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Mesocarp discoloration in the Pinkerton cultivar

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ABSTRACT

The avocado cultivar 'Pinkerton' is susceptible to an intense blackening of the mesocarp after fruit softening. This disorder has proven to be complex, requiring a better understanding of the fruit's physiology. The purposes of our studies were to identify the role of pre- and postharvest factors or their interactions in the development of the problem. This was done by obtaining fruit from several production areas of varying mesocarp discoloration histories during the 1999 and 2000 seasons. Fruit were stored at 8°C, 6°C, and 2°C for 30 days, as well as ambient (20°C). Evaluations of fruit quality, membrane stability and internal blackening were made before and after storage, as well as after softening. Once removed from storage, fruit softness and respiration rates were monitored daily. While mesocarp discoloration was not as prevalent in the 2000 season, severe cases of anthracnose and stem end rot proved to be problematic in many areas. Membrane integrity studies showed some interaction between temperature and fruit maturity. At the beginning of the season solute leakage was highest in 6°C treatment, but with time the 8°C treatment proved to be worse. This trend was noticeable in fruit from more than one origin. Although chilling injury was observed in some of the treatments, this was not found to be synonymous with mesocarp discoloration. Some interaction was, however, found to exist between the prevalence of the disorder and the origin of the fruit, therefore highlighting the important role of preharvest conditions.

INTRODUCTION

The 'Pinkerton' cultivar initially showed great promise in South Africa giving higher yields than many of the other green skin cultivars. Unfortunately the cultivar has proved to have a few drawbacks, often developing physiological disorders when shipped for any extended time under low temperature storage. The use of low temperature storage is, however, the major means by which the physiology of the highly climateric and rapidly softening avocado fruit can be slowed down so as to arrive at their destination with sufficient shelf life to allow for sale. Once the rate of softening has been controlled, an additional two factors become important to the avocado exporter; that of pathological infections and/or physiological disorders. A physiological disorder particularly severe in the Pinkerton cultivar, is characterized by an intense blackening of the mesocarp after fruit softening. This is unacceptable when shipping to European countries and results in general consumer reluctance to buy this cultivar. Previous studies have indicated that the intensity of the disorder varies from area to area (Bower *et al*., 2000), and even between growers. Growers from high risk areas have thus been discouraged from exporting in an attempt to improve the general impression of the fruit.

Comprehensive studies have already been conducted to ascertain the cause(s) of the disorder, but to date results have shown that the problem is complex and requires a better understanding of the fruit's physiology. Factors that have previously been implicated at a macro level, include preharvest water stress, poor calcium uptake (affecting the structural stability of plant tissue) and the postharvest effects of chilling, oxygen and carbon dioxide ratios and fruit age postharvest, as well as overall maturity.

Fruit blackening is caused by the oxidation of o-diphenols which are catalyzed by the enzyme polyphenol oxidase (PPO) (Van Rensburg and Engelbrecht, 1986). This enzyme is considered to be situated in the thylakoid membranes of the chloroplasts or closely associated microsomes (Vaughan and Duke, 1984), and is primarily membrane bound and non active (Kahn, 1977). Thus, in order for the browning reaction to occur, the enzyme (PPO) and phenolic substrate must come into contact implying some loss of membrane integrity.

The purpose of this work was to elucidate the roles of postharvest shipping temperatures and preharvest factors as determined by fruit origin, on the incidence of mesocarp discoloration. In order to indicate possible mechanisms through which the macro factors may initiate cellular damage, membrane stability and fruit respiration studies were included in the evaluations.

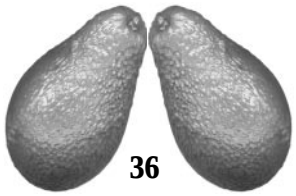
MATERIALS AND METHODS

Fruit were obtained from three different production areas of varying mesocarp discoloration histories during the 1999 and 2000 seasons. Fruit were subjected to normal packhouse procedures, and dispatched by overnight courier to Pietermaritzburg for further treatment and analysis. On arrival the fruit were divided into eight treatments (five fruit per treatment), see Table 1.

Evaluations of fruit quality, membrane stability and internal blackening were made before and after storage as well as after softening. Once removed from storage, fruit softness and respiration rates were monitored daily. Eating ripeness was determined with a hand-held densimeter.

Membrane integrity tests

Solute leakage tests were conducted on all eight treatments to establish the membrane integrity of fruit on arrival as well as before and after storage at the various treatments. This was determined using the technique outlined by Venkatarayappa, Fletcher & Thompson (1984).



A Hansatech plant efficiency analyser was used, as per manufacturers directions, to determine the ratio of variable to maximum fluorescence yield (Fv/Fm ratio) (DeEll *et al.*, 1999) on small pieces of excised mesocarp and exocarp tissue.

Discoloration ratings

During sampling fruit were cut in half (longitudinally) and mesocarp discoloration rated on a scale of 0 to 5, where 0 indicated no discoloration and 5 extensive blackening. The presence of anthracnose (*Colletotrichum gloeosporioides* Penz.) and stem end rot (*Diplodia natalensis* Pole-Evans.) was also noted and scored (0 = none, 1 = present).

Respiration

The rate of carbon dioxide (CO_2) release was determined every day using infra red gas analysis. Fruit were placed in a jar through which air was circulated, at a set flow rate. The jar had both an inlet and an outlet to prevent the accumulation of CO_2.

Maturity

Maturity was ascertained using a freeze drier to determine the moisture content of the fruit.

RESULTS AND DISCUSSION

Compared to the poor quality of the 'Pinkerton' cultivar in 1999, fruit in the 2000 season were remarkably improved in terms of mesocarp discoloration. This was surprising as the heavy rains early in 2000 had threatened to bring with it disaster. Unfortunately the high degree of mesocarp discoloration experienced in 1999 was replaced by very bad infections of anthracnose and stem end rot. Lenticel damage was also a lot more noticeable.

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

Treatment	Storage temperature	Storage period
1*	-	-
2	20°C	until soft
3	8°C	30 days
4	8°C	30 days
	20°C	till soft
5	6°C	30 days
6	6°C	30 days
	20°C	till soft
7	2°C	30 days
8	2°C	30 days
	20°C	till soft

Treatment 1 was sampled on arrival.

Temperature

The role of temperature in the development of mesocarp discoloration, during the 2000 season, was not very clear. Individual growers were found to be affected differently, and considerable differences were found between the intensity of the discoloration in one temperature treatment and the actual number of fruit showing the disorder (Figure 1(a) and 1(b)). Thus, while it generally appeared that the intensity of the disorder was more severe in fruit stored at higher temperatures (8°C and 6°C), a greater number of fruit were affected in the 2°C treatments. During the 1999 season the intensity of the disorder was generally worse at the higher storage temperatures, with the 2°C treatments showing the least discoloration (Bower *et al.*, 2000).

Fruit origin

As in the 1999 season (Bower *et al.*, 2000), fruit origin proved to play an important role, with certain growers having more severe cases of both mesocarp discoloration and pathogen infections (Figure 2(a) and 2(b)).

Membrane integrity

Although the significance of solute leakage differences were not very clear with respect to mesocarp discoloration, fruit from all three production areas showed similar trends during the course of 2000. Initially the 6°C treatment gave the highest solute leakage (Figure 3a), being replaced later by the 8°C treatment (Figure 3b). This indicates that temperature sensitivity changes as the fruit matures, which is to be expected as membrane structure and oil content changes as the fruit mature. It has, for example, previously been found that fruit are more sensitive to very low temperature storage earlier in the season (Toerien, 1986).

In theory, treatments showing high solute leakage immediately after storage would indicate which fruit would potentially show problems upon ripening. High leakage may imply some membrane breakdown during storage leading to the enzyme PPO coming into contact with it's phenolic substrate, normally resulting in a browning reaction. No link was, however, found between unripened treatments displaying high solute leakage and treatments showing some mesocarp discoloration. Furthermore, treatments sampled on ripening all showed high solute leakage regardless of the presence of mesocarp discoloration, with no visible differences being seen between the various temperature treatments. When comparing the actual solute leakage figures between the various fruit origins it was noted that fruit from certain growers gave a higher percent leakage irrespective of disorder or pathogen infection. Fruit with better overall quality often showed higher leakage than fruit from areas with histories of poor quality. Cultural differences perhaps play a large role, as soil and fruit mineral contents would have differed according to fertilizer application rates. Mineral studies will be needed to help elucidate the role of preharvest mineral content on solute leakage and membrane integrity.

Chlorophyll fluorescence technology was used to try and establish the integrity of the chloroplast membrane under different storage temperatures and through different stages of senescence. When

plants become stressed they produce free radicals which result in membrane breakdown and the release of soluble polyphenol oxidase, which normally leads to mesocarp discoloration. The thylakoid membranes of chloroplasts have been shown to be very sensitive to oxidative stress, as is the quantum yield of photosystem II (PSII) (Tijskens *et al*., 1994).

Chlorophyll work done on excised fruit exocarp tissue revealed that Fv/Fm ratios decrease as storage temperatures are lowered (Figure 4). These results agree with work done on chilling injury in cucumbers, stored at low temperatures in the dark, where a decrease in PSII quantum yield was found in fruit stored at low temperatures in the dark (Tijskens *et al*., 1994). Chilling injury is surmised to be the result of a low-temperature-induced irreversible inhibition of enzymes (Graham and Patterson, 1982) and a redistribution of cellular calcium (Minorsky, 1985), leading to the formation of free radicals. The degree of oxidative stress experienced by the cell is a function of the activity of the free-radical generating reactions on the one hand, and the activity of free-radical-scavenging systems on the other. Oxidative stress results in lipid peroxidation (Halliwell, 1987) eventually leading to the break-down of membranes and concomitantly a breakdown in PSII (Gounaris and Selkirk, 1992). No relationship between external chilling injury and internal mesocarp discoloration was, however, found during the 2000 season.

Photosynthetic efficiency ratios taken of mesocarp tissue showed some inconsistent trends with the chlorophyll content being very low in the flesh and other pigments perhaps playing a role. Abbott *et al*. (1993) found chilling injury in eggplant (*Solanum melongena* L.) could not be detected using chlorophyll fluorescence measurements because of high concentrations of red pigments in the skin.

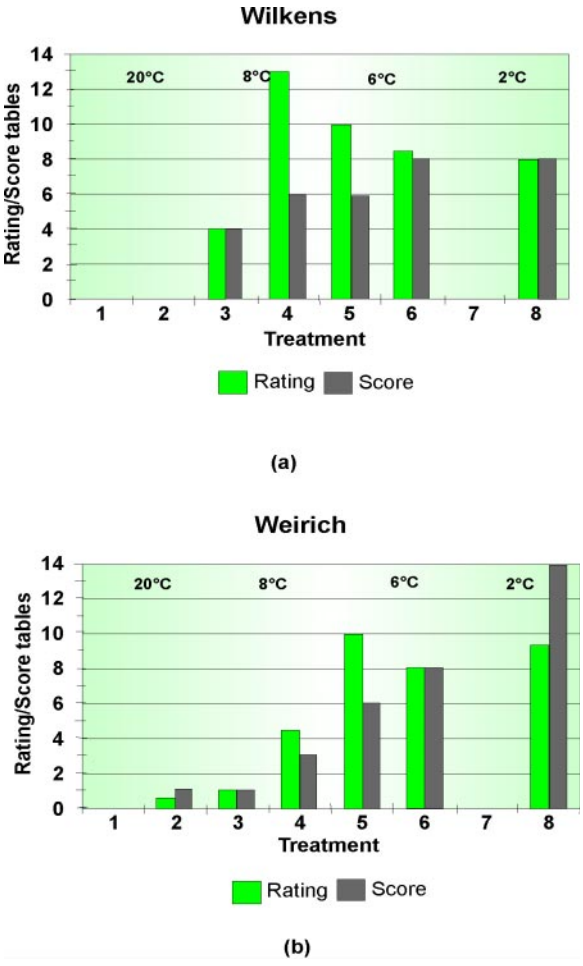
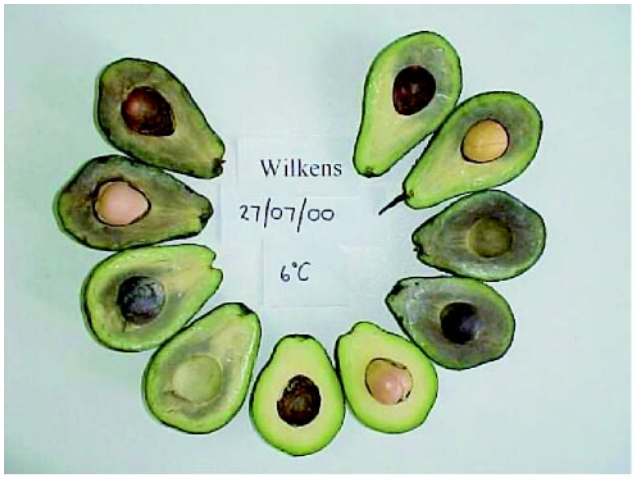
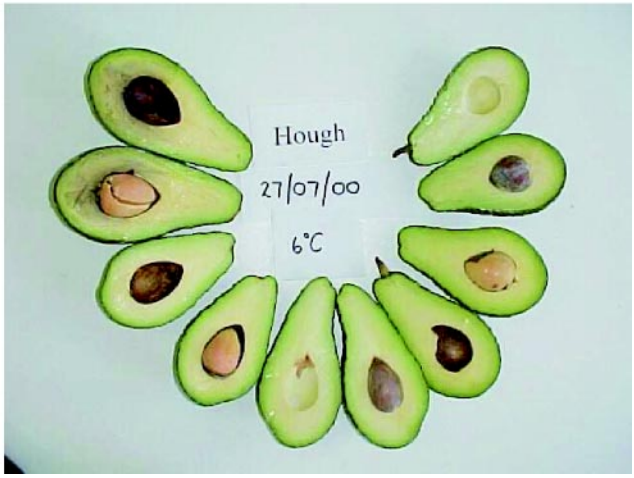


Figure 1 Comparison of the relationship between temperature and mesocarp discoloration in terms of intensity of the disorder (Rating) and the number of fruit affected (Score) between fruit from two different origins; (a) Nelspruit (b) Kiepersol. Treatment 1 = fruit sampled on arrival; 2 = sampled on softening (without storage); 3,5,7 = sampled after 30 days storage at designated temperatures; 4, 6, 8 = sampled on saftening after storage at respective temperatures. Where no data is indicated, a zero rating and score was recorded.



(a)



(b)

Figure 2 Difference in mesocarp discoloration intensity between fruit from a 'high risk' area (a) and a 'low risk' area (b). Fruit from both areas were stored at 6°C.

The role of antioxidants is not confined to free radical scavenging as some researchers have found antioxidants, particularly α-tocopherol (vitamin E), to have the ability to delay the onset of anthracnose and stem end decay in avocados after harvest (Prusky, 1988), further illustrating the importance of antioxidants in fruit quality.

Respiration

Measuring respiration proved to be a very laborious and unrewarding task. While storage temperatures seemed to have an affect on the rate of respiration during the softening phase at 20°C, the relationship between mesocarp discoloration and respiration rate could not be ascertained. This is due to the many factors affecting CO_2 exchange namely; the degree of lenticel damage, the presence and degree of pathogenic infection, and the maturity of individual fruit. The obvious lack in the incidence of the disorder not helping much. In addition, not all fruit within a treatment were affected to the same degree and the small sample size (five fruit per treatment) made interpreting results very difficult. Nonetheless, it was noted that the 'Pinkerton' cultivar seems to have two carbon dioxide peaks when stored at ambient temperature (treatment 2), but not after low temperature storage. Further studies to confirm this, and the possible significance, are needed.

Maturity

As in 1999, no relationship was found between fruit maturity, as indicated by moisture content, and overall mesocarp discoloration. Moisture content was determined on all treatments after sampling. Some interesting trends were, nonetheless, noted through 2000 with different treatments showing different percentages moisture content at sampling (Figure 5). Storage temperatures appear to affect the final moisture content of the fruit. It was noted in fruit from all three packhouses that treatment 5 and often 6 had lower moisture contents at sampling than the others. This, indicates that warmer temperatures alone are not responsible for higher

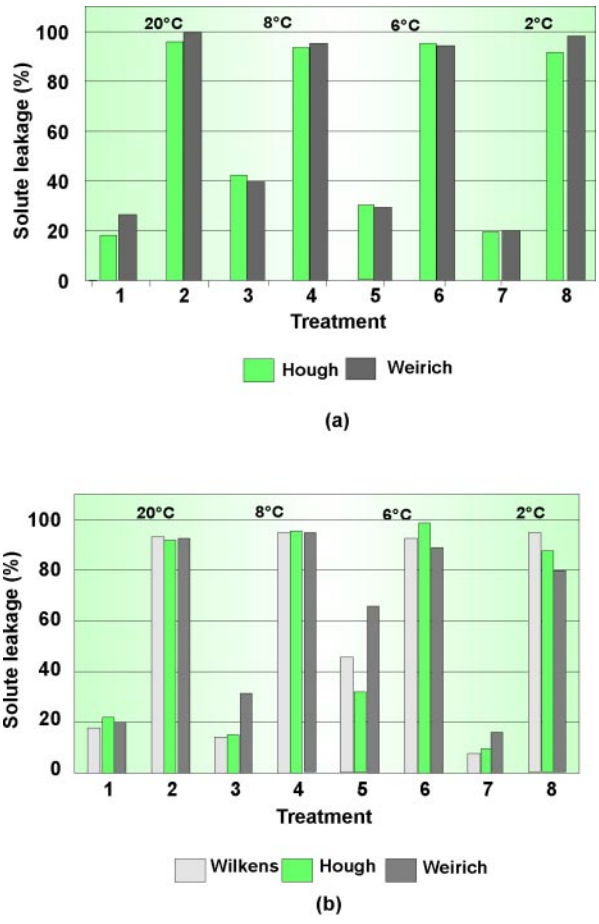


Figure 3 Difference in solute leakage of fruit harvested on the 30/05/00 (a) and the 20/06/00 (b). Treatment 1 = fruit sampled on arrival; 2 = sampled on softening (without storage); 3,5,7 = sampled after 30 days storage at designated temperatures; 4,6,8 = sampled on saftening after storage at respective temperatures.

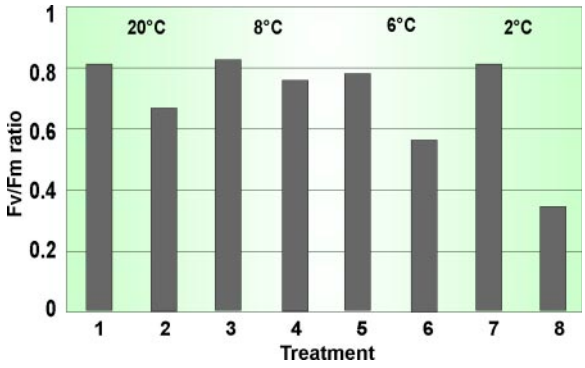


Figure 4 An example of the ratio variable to maximum fluoresence yeild (Fv/Fm ratio) on excised fruit exocarp tissue. Treatment 1 = fruit sampled on arrival; 2 = sampled on softening (without storage); 3,5,7 = sampled after 30 days storage at designated temperatures; 4,6,8 = sampled on saftening after storage at respective temperatures.

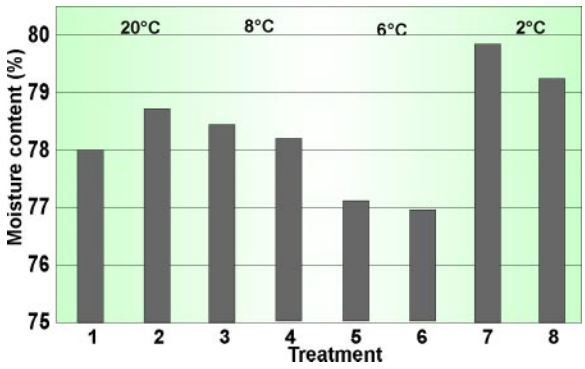
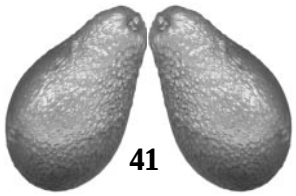


Figure 5 Example of the differences in moisture content, as determined by a freeze drier, in fruit harvested on 17/05/00. Treatment 1 = fruit sampled on arrival; 2 = sampled on softening (without storage); 3,5,7 = sampled after 30 days storage at designated temperatures; 4,6,8 = sampled on saftening after storage at respective temperatures.



moisture loss. One notable difference, besides actual temperature, was that the 6°C storage room showed small variations in air temperature throughout the day, whereas the air temperature in the 8°C storage room was very stable. While thermocouples inserted in the flesh of fruit stored at 6°C showed that pulp temperature remained fairly stable the air temperature was not. Fruit moisture loss appears to be exacerbated by changes in air temperature, highlighting the importance of stable shipping temperatures. The higher solute leakage results in the fruit stored at 6°C could perhaps be indirectly linked to the lower moisture contents at sampling, although this trend was not visible throughout the season.

The high moisture content observed in fruit stored at 2°C had not been expected, but could probably be explained by water condensing on the surface of the fruit, once removed from cold storage, and being taken up through the stomata.

CONCLUSIONS

Although more care was taken by the industry during 2000 to ensure correct shipping conditions, to improve postharvest calcium contents by applying postharvest calcium dips, it is still strongly believed that the disorder is initiated preharvest. Fruit calcium levels, may indeed be very important, and should be improved preharvest by ensuring that tree vigor is controlled (especially during fruit growth). This can be done by controlling nitrogen levels as a vegetative flush will compete with growing fruit for the much needed calcium. The importance of the other minerals should, however, not be overlooked as calcium, magnesium and potassium ratios and contents also play a role in fruit quality (Koen *et al*., 1990). High potassium and low calcium have been shown to aggravate mesocarp discoloration .

Climatic conditions through the growing season will also play a large role. Kruger, Kritzinger and Malumane (2000) found that fruit from cooler production areas were less susceptible to mesocarp discoloration. Temperature has been found to affect lipid saturation (Woolf *et al.*, 1999) which will affect how fruit respond to different storage temperatures. Rainfall patterns may also be important as there was a considerable difference between 1999 and 2000. Compared to 1999 the fruit grown in 2000 would have experienced very little, if any, water stress. The relationship of preharvest stresses, like water stress, perhaps requiring further investigation as preharvest water stress has been found to predispose fruit to higher postharvest levels of soluble polyphenol oxidase levels (Cutting *et al.*, 1989).

Any form of preharvest stress will, therefore, result in the formation of free radicals. Thus, an investigation into the role of antioxidant levels, and factors affecting their concentration should prove useful. Antioxidant levels can be improved culturally and might make a significant difference to overall fruit quality.

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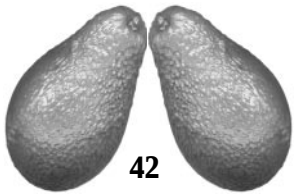

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