Horticulturae*, **51**, 83-95.
- SMIRNOFF, N. and CUMBES, Q.J. (1989). Hydroxyl radicals scavenging activity of compatible solutes. *Phytochemistry*, **28**, 1057-1060.
- SMITH, J.H.E. and LUNT, R.E. (1984). Storage temperature studies. *South African Avocado Growers' Association Yearbook*, **7**, 36-37.
- SMITH, T.E., ASHER, C.J. STEPHENSON, R.A. and HETHERINGTON, S.E. (1997). Boron deficiency of avocado. 2. Effects on fruit size and ripening. In: *Boron in soils and plants* (Bell, R.W. and Rerkasem, B., Eds). Kluwer Academic Publishers, Dordrecht, The Netherlands. 135-137.
- SNIJDER, B., MATHUMBU, J.M. and KRUGER, K.F. (2003). Development of fruit maturity and mineral content norms for export avocado cultivars from different South African avocado growing regions. *South African Avocado Growers' Association Yearbook*, **26**, 51-54.
- SNIJDER, B., PENTER, M.G., MATHUMBU, J.M. and KRUGER, K.F. (2002). Further refinement of 'Pinkerton' export parameters. *South African Avocado Growers' Association Yearbook*, **25**, 50-53.
- SOLER, A. (1994). Enzymatic characterization of stress induced translucence of pineapple flesh in the Ivory Coast. *Acta Horticulturae*, **334**, 295-304.

- SOLOMOS, T. and LATIES, G.G. (1974). Similarities between the actions of ethylene and cyanide in initiating the climacteric and ripening of avocados. *Plant Physiology*, **54**, 506-511.
- SOLOMOS, T. and LATIES, G.G. (1976). Effect of cyanide and ethylene on the respiration of cyanide-sensitive and cyanide-resistant plant tissues. *Plant Physiology*, **58**, 47-50.
- SPALDING, D.H. and REEDER, W.F. (1983). Conditioning 'Tahiti' limes to reduce chilling injury. *Proceedings of the Florida State Horticultural Society*, **96**, 231-232.
- SRIVASTAVA, G.C. and FOWDEN, L. (1972). The effect of growth temperature on enzyme and amino-acid levels in wheat plants. *Journal of Experimental Botany*, **23**, 921-929.
- STANLEY, D.W. (1991). Biological membrane deterioration and associated quality losses in food tissues. *Critical Reviews in Food Science and Nutrition*, **30**, 487-553.
- STEWART, C.R. and HANSON, A.D. (1980). Proline accumulation as a metabolic response to water stress. In: *Adaptation of plants to water and high temperature stress* (Turner, N.C. and Kramer, P.J., Eds). John Wiley and Sons, New York, NY, USA. 173-189.
- STEWART, R.J., SAWYER, B.J.B., BUCHELI, C.S. and ROBINSON, S.P. (2001). Polyphenol oxidase is induced by chilling and wounding in pineapple. *Australian Journal of Plant Physiology*, **28**, 181-191.
- STRACK, D. RUHOFF, R. and GRAWE, W. (1986). Hydroxycinnamoylcoenzyme-A: tartronate hydroxycinnamoyltransferase in protein preparations from mung bean. *Phytochemistry*, **25**, 833-837.
- SWARTS, D.H. (1980). Die invloed van abnormale lae temperature op die ontwikkeling van doueskade by Fuerte avokado's. *Subtropica*, **1**, 16-18.
- SWARTS, D.H. (1982). 'n Nuwe benadering vir die verkoeling van uitvoeravokados. *South African Avocado Growers' Association Yearbook*, **5**, 48-50.
- SWARTS, D.H. (1984). Post harvest problems of avocados – let's talk the same language. *South African Avocado Growers' Association Yearbook*, **7**, 15-19.
- TALEISNIK, E. and GRUNBERG, K. (1994). Ion balance in tomato cultivars differing in salt tolerance. I. Sodium and potassium accumulation and fluxes under moderate salinity. *Physiologia Plantarum*, **92**, 528-534.
- TAYLER, C.B. (1996). Proline and water deficit: ups, downs, ins, and outs. *The Plant Cell*, **8**, 1221-1224.
- TERBLANCHE, J.H. (1972). *Seisoens' opname en verspreiding van tien voedingselemente by jong appelbome gekweek in sandkulture*. PhD Thesis, University of Stellenbosch, South Africa.

- TESTONI, A. and PIZZOCARO, F. (1980). Calcium absorption in apples and pears treated postharvest with calcium, zinc or urea. In *Mineral Nutrition of Fruit Trees* (Atkinson, D., Jackson, J.E., Sharples, R.O. and Waller, W.M., Eds). Butterworths, London-Boston. 329-331.
- THOMPSON, J.E. (1988). The molecular basis for membrane deterioration during senescence. In: *Senescence and Aging in Plants* (Nooden, L.D. and Leopold, A.C., Eds). Academic Press, San Diego, CA, USA. 51-83.
- THOMPSON, A.K. (1996). *Postharvest Technology of Fruit and Vegetables*. Blackwell Science Ltd, Oxford, UK. 410 pp.
- THORP, T.G., HUTCHING, D., LOWE, T. and MARSH, K.B. (1997). Survey of fruit mineral concentrations and postharvest quality of New Zealand-grown 'Hass' avocado (*Persea americana* Mill.). *New Zealand Journal of Crop and Horticultural Science*, **25**, 251-260.
- TIJSKENS, L.M.M., OTMA, E.C. and VAN KOOTEN, O. (1994). Photosystem II quantum yield as a measure of radical scavengers in chilling injury in cucumber fruits and bell peppers. *Planta*, **194**, 47-486.
- TINGWA, P.O. and YOUNG, R.E. (1974). The effect of calcium on the ripening of avocado (*Persea americana* Mill.) fruits. *Journal of the American Society for Horticultural Science*, **99**, 540-542.
- TINGWA, P.O. and YOUNG, R.E. (1975). Studies on the inhibition of ripening in attached avocado (*Persea americana* Mill.) fruits. *Journal of the American Society for Horticultural Science*, **100**, 447-449.
- TORRES, A.M., MAU-LASTOVICKA, T. and REZAAIYAN, R. (1987). Total phenols and high-performance liquid chromatography of phenolic acids of avocado. *Journal of Agricultural and Food Chemistry*, **35**, 921-925.
- TUCKER, G.A. (1993). Introduction. In: *Biochemistry of Fruit Ripening* (Seymour, G.B., Taylor, J.E. and Tucker, G.A., Eds). Chapman and Hall, London, UK. 1-51.
- ULRICH, R. (1958). Postharvest physiology of fruits. *Annual Review of Plant Physiology*, **9**, 385-416.
- VAKIS, N.J. (1982). Storage behaviour of 'Ettinger', 'Fuerte' and 'Hass' avocados grown on Mexican rootstocks in Cyprus. *Journal of Horticultural Science*, **57**, 221-226.
- VAN DER MESCHT, A. and DE RONDE, J.A. (1993). Drought related protein synthesis in cotton. *South African Journal of Plant and Soil*, **10**, 50-51.

- VAN LELYVELD, L.J. and BOWER, J.P. (1984). Enzyme reactions leading to avocado mesocarp discoloration. *Journal of Horticultural Science*, **59**, 257-263.
- VAN LELYVELD, L.J. and DE BRUYN, J.A. (1977). Polyphenols, ascorbic acid and related enzyme activities associated with Blackheart in Cayenne Pineapple fruit. *Agrochemphysica*, **9**, 1-6.
- VAN LELYVELD, L.J., GERRISH, C. and DIXON, R.A. (1984). Enzyme activities and polyphenols related to mesocarp discoloration of avocado fruit. *Phytochemistry*, **23**, 1531-1534.
- VAN RENSBURG, E. and ENGELBRECHT, A.H.P. (1985). The effect of calcium salts on the components causing browning of avocado fruit. *South African Avocado Growers' Association Yearbook*, **6**, 64-65.
- VAUGHN, K.C. and DUKE, S.O. (1984). Function of polyphenol oxidase in higher plants. *Physiologia Plantarum*, **60**, 106-112.
- VAUGHN, K.C., LAX, A.R. and DUKE, S.O. (1988). Polyphenol oxidase: The chloroplast oxidase with no established function. *Physiologia Plantarum*, **72**, 659-665.
- VENDRELL, M. and PALOMER, X. (1997). Hormonal control of fruit ripening in climacteric fruits. *Acta Horticulturae*, **463**, 235-334.
- VENKATARAYAPPA, T., FLETCHER, R.A. and THOMPSON, J.E. (1984). Retardation and reversal of senescence in bean leaves by benzyladenine and decapitation. *Plant and Cell Physiology*, **25**, 407-418.
- VICKERY, R.S. and BRUINSMA, J. (1973). Compartments and permeability of potassium in developing fruits of tomato *Lycopersicon esculentum* Mill. *Journal of Experimental Botany*, **24**, 1261-1270.
- VOISINE, R., VEZINA, L.P. and Willemot, C. (1991). Induction of senescence-like deterioration of microsomal membranes from cauliflower by free radicals generated during gamma irradiation. *Plant Physiology*, **97**, 545-550.
- VOISINE, R., VEZINA, L.P. and WILLEMOT, C. (1993). Modification of phospholipid catabolism in microsomal membranes of γ -irradiated cauliflower (*Brassica oleracea* L.). *Plant Physiology*, **102**, 213-218.
- VORSTER, L.L. and BEZUIDENHOUT, J.J. (1988). Does Zinc play a role in reducing pulp spot? *South African Avocado Growers' Association Yearbook*, **11**, 60.
- VORSTER, L.L., TOERIEN, J.C. and BEZUIDENHOUT, J.J. (1987). A storage temperature regime for South African export avocados. *South African Avocado Growers' Association Yearbook*, **10**, 146-149.

- VORSTER, L.L., TOERIEN, J.C. and BEZUIDENHOUT, J.J. (1989). Factors involved in fruit quality. *South African Avocado Growers' Association Yearbook*, **13**, 76-78.
- WALTON, D.C. (1980). Biochemistry and physiology of abscisic acid. *Annual Review of Plant Physiology*, **31**, 453-489.
- WANG, C.Y. (1990). Alleviation of chilling injury of horticultural crops. In: *Chilling Injury of Horticultural Crops* (Wang, C.Y., Ed). CRC Press, Boca Raton, FL, USA. 281-302.
- WANG, C.Y. (1991). Effect of Abscisic acid on chilling injury of zucchini squash. *Journal of Plant Growth Regulators*, **10**, 101-105.
- WANG, C.Y. (1993). Approaches to reduce chilling injury of fruits and vegetables. *Horticultural Reviews*, **15**, 63-95.
- WANG, C.Y. (1994). Combined treatment of heat shock and low temperature conditioning reduces chilling injury in zucchini squash, *Postharvest Biology and Technology*, **4**, 65-73.
- WANG, C.Y. 2001. Postharvest techniques for reducing low temperature injury in chilling-sensitive commodities. *Proceedings of the International Symposium on Improving Postharvest Technology in Fruits and Vegetables*, 467-473.
- WANG, C.Y. and QI, L. (1997). Modified atmosphere packaging alleviates chilling injury in cucumbers. *Postharvest Biology and Technology*, **10**, 195-200.
- WANG, C.Y., KRAMER, G.F., WHITAKER, B.D. and LUSBY, W.R. (1992). Temperature preconditioning increases tolerance to chilling injury and alters lipid composition in zucchini squash. *Journal of Plant Physiology*, **140**, 229-245.
- WANG, C.Y., WANG, S.Y. and MELLETHIN, W.M. (1972). Identification of abscisic acid in Bartlett pears and its relationship to premature ripening. *Journal of Agricultural and Food Chemistry*, **20**, 451-453.
- WARDOWSKI, W.F., GRIERSON, W. and EDWARDS, G.J. (1973). Chilling injury of stored limes and grapefruit as affected by differentially permeable packaging films. *HortScience*, **8**, 173-175.
- WHEATON, T.A. and MORRIS, L.L. (1967). Modification of chilling sensitivity by temperature conditioning. *Proceedings of the American Society for Horticultural Science*, **91**, 529-533.
- WHILEY, A.W., SMITH, T.E., WOLSTENHOLME, B.N. and SARANAH, J.B. (1996). Boron nutrition of avocados. *South African Avocado Growers' Association Yearbook*, **19**, 1-7.
- WILLS, R.B.H. and TIRMAZI, S.I.H. (1982). Inhibition of ripening of avocados with calcium. *Scientia Horticulturae*, **16**, 323-330.

- WILLS, R.B.H., MCGLOSSON, W.B., GRAHAM, D., LEE, T.H. and HALL, E.G. (1989). *Postharvest: An Introduction to the Physiology and Handling of Fruit and Vegetables*. BSP Professional Books, Oxford, UK. 174 pp.
- WITNEY, G.W., HOFMAN, P.J. and WOLSTENHOLME, B.N. (1990). Effect of cultivar, tree vigour and fruit position on calcium accumulation in avocado fruits. *Scientia Horticulturae*, **44**, 279-291.
- WOLSTENHOLME, B.N. (2003). Avocado rootstocks: What do we know; are we doing enough research? *South African Avocado Growers' Association Yearbook*, **26**, 106-112.
- WOLSTENHOLME, B.N. (2004). Nitrogen – the manipulator element: Managing inputs and outputs in different environments. *South African Avocado Growers' Association Yearbook*, **27**, 62-78.
- WOOD, W.M. (1984). Avocado cultivars. *South African Avocado Growers' Association Yearbook*, **7**, 10-14.
- WOOLF, A.B. and LAING, W.A. (1996). Avocado fruit skin fluorescence following hot water treatments and pretreatments. *Journal of the American Society for Horticultural Science*, **121**, 147-151.
- WOOLF, A. B., BOWEN, J. H. and FERGUSON, I. B. (1999). Preharvest exposure to the sun influences postharvest responses of 'Hass' avocado fruit. *Postharvest Biology and Technology*, **15**, 143-153.
- WOOLF, A.B., COX, K.A., WHITE, A. and FERGUSON, I.B. (2003). Low temperature conditioning treatments reduce external chilling injury of 'Hass' avocados. *Postharvest Biology and Technology*, **28**, 113-122.
- WOOLF, A.B., WEXLER, A., PRUSKY, D., KOBILER, E. and LURIE, S. (2000). Direct sunlight influences postharvest temperature responses and ripening of five avocado cultivars. *Journal of the American Society for Horticultural Science*, **125**, 370-376.
- WYN JONES, R.G. and STOREYS, R. (1978). Salt stress and comparative physiology in the Gramineae. II. Glycinebetaine and proline accumulation in two salt- and water-stressed barley cultivars. *Australian Journal of Plant Physiology*, **5**, 817-829.
- WYN JONES, R.G., STOREY, R., LEIGH, R.A., AHMAD, N. and POLLARD, A. (1977). A hypothesis on cytoplasmic osmoregulation. In: *Regulation of Cell Membrane Activities in Plants* (Marrá, E. and Ciferri, O., Eds). Elsevier/North-Holland Biomedical Press, Amsterdam, The Netherlands. 121-136.

- XIN, Z. and LI, P.H. (1991). Abscisic acid-induced chilling tolerance in cell-suspension-cultured maize: A study of *De Novo* protein synthesis. *Plant Physiology*, **96**, 29.
- YELENOSKY, G. (1978). Cold hardening 'Valencia' orange trees to tolerate -6.7°C without injury. *Journal of the American Society for Horticultural Science*, **103**, 449-452.
- YELENOSKY, G. (1979). Water stress-induced cold hardening of young citrus trees. *Journal of the American Society for Horticultural Science*, **104**, 279-273.
- YUEN, C.M.C., CAFFIN, N. and BOONYAKIEAT, D. (1994). Effect of calcium infiltration on ripening of avocados of different maturities. *Australian Journal of Experimental Agriculture*, **34**, 123-126.
- ZAUBERMAN, G. and JOBIN-DÉCOR, M.P. (1995). Avocado (*Persea americana* Mill.) quality changes in response to low-temperature storage. *Postharvest Biology and Technology*, **5**, 235-243.
- ZAUBERMAN, G. and SCHIFFMAN-NADEL, M. (1972). Respiration of whole fruit and seed of avocado at various stages of development. *Journal of the American Society for Horticultural Science*, **97**, 313-315.
- ZAUBERMAN, G., and SCHIFFMAN-NADEL, M. (1977). The response of avocado fruit to different storage temperatures. *HortScience*, **12**, 353-354.
- ZHOU, H-W., SONEGO, L., KHALCHITSKI, A., BEN-ARIE, R., LERS, A. and LURIE, S. (2000). Cell wall enzymes and cell wall changes in 'Flavortop' nectarines: mRNA abundance, enzyme activity, and changes in pectic and neutral polymers during ripening and in woolly fruit. *Journal of the American Society for Horticultural Science*, **125**, 630-637.

LIST OF PUBLICATIONS

- Bower, J.P., Van Rooyen, Z. and Allwood, G.A. (2000). Progress in identifying the primary causes of mesocarp discoloration in cv 'Pinkerton'. *South African Avocado Growers' Association Yearbook*, **23**, 15-20
- Van Rooyen, Z. and Bower, J.P. (2001). Mesocarp discoloration in the Pinkerton cultivar. *South African Avocado Growers' Association Yearbook*, **24**, 35-42.
- Van Rooyen, Z. and Bower, J.P. (2002). Tackling the Pinkerton problem. *South African Avocado Growers' Association Yearbook*, **25**, 64-71.
- Van Rooyen, Z. and Bower, J.P. (2003). An investigation of the role of temperature in mesocarp discoloration of 'Pinkerton' avocados. *Acta Horticulturae*, **628**, 575-581.
- Van Rooyen, Z. and Bower, J.P. (2003). The role of fruit mineral composition, phenolic concentration and polyphenol oxidase activity on mesocarp discoloration in 'Pinkerton'. *South African Avocado Growers' Association Yearbook*, **26**, 72-82.
- Van Rooyen, Z. and Bower, J.P. (2005). The role of fruit mineral composition on fruit softness and mesocarp discoloration in 'Pinkerton' avocado (*Persea americana* Mill.). *The Journal of Horticultural Science and Biotechnology*, **80**, 793-799.
- Van Rooyen, Z. and Bower, J.P. (2006). Effects of storage temperature, harvest date and fruit origin on post-harvest physiology and the severity of mesocarp discoloration in 'Pinkerton' avocado (*Persea americana* Mill.). *The Journal of Horticultural Science & Biotechnology*, **81**, 89-98.

RESEARCH PAPERS PRESENTED

- Van Rooyen, Z. and Bower, J.P. and Allwood, G.A. (2000). Mesocarp discoloration of avocado cv 'Pinkerton'. 9th Congress of the Southern African Society for Horticultural Science, Nelspruit, South Africa.
- Van Rooyen, Z. and Bower, J.P. (2001). Mesocarp discoloration in the 'Pinkerton' cultivar. South African Avocado Growers' Association Research Symposium, Nelspruit, South Africa.
- Van Rooyen, Z. and Bower, J.P. (2001). Internal Disorders. Natal Avocado Symposium, Wartburg, South Africa.
- Van Rooyen, Z. and Bower, J.P. (2002). A study of mesocarp discoloration in the 'Pinkerton' avocado cultivar. Combined Congress of the Southern African Society for Horticultural Science and the South African Society of Crop Production, Cedara Agricultural College, Pietermaritzburg, South Africa.
- Van Rooyen, Z. and Bower, J.P. (2002). Tackling the 'Pinkerton' problem. South African Avocado Growers' Research Symposium, Magoebaskloof, South Africa.
- Van Rooyen, Z. and Bower, J.P. (2002). An investigation of the role of temperature in mesocarp discoloration of 'Pinkerton' avocados. XXVIth International Horticultural Congress and Exhibition, Toronto, Canada.
- Van Rooyen, Z. and Bower, J.P. (2002). The effect of shipping temperature and mineral analysis on fruit quality. Natal Avocado Symposium, Wartburg, South Africa.
- Van Rooyen, Z. and Bower, J.P. (2003). The effect of mineral status and storage temperature on mesocarp discoloration in 'Pinkerton' avocados. Golden Jubilee Congress: Combined Congress of the Soil Science Society of Southern Africa with South African Society for Horticultural Sciences and the South African Society of Crop Production, Stellenbosch, South Africa.
- Van Rooyen, Z. and Bower, J.P. (2003). 'Pinkerton' do's and don'ts. South African Avocado Growers' Association Research Symposium, Magoebaskloof, South Africa.
- Van Rooyen, Z. and Bower, J.P. (2005). A new approach to preventing chilling injury. South African Avocado Growers' Association Research Symposium, Magoebaskloof, South Africa.