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 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. 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.

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

Mesocarp discolouration

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 180^0 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 cm^3), 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 discolouration 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 discolouration, the rating scale (0/10) was converted into a binomial variable (i.e., 0 = no discolouration; 1 = any discolouration) allowing use of logistic regression to analyse the data. This form of regression uses chi-square (Π^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^{o}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 %
Low risk									
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
Medium risk									
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
High risk									
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

[1]LSD = 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

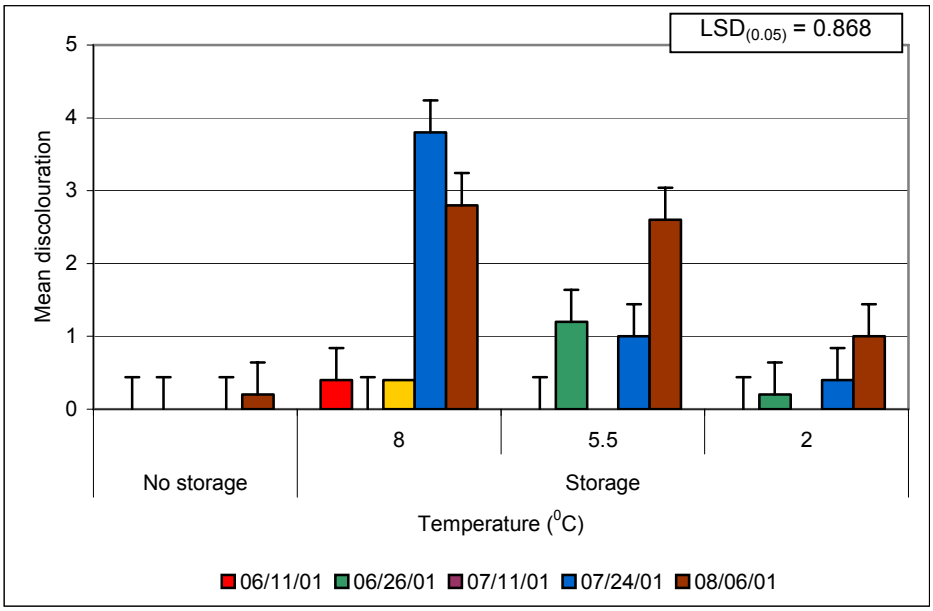

FIG. 1

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 (± SE). LSD = Date x Temperature.

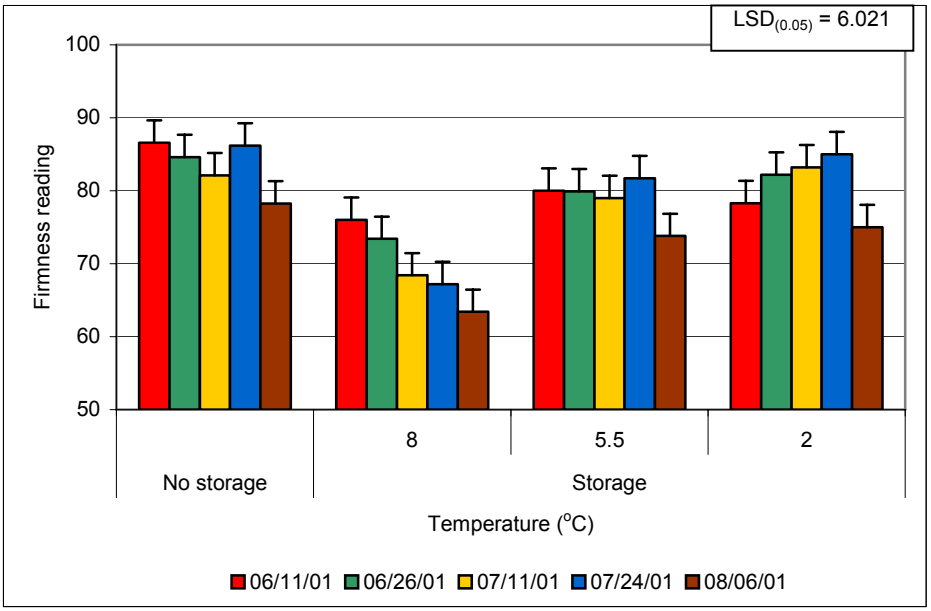

FIG. 2

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 (± SE). LSD = Date x Temperature.

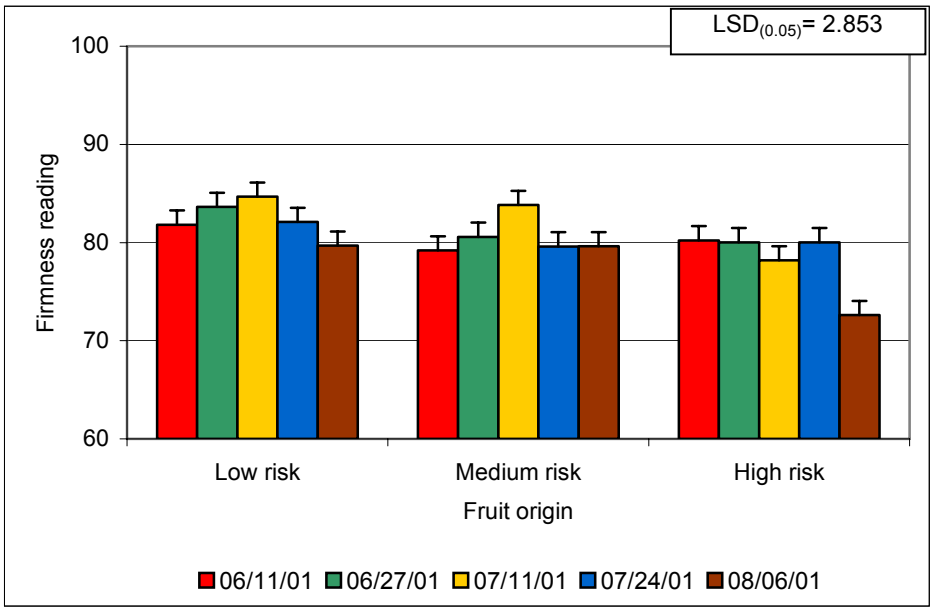

FIG. 3

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

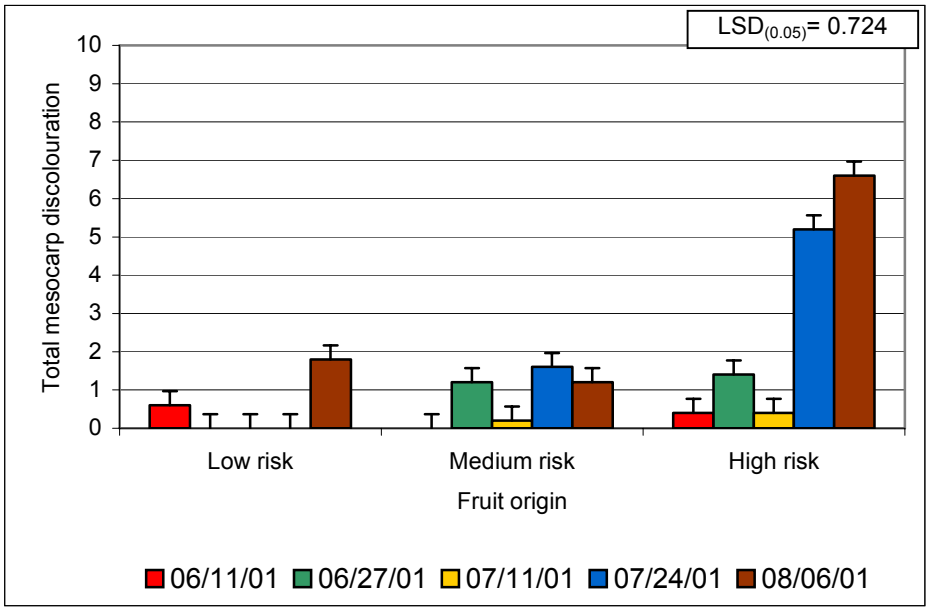

FIG. 4

Mesocarp discolouration 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 (± SE). LSD = Date x Fruit origin.