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 (CO_2) production rates, monitored daily following storage, showed that fruit harvested later in the season had a slightly higher CO_2 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^{o}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^{o}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.; 1ℓ $tonne^{-1}$ 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 cm^3), 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 cm^3) 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^o 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 (CO_2), 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^{o}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 CO_2 concentration (μℓ $ℓ^{-1}$) was determined and the results calculated as a rate of CO_2 production (mℓ kg^{-1}FW hr^{-1}), taking into account the fruit mass and volume, free space in the jar and the ambient room CO_2 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 mℓ 100% chloroform and 10 mℓ 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 mℓ 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 μg $mℓ^{-1}$ dilution series of gallic acid. For all the replicates and the standard curve, 0.1 mℓ aliquots were placed into 20 mℓ test tubes in duplicates. A spectrophotometer (Anthelie Advanced, Secoman, Domont Cedex, France) was calibrated using a blank of 0.1 mℓ distilled water. Six mℓ distilled water and 0.5 mℓ Folin-Ciocalteu reagent were added to each tube, which was vortexed thoroughly and allowed to stand for 5 min. Sodium carbonate (1.5 mℓ 20% w/v) was then added, followed by 1.9 mℓ distilled water to bring the total volume to 10 mℓ. 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 polyvinylpolypyrrolidone (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 min^{-1} 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 :ℓ 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% $CuSO_4.5H_2O$ in 5% sodium-citrate. Folin B was made up of 2% Na_2CO_3 in 0.1 M NaOH. After 10 min 100 :ℓ 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 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^{o}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 CO_2 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 CO_2 production rates of unstored fruit between harvest dates (Table V). Nevertheless, maximum CO_2 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 CO_2 production rate was not consistent, with maximum CO_2 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 CO_2 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 CO_2 production rate after storage (Table VI). Storage at 2°C delayed the number of days taken to reach the maximum CO_2 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 affect 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 affect 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 CO_2 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 CO_2 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^{o}C and 8^{o}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^{o}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 CO_2 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)

TABLE I

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
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										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										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.

TABLE II

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				

TABLE III

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 CO_2 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 (P = 0.05); * = significant; n.s = not significant; n = 5					LSD (P = 0.05); * = significant; n.s = not significant; n = 5				

TABLE VI

Days to reach maximum CO_2 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 (μg g^{-1}FW)	*Total PPO activity (ΔOD 420 min^{-1} mg^{-1} protein)	*Soluble PPO activity (ΔOD 420 min^{-1} mg^{-1} 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 (μg g^{-1}FW)		Total PPO activity (ΔOD 420 min^{-1} mg^{-1} protein)		Soluble PPO activity (ΔOD 420 min^{-1} 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 (μg g^{-1}FW)	Total PPO activity (ΔOD 420 min^{-1} mg^{-1} protein)	Soluble PPO activity (ΔOD 420 min^{-1} mg^{-1} 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