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^{o}C. The remaining fruit were then placed into storage at 2^{o}C, 5.5^{o}C or 8^{o}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 mesocarp discolouration were

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

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. 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^{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].

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

Mesocarp discolouration

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^{o}C and 5.5^{o}C treatments than at 2^{o}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^{o}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 (^{o}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
	04/05/0	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				

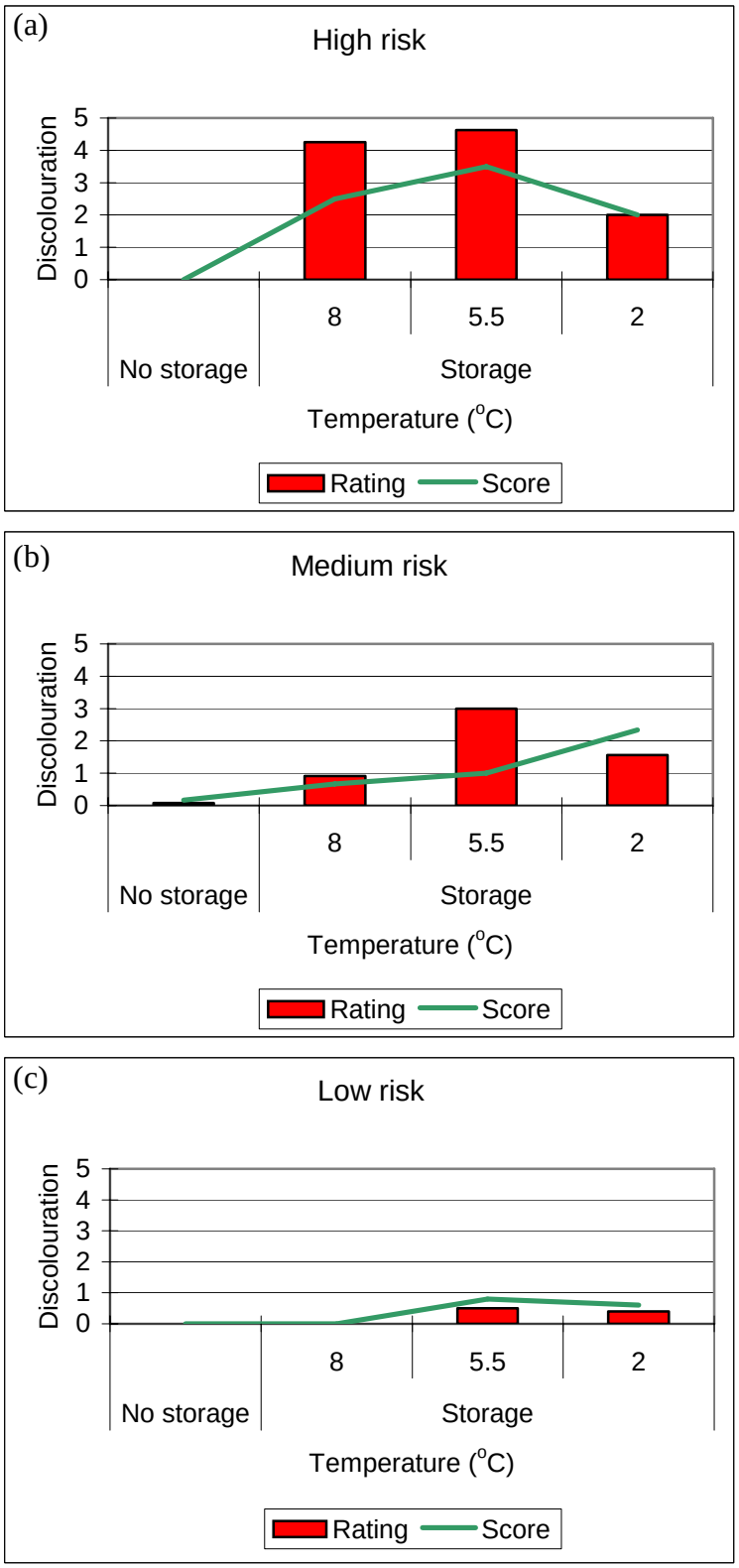

Fig. 1

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.

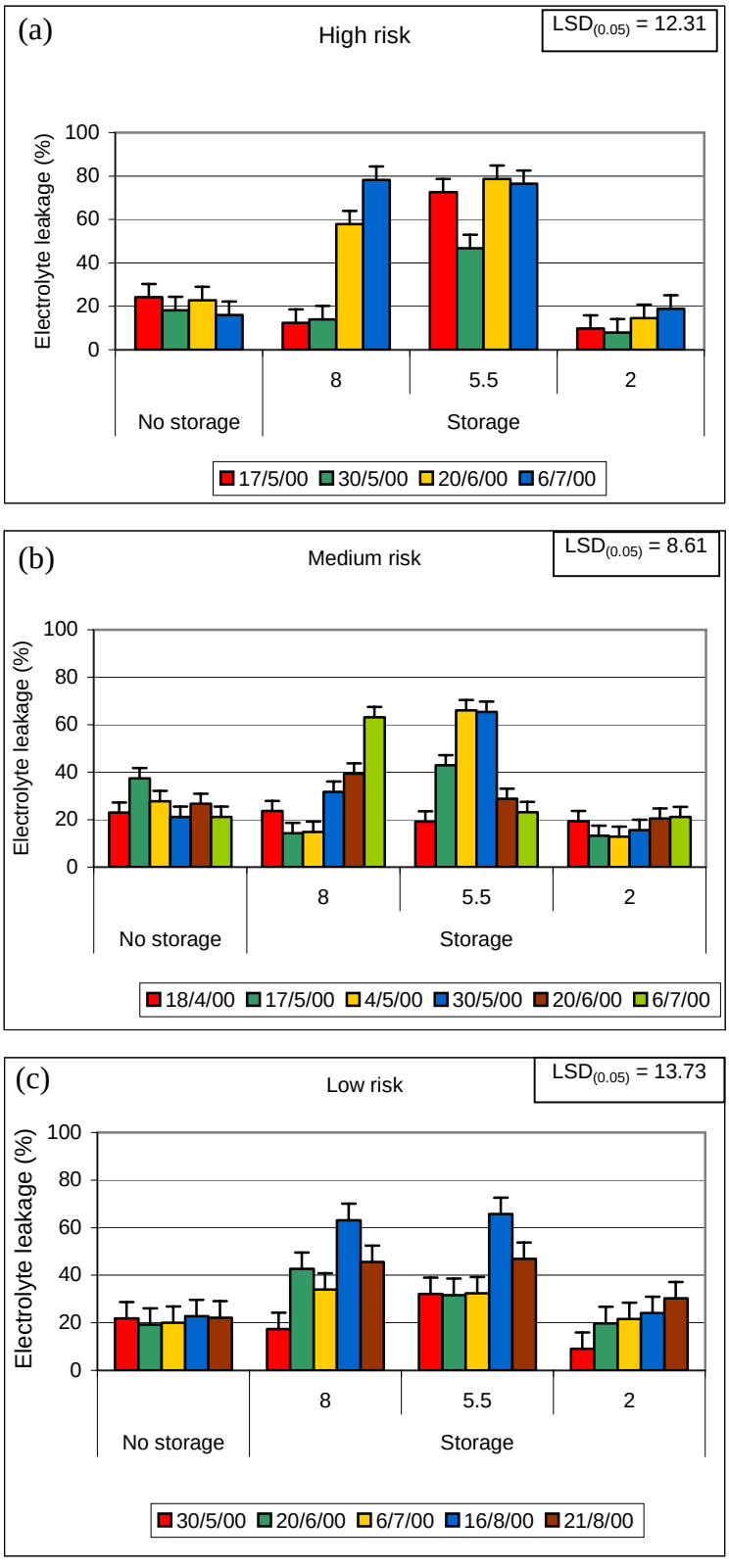

Fig. 2

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 (^{o}C). LSD = Temperature x Date; n = 5.

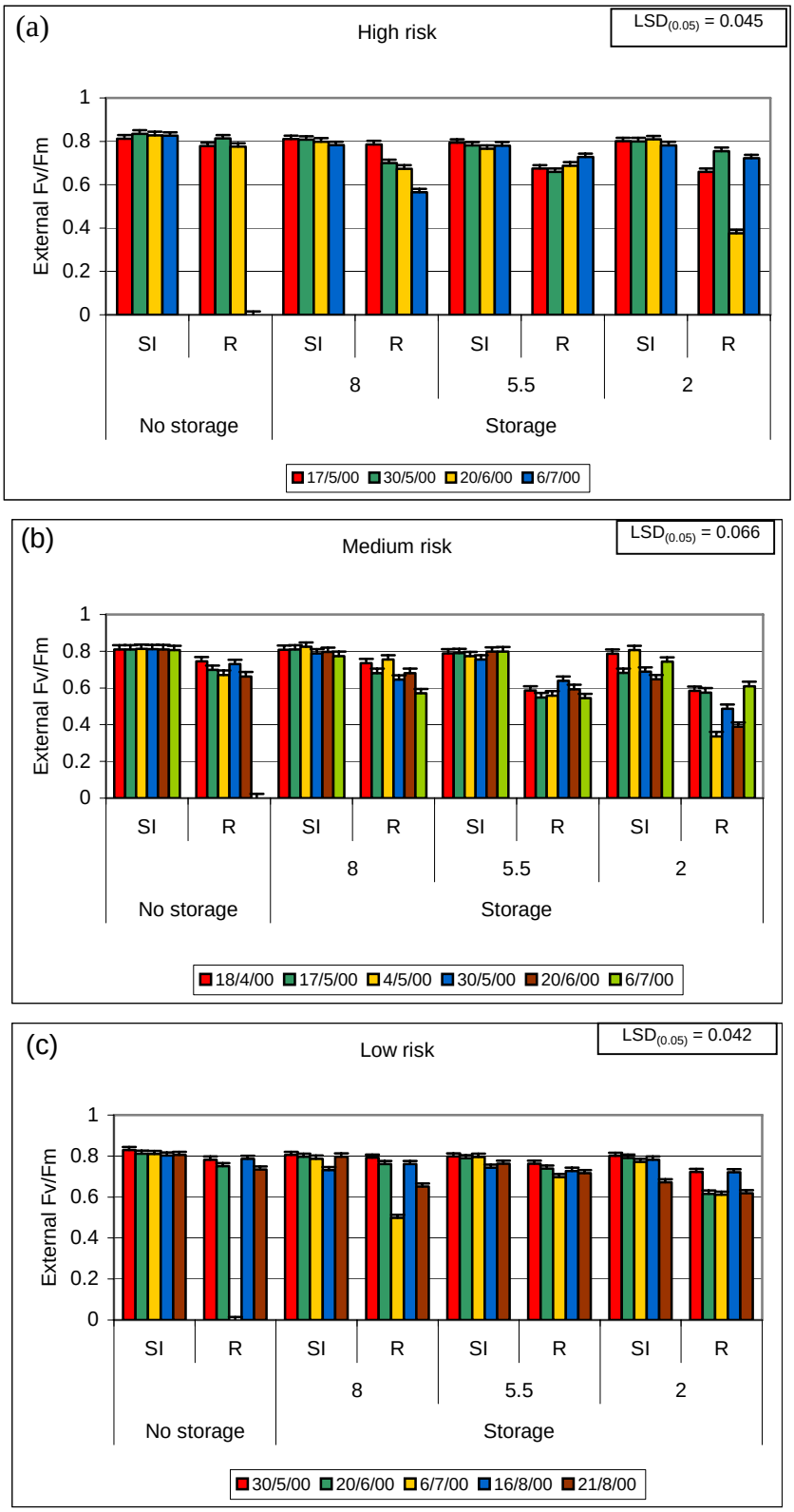

Fig. 3

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 (^{o}C). SI = sampled immediately; R = allowed to ripen. LSD = Temperature; n= 5.

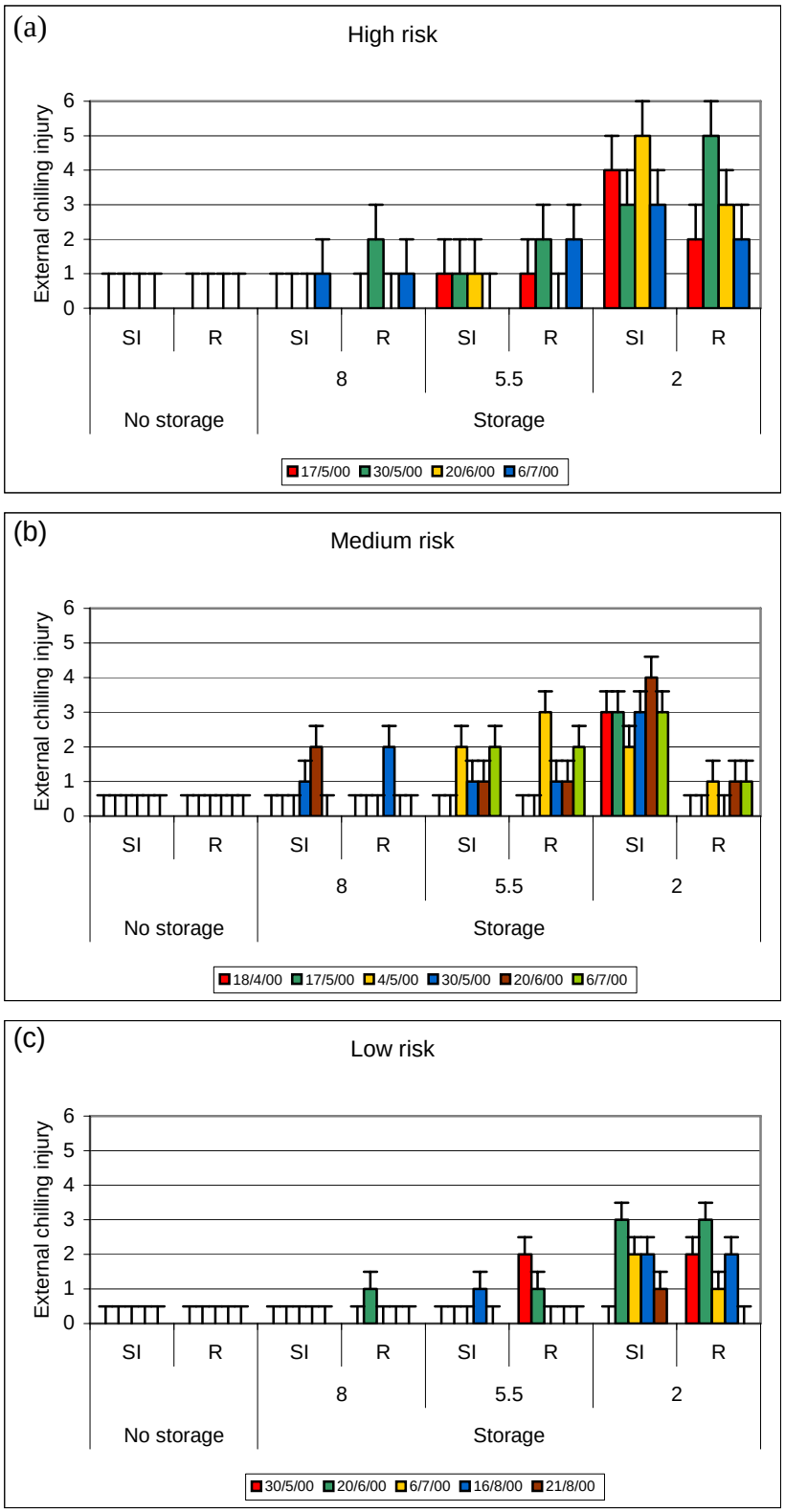

Fig. 4

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 (^{o}C). SI = sampled immediately; R = allowed to ripen. n = 5.

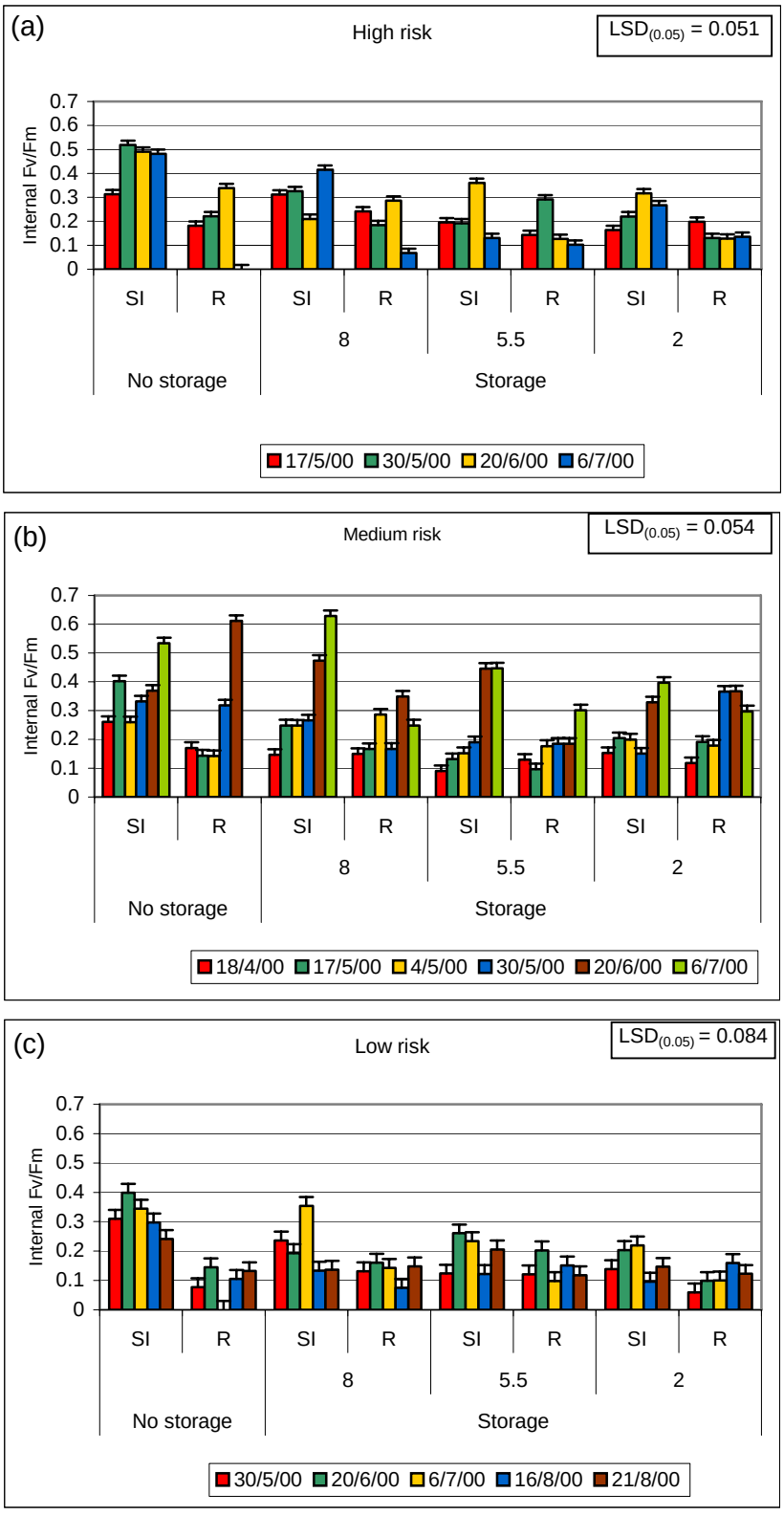

Fig. 5

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 (^{o}C). SI = sampled immediately; R = allowed to ripen. LSD = Temperature; n = 5.