

The role of fruit mineral composition, phenolic concentration and polyphenol oxidase activity on mesocarp discolouration in 'Pinkerton'

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ABSTRACT

The 'Pinkerton' avocado was introduced into South Africa as a high yielding green skin cultivar. However, the susceptibility of this cultivar to mesocarp discolouration after storage has threatened its export. Initial studies showed that chilling injury may have been responsible for the internal blackening which resulted in storage trials being done to ascertain the role of temperature. Fruit were obtained from areas varying in discolouration severity histories and then stored at 8°C, 5.5°C and 2°C for 30 days, as well as 20°C. Various physiological responses were monitored and it was found that the disorder was not the result of too low shipping temperatures and that in fact temperatures could be decreased to as low as 2°C. Fruit at 2°C had the best internal quality and had better membrane stability. Differences in quality found between the various growers indicated that pre-harvest factors play a vital role. Biochemical analyses to determine the concentration of total phenols and the activity of the enzyme polyphenol oxidase also showed that the potential for browning was initiated pre-harvest. Mineral analysis done on the fruit showed that certain key elements played a significant role in the severity of the disorder, with excessive nitrogen and deficient boron levels appearing to be the most influential.

INTRODUCTION

Van Rooyen and Bower (2002) previously reported on the effect of temperature on mesocarp discolouration in 'Pinkerton'. It was found that decreasing shipping temperatures to below the accepted norm (i.e. from 5.5°C to 2°C) was beneficial in reducing the severity of the disorder. Membrane stability was

also found to be the best at this temperature, with fruit after 30 days storage still being firm and ripening normally. Generally, fruit quality deteriorated as the season progressed. The study also revealed that fruit origin had an important affect of the final fruit quality, with significant differences being noted between fruit receiving the same postharvest treat-

ments.

Additional studies were therefore needed to establish what these pre-harvest differences were and how they affected the final fruit quality. Fruit and vegetables have often been shown to exhibit various browning symptoms that have been attributed to the mineral constituents of the produce and this was investigated in this study. The authors believe that the ultimate method for the prevention of a disorder is to try and understand the metabolic sequences that lead to the development of the disorder. Fruit browning has been directly related to the polyphenol oxidase (PPO) activity and the phenol content of the fruit (Kahn, 1975; Golan *et al.*, 1977). This study was, therefore, aimed at establishing what the differences were between fruit origins, different harvest dates and different shipping temperatures.

MATERIALS AND METHODS

Pinkerton avocado fruit were obtained from different production areas of varying mesocarp discolouration histories throughout the harvest season (termed "high", "medium" or "low risk" areas). Fruit were subjected to normal packhouse procedures, and dispatched by overnight courier to the University of Natal for further treatment and analysis. On arrival the fruit were randomly divided into eight treatments and stored at 8°C, 5.5°C or 2°C for 30 days and then allowed to ripen at 20°C (Table 1). Each treatment consisted of 5 individual fruits. Evaluations of fruit quality and internal blackening were made before and after storage as well as after softening. Once removed from storage fruit softness was monitored. On sampling, the severity of the disorder was subjectively rated on a scale of 0 to 10 (with 0 indicating no discolouration and 10 indicating severe discolouration). Fruit were then peeled, the seed removed and the mesocarp cut into small pieces. The mesocarp tissue was then rapidly frozen with liquid nitrogen and stored at -20°C until further analysis could be done.

Table 1. Treatments used to establish the role of temperature in disorder development.

Treatment	Storage temperature	Storage period
1	*(not stored)	(not stored)
2	20°C	Until soft
3	8°C	30 days
4	8°C	30 days
	20°C	Until soft
5	5.5°C	30 days
6	5.5°C	30 days
	20°C	Until soft
7	2°C	30 days
8	2°C	30 days
	20°C	Until soft

* Sampled on arrival

Total phenolic compounds

Total phenolics were determined colorimetrically using a modified method of Donkin (1995). In this study frozen mesocarp tissue was used instead of freeze-dried tissue. Before analysis, samples were finely ground with a mortar and pestle, in liquid nitrogen, and 2 g of each sample was then used for the analysis. Each sample had two replicates.

Enzyme activities

Crude enzyme extraction for PPO analysis was done using a modified method of Bower and Van Lelyveld (1985). The supernatant was used immediately for soluble PPO assay. Total PPO was extracted by the same method, except that the detergent SDS at a 0.1% concentration was added during the initial grinding (Kahn, 1977). The PPO assay was as described by Van Lelyveld, Gerrish and Dixon (1984). The PPO activity was expressed as the OD change at 420 nm min⁻¹ mg⁻¹ protein at 24°C. The protein content of the extracts used for the soluble PPO assay was determined using the Bradford method (Bradford, 1976), and that of the extracts for the total PPO determination done following precipi-

tation of protein by 10% trichloroacetic acid, by a modified method of Lowry, Rosebrough, Farr and Randall (1951) due to SDS being incompatible with the dye-binding reagent used in the Bradford method (Bollag and Edelstein, 1991).

Mineral analysis

Fruit mineral analysis was done at Cedara Agricultural College, KwaZulu-Natal. Five grams of finely milled freeze-dried samples were submitted, and mineral content was determined using atomic absorption spectrometry.

Statistical analysis

Statistical analysis was carried out on data using GENSTAT (Rothamsted, UK). Where applicable means were compared using least significant differences (LSD's) at $P = 0.05$. Logistic regression was used to determine the effect of minerals on mesocarp discolouration severity. This form of regression uses chi-square probabilities and works with deviance ratios.

RESULTS AND DISCUSSION

Total phenolic compounds

The total phenolic content of the fruit was found to increase as the season progressed (Figure 1). This agrees with work done by Cutting *et al.* (1992) who associated this increase with declining fruit quality later in the season.

Storage temperature also appeared to affect the total phenolic content as concentrations decreased with decreasing temperatures (Figure 2). A possible explanation is that fruit stored at 2°C lost less water during storage than the other treatments and this would affect membrane

stability. Slight differences in total phenolic content were also found between growers.

Enzyme activities

It was found that fruit from historically poor quality areas had much higher PPO activities than fruit from "low risk" areas. Soluble PPO activity appeared to increase slightly as the season progressed (Figure 3) and fruit quality deteriorated, which supports the theory that PPO is involved in fruit browning. The total PPO activity was, however, seen to decrease slightly as the season progressed (Figure 3). This may be explained by the fact that PPO is essentially membrane bound and that membrane stability decreases as the season progresses (Van Rooyen and Bower, 2002). PPO activity is also qualitatively and quantitatively substrate dependent (Vaughn, Lax and Duke, 1988). Thus, with the increasing total phenolic content and decrease in membrane stability as the season progressed perhaps most of the PPO had already been activated and thus very little latent PPO was released.

Storage temperature had an effect on PPO activity in that the highest activity was seen at the lowest temperature (Figure 4). Whether this is due to a reduction in substrate for the enzyme to act on or rather due to membranes being more intact at this temperature still needs to be elucidated.

Table 2. Contribution of individual elements to mesocarp discolouration.

	Mesocarp discolouration	
	Deviance ratio	Chi prob
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	4.51	0.034
Potassium (%)	4.09	0.043
Calcium (%)	3.01	0.083

Mineral analysis

Significant ($P < 0.001$) differences in mineral concentrations were found between the various fruit origins and harvest dates. Interactions between minerals were found to confound statistical analysis and on account of these findings each element was evaluated individually to negate the interaction effects (Table 2).

High fruit nitrogen concentrations no doubt played a very significant role in disorder development ($P < 0.001$). This was to some extent expected due to the nature of the soil the two "high risk" orchards were situated on. These 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. High nitrogen contents in soils have previously been found to result in an increase in fruit nitrogen, with studies in 'Hass' finding that this resulted in faster ripening and more internal disorders (Arpaia *et al.*, 1996). In addition, high flesh nitrogen concentra-

tions have also been positively correlated with mesocarp discoloration in 'Fuerte' (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 (Solier, 1994; Paul and Reyes, 1996) also being related to high nitrogen concentrations.

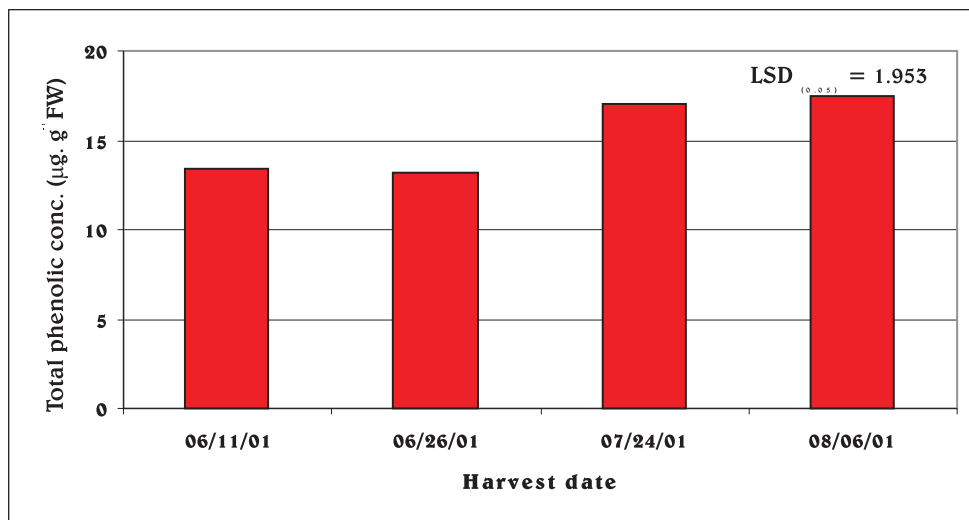


Figure 1. Total phenolic concentration in mesocarp sample from 'Pinkerton' avocado at different harvest dates.

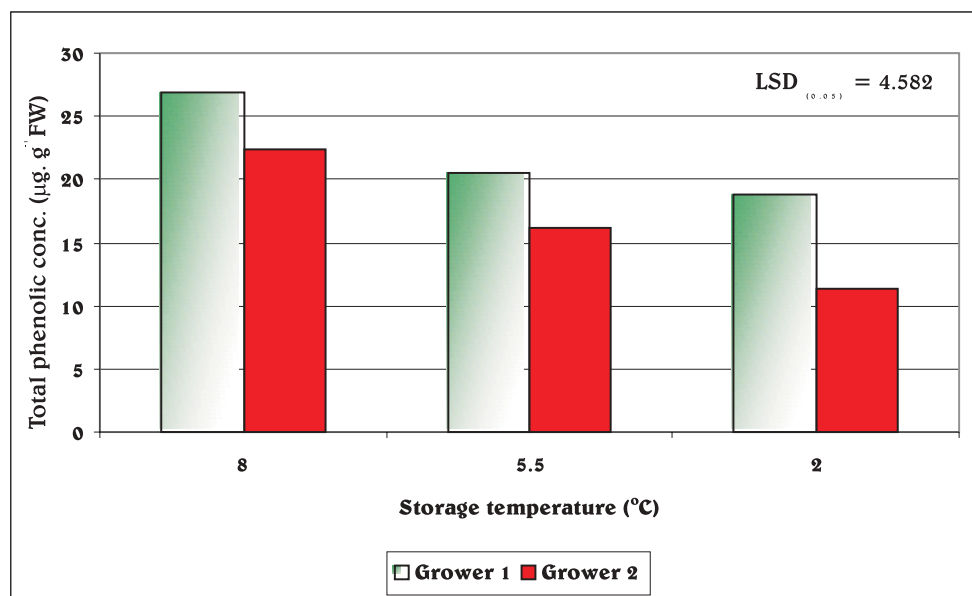


Figure 2. Total phenolic concentration in mesocarp sample from 'Pinkerton' avocados from two different origins after storage at various storage temperatures.

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. This would have many negative spin offs. For example, calcium moves in the transpiration stream of the tree and would thus be directed to the actively respiring 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 concentration in plant tissues may re-

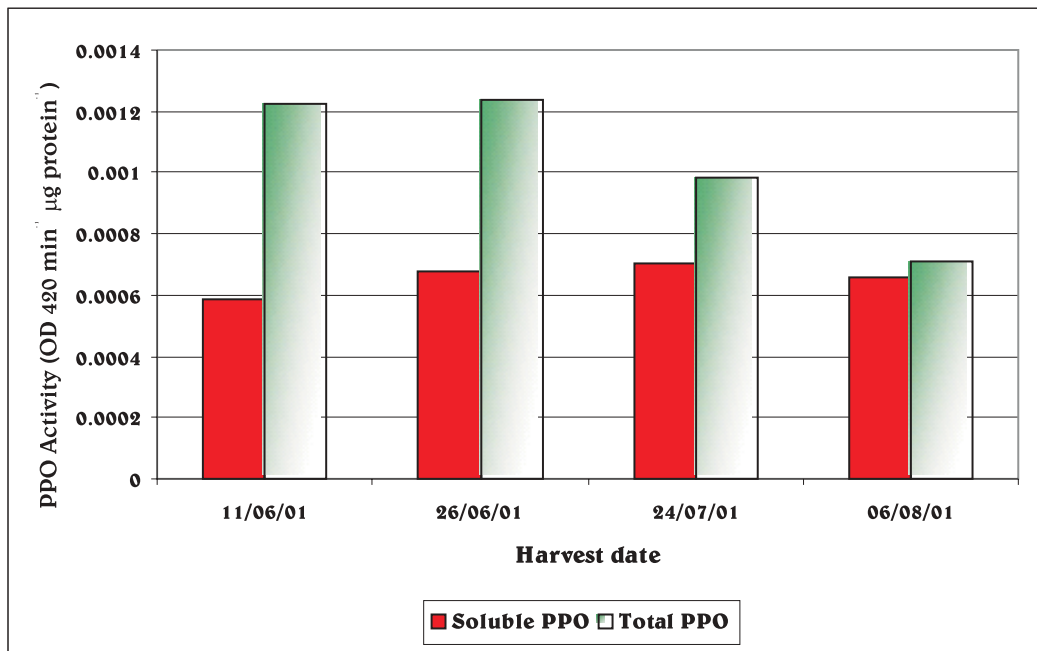


Figure 3. PPO activity of fruit sampled (without storage) during the harvest season.

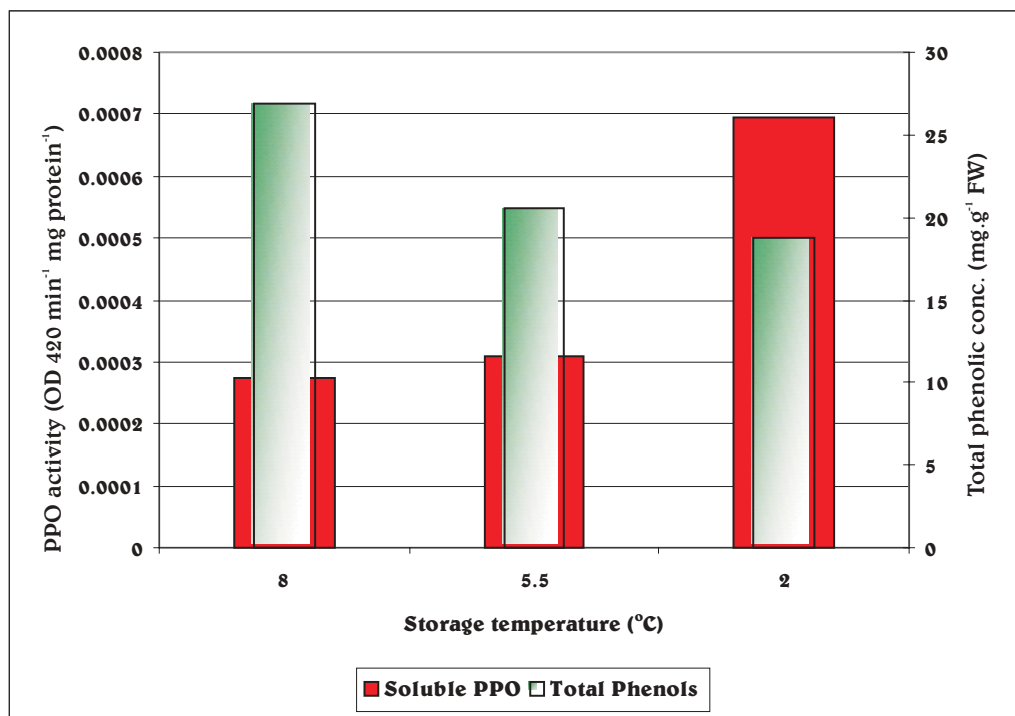


Figure 4. PPO activity and phenol content of fruit after storage at the various temperatures.

sult in thinner cell walls. Snijder *et al.* (2002) recommended that fruit nitrogen concentrations be less than 1% by March to reduce mesocarp discolouration development and in

this study nitrogen concentrations were above 1% throughout the season in the poor quality areas (Figure 5).

Copper is known to activate a group of oxidising enzymes such as polyphenol oxidase, monophenol oxidase, laccase and systems of oxidising ascorbic acid (Marschner, 1995). It acts as a catalyst for the enzymic systems that lead to enzymic browning, and is thought to be interrelated with zinc in various oxidation-reduction reactions. It is also 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 (Figure 5) and copper (Figure 6) to decrease during the season, maintaining an adequate supply of copper could prove to be vital to fruit quality.

Manganese concentrations were seen to gradually decrease as the season progressed in two of the fruit origins. Generally zinc, copper, iron and manganese are all important components of detoxifying enzymes,

with some elements being directly involved in the photosynthetic electron transport chain. Furthermore, manganese is thought to play an important role in sugar formation and sugar metabolism (Lal and Subba Rao, 1954).

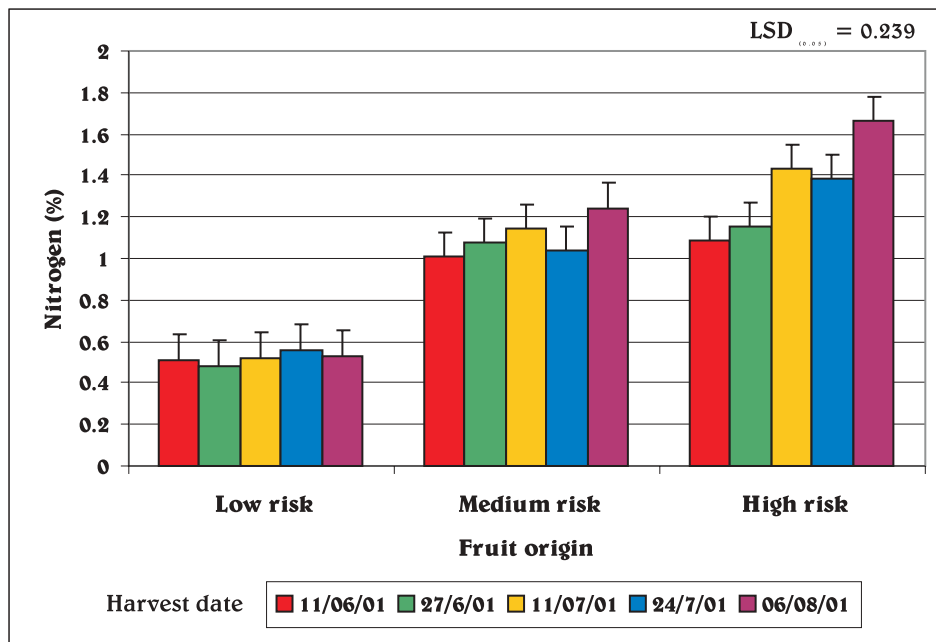


Figure 5. Nitrogen concentrations of fruit from various origins throughout the 2001 season. $LSD = Date * Fruit\ origin$

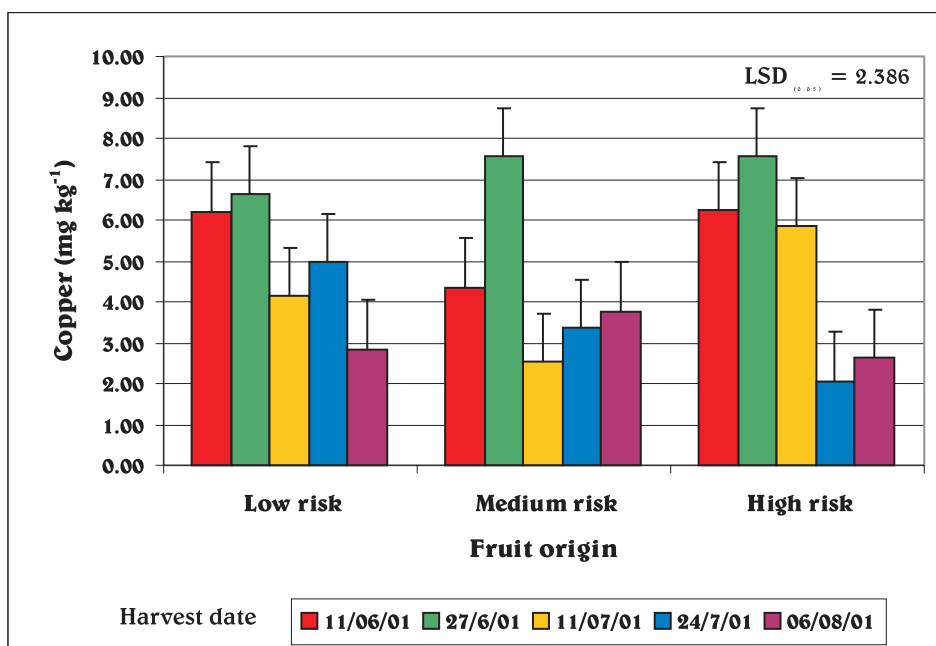


Figure 6. Copper concentrations of fruit from different fruit origins throughout the 2001 season. $LSD = Date * Fruit\ origin$

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 (IAA). While boron proved to be a significant element it was not clear from the data 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 ($r = 0.797$) could be important. Starch synthesis can be inhibited by high concentrations of phosphorus and this could be detrimental to energy generation.

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. Results from the study showed a significant ($P < 0.001$) decrease in zinc during the season in fruit from the "high risk" area. Zinc may also be connected with phosphorylation of glucose and may be necessary for cellulose formation (Lal and Subba Rao, 1954). The poor quality found in fruit from the "high risk" area could thus be due to the fact that these fruit came from highly vegetative trees and thus already had lower carbohydrate concentrations.

Calcium has been associated with more physiological disorders than any other mineral (Bangerth, 1979; Wills *et al.*, 1989). Reduced concentrations of calcium 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. 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.

CONCLUSIONS

Few postharvest disorders of fruits are completely independent of pre-harvest factors. Even those disorders, which are induced specifically by storage conditions such as low temperature, will be modified by pre-harvest environmental conditions and orchard practice. 'Pinkerton' have large fruit and are heavy bearing. The ability of fruit to reach their full potential will therefore depend on the ability of fruit to effectively compete with other metabolic sinks for available reserves. This study supported the fact that fruit mineral composition plays a vital role in determining the susceptibility of fruit to mesocarp discolouration. High fruit nitrogen concentrations appeared to be the driving force behind disorder development. Ensuring good membrane stability is a key factor in ensuring good fruit quality and mineral nutrition can be vital in this regard. Certain minerals also affect the concentration of total phenols and the activity of polyphenol oxidase. Fruitlet analysis could therefore prove to be a very useful tool as deficiencies/toxicities can be picked up as early as a few weeks after fruit set. Further work should be aimed at establishing norms for fruit so that remedial action can be taken ahead of time.

The advantage of low temperature storage (2°C) was supported further by this study. Van Rooyen and Bower (2002) showed that membrane stability was best after storage at 2°C rather than at 5.5 °C or 8°C. Mesocarp discolouration was also found to be more severe later in the season. Results from this study showed that the total phenolic concentration (the browning substrate) was higher at the highest storage temperature (8°C) and

that concentrations increased as the season progressed. Polyphenol oxidase activity (the browning enzyme) was also found to increase slightly as the season progressed. Maintaining membrane integrity will therefore prove to be vital as any membrane collapse will result in PPO coming into contact with its phenolic substrate. The potential for browning is therefore greater at the higher storage temperatures and later in the season, and storing fruit at 2°C can reduce this risk. Work is, however, still being conducted on conditioning fruit for such low temperature storage as external chilling injury still poses a threat.

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