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Progress in identifying the primary causes of mesocarp discoloration in cv Pinkerton

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ABSTRACT

Extreme mesocarp discoloration of Pinkerton fruit on arrival and after softening in Europe, has resulted in considerable financial loss, and threatened the future of the cultivar. Attempts to solve the problem by increasing shipping temperature have not been successful. The aim of this study was to investigate the role of shipping temperature, as well as fruit origin, as contributing factors to the development of the disorder. Potential physiological processes leading to breakdown of cellular integrity, such as the development of free radical damage, membrane leakiness and fruit respiration were studied. Results showed that the disorder is not chilling injury *per se*, and that in fact, shipping fruit at 8°C resulted in more intense mesocarp discoloration on softening after 30 days storage, than did 2°C. Some differences in sensitivity to chilling did occur, with fruit origin being implicated. Fruit origin strongly influenced the development of mesocarp discoloration, indicating that this is a preharvest disorder manifested postharvest after storage. A positive correlation existed between the rate of fruit softening and the manifestation of mesocarp discoloration. However, there was not a clear trend between respiration rate and discoloration. Membrane leakiness measurements and attempts to measure possible free radical damage by fluorescence technology were unsuccessful, possibly due to methodology resulting in high variation. It was concluded that the potential for the disorder is determined by preharvest cultural and environmental factors, which interact with postharvest conditions resulting in membrane alteration which allows for the reactions necessary to cause mesocarp discoloration.

INTRODUCTION

The postharvest quality of avocados is variable, especially when shipped for long periods at low temperature. A number of physiological disorders may occur, including a general discoloration of the mesocarp, often referred to as mesocarp discoloration. This discoloration may be present on cutting the fruit, but normally intensifies with exposure of the mesocarp to the atmosphere.

The blackening of the fruit is caused by the oxidation of o-diphenols, catalysed by the enzyme polyphenol oxidase (PPO) (Van Rensburg & Engelbrecht, 1986). The enzyme is considered to be situated in the thylakoid membranes of the chloroplasts or closely associated microsomes (Vaughan & Duke, 1984), and is primarily membrane bound

and non active (Kahn, 1977). The enzyme will not come into contact with the phenolic substrate unless solubilisation and activation occur. This implies (Vaughan & Duke, 1984) some loss of membrane integrity. While some solubilisation will occur during normal ripening, this is usually not of the magnitude required to cause severe mesocarp blackening.

Both pre- and postharvest factors have been implicated in the disorder by work done on other cultivars (Bower & Cutting, 1988). The factors implicated have primarily been at a macro level, such as preharvest water stress, poor calcium uptake (surmised to affect membrane stability under postharvest chilling stress) and postharvest effects of chilling, oxygen and carbon dioxide ratio and fruit age postharvest, as well as overall maturity.

Knowledge of these factors has helped reduce the incidence of the disorder in other cultivars, particularly through the use of managed shipping temperatures and controlled atmosphere storage. The same approach has, however, not had the desired effect in the case of cv Pinkerton (Eksteen, Bezuidenhout, Keevy, Nelson, Reay & Robinson, 1998). Incidence of the disorder to the extent that severe market resistance has occurred, is evident, despite increasing shipping temperatures to limit chilling injury, use of controlled atmosphere storage and attempts to increase calcium content of fruit.

Circumstantial evidence has indicated that fruit from different origins vary in their potential to exhibit the disorder, that controlled atmosphere, while having some positive effect, has not been a solution, and that fruit which ripen rapidly or are soft on arrival after shipment, are more prone to mesocarp discoloration. Shipping at the warmest temperature possible without fruit softening occurring, has also not solved the problem. There is thus confusion over the factors responsible for initiating the apparent cellular collapse resulting in mesocarp discoloration.

The purpose of this work was to elucidate the roles of postharvest temperature during shipment 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 were included in the evaluations.

MATERIALS AND METHODS

Fruit was evaluated from a number of sites, determined from historical data to have differing potentials for disorder development, using more than one picking date, to take account of maturity differences. The areas consisted of Kiepersol (two dates and two packhouses within the same geographical area), White River (two dates) and Schagen. While all these areas are situated within approximately 50 km of Nelspruit, considerable microclimate differences are evident, due primarily to altitude.

Fruit was subjected to normal packhouse procedures, and dispatched by overnight courier to Pietermaritzburg for further treatment and analysis.

The following storage treatments were applied:

20°C until fruit softened (no cold temperature storage)

8°C for 30 days followed by softening at 20°C

4.5°C for 30 days followed by softening at 20°C

2°C for 30 days followed by softening at 20°C

In all cases, evaluations of fruit quality parameters (as outlined later) were conducted on a sub-sample on arrival before softening, and immediately after removing fruit from the respective cold rooms. Fruit was also evaluated when deemed soft as determined by a fruit firmness tester. Each sample consisted of 20 randomly selected and labeled (on arrival) fruit, with 10 used for testing before and 10 after softening.

The following evaluations were carried out:

Fruit was cut, and mesocarp discoloration determined visually on a 1-5 scale, where 1 indicated no discoloration and 5 extensive blackening and not fit for consumption.

Membrane leakiness was determined using the technique outlined by Venkatarayappa, Fletcher & Thompson (1984).

Chlorophyll fluorescence technology has recently been used to indicate plant stress, including chilling, heat, water, and atmospheric stress. In addition, DeEll, Kooten, Prange and Murr (1999) report usefulness in determining shelf life, ripening and senescence of fruits. As the PPO related to mesocarp discoloration is normally in or associated with chloroplasts, it is possible that evaluation of the fluorescence parameters will provide an early indication of chloroplast membrane integrity and/or the possibility of free radical damage which could result in release of soluble PRO, and thus mesocarp discoloration. A Hansatech plant efficiency analyser was used in accordance with the manufacturers instructions, and the F_v/F_m ratio (DeEll *et al*., 1999) determined on a small piece of excised mesocarp tissue.

During the fruit softening phase, CO_2 evolution, as an indication of respiratory activity, was measured on a daily basis with a PP systems CO_2 analyser. Fruit were placed in glass jars and sealed for 10 minutes, after which the head space CO_2 concentration was determined and results calculated as rate of CO_2 evolution, taking into account fruit mass and free space in the jar.

RESULTS AND DISCUSSION

FRUIT ORIGIN

Evaluation of fruit at the soft stage, all treatments combined, showed that origin played a considerable role in mesocarp discoloration. Figure 1 indicates that the packhouses in the Kiepersol (Bella Nova and Kiepersol) area had the fruit with the highest discoloration index.

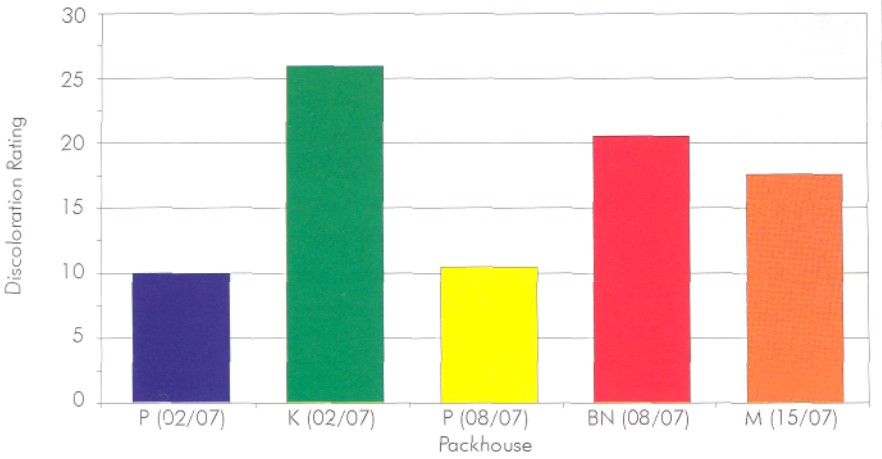

Figure 1 *Discoloration rating of fruit from each of the various packhouses (cumulative for all treatments). P = Pienaar, K = Kiepersol, BN = Bella Nova, M = Mayo. Date of harvest indicated in brackets.*

The best quality fruit (least discoloration) came from the Pienaar packhouse, which is situated closer to White River, and where the climate is said to be slightly cooler as well as the soils being different. The two picking dates at this packhouse did not have any effect. Fruit from the Mayo packhouse at Schagen, which is cooler than the other areas, had fruit of intermediate quality. Thus, while there was a clear effect of fruit origin, the reasons for the difference are not as evident, with the climatic conditions, particularly temperature, interacting with other factors. Many of the avocado orchards in the Kiepersol area are planted on old banana plantation soils, where nitrogen and potassium contents are very high. Fruit maturity, (as measured by percentage moisture content), while possibly playing a role in the overall development of the disorder (Kruger, personal communication, Institute for Tropical and Subtropical Crops), did not appear to play any definitive role within the group of samples tested. This is similar to findings of Zauberman & Jobin-Decor (1995) in their work on Hass.

STORAGE TEMPERATURE

Overall mesocarp discoloration (combination of all sites and harvest dates) showed a tendency for decreased discoloration as temperature of storage was decreased (Figure 2). This was evident in fruit cut immediately after removal from storage as well as after softening. Storage at 2°C was thus optimum overall. This accords well with the work of Zauberman & Jobin-Decor (1995), who found that Hass fruit could be stored at 2°C for up to five weeks without injury, while that at 7°C developed significant discoloration.

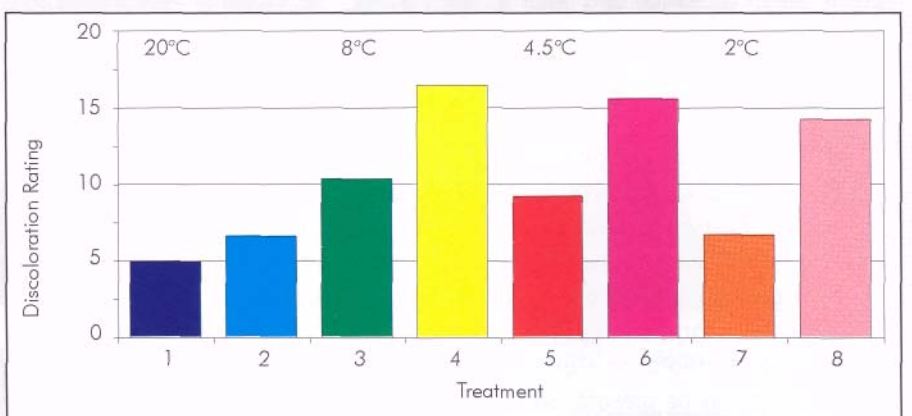

Figure 2 ***Discoloration rating of fruit within each of the various treatments (cumulative for all packhouses). Treatment 1 = fruit sampled on arrival; 2 = sampled on softening (without storage); 3, 5, 7 = sampled after 30 days storage at designated temperatures; 4, 6, 8 = sampled on softening after storage at respective temperatures.***

However, analysis of fruit from individual consignments, representing various sites and harvest dates, show a varying sensitivity to temperature. Figure 3a shows Kiepersol fruit harvested on 2 July, to have an optimal storage temperature of 4.5°C, while that from a different packhouse picked a week later, had the previously described trend whereby 2°C was optimum, with 8°C the most affected by mesocarp discoloration. However, the overall quality of the fruit was poor as was evident by the mean rating, and caution should be exercised in interpreting the temperature sensitivity of the earlier harvested fruit.

Fruit from the Pienaar packhouse was of considerably better overall quality than that from the Kiepersol area, and again was of optimal quality when stored at 2°C in the case of the first consignment. The second consignment, one week later, showed the greatest discoloration at 4.5°C (Figure 3b). The reason for this change in sensitivity is unknown, but illustrates the complexity of the situation, and strongly emphasises the role of preharvest factors in the development of postharvest mesocarp discoloration.

In the case of fruit from Mayo in the Schagen area, (Figure 3c) overall quality was again fairly poor on softening after storage. Fruit examined after storage but before softening, again showed the trend of decreasing disorders with decreasing temperatures. However, fruit at the warmer temperature may have started softening in storage and could therefore be expected to show such a trend. The fruit at soft stage showed 4.5°C to be the best temperature of storage, but as with the Kiepersol fruit, overall quality was poor with storage temperature appearing to have little influence.

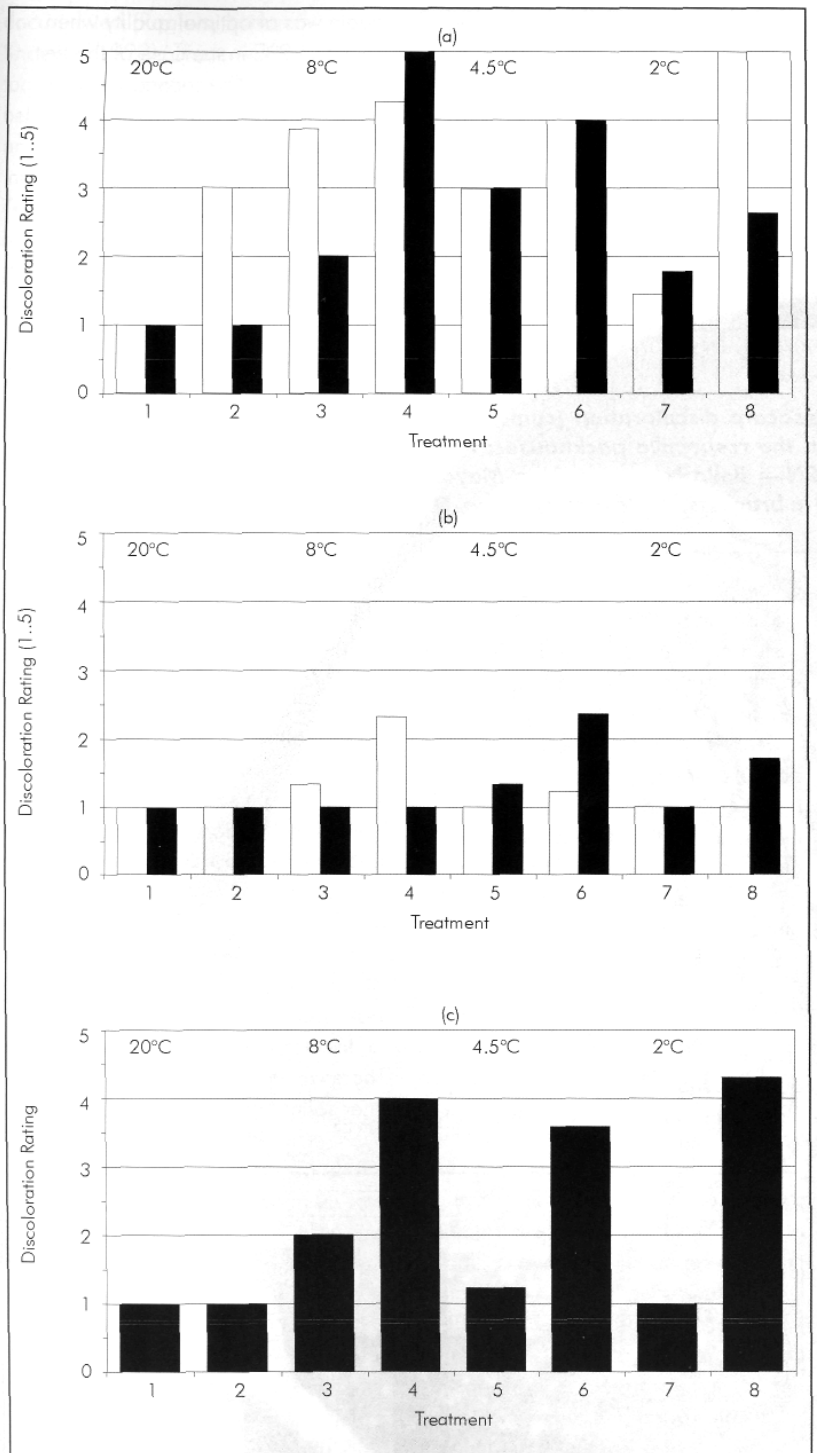

Figure 3 ***Comparison of the extent of mesocarp discoloration between the different packhouses (a) Kiepersol □ (02/07) ■ (08/07), (b) Pienaar □ (02/07) ■ (08/07) and (c) Mayo (15/07). Treatment 1 = fruit sampled on arrival; 2 = sampled on softening (without storage); 3, 5, 7 = sampled after 30 days storage at designated temperatures; 4, 6, 8 = sampled on softening after storage at respective temperatures***

MEMBRANE DAMAGE

Attempts to identify membrane leakiness or instability as a precursor to discoloration were not successful. There was a trend to higher leakiness after softening, which has been well documented (Rogiers, Kumar & Knowles, 1998). There was, however, too much variability in results both before and after softening, to make any conclusions.

The fluorescence ratio of F_v/F_m, which has been found useful in determining stress in a number of fruit where chloroplasts are found (DeEll *et al.,* 1999), showed a tendency for the ratio to decrease after storage. The magnitude of the decrease showed some relation to a higher incidence of mesocarp discoloration. However, the results were again not sufficiently consistent. This may have been due to sample variability, and with improvement in technique, may be more useful. Nevertheless, the results may imply a disruption in chloroplast activity.

FRUIT SOFTENING AND RESPIRATION

Considering all packhouses harvest times and storage temperatures (Figure 4), a trend was evident whereby the more rapidly the fruit softened, the more intense the mesocarp discoloration. This supports the field observations. There would therefore appear to be higher metabolic activity in the fruits most susceptible to the disorder.

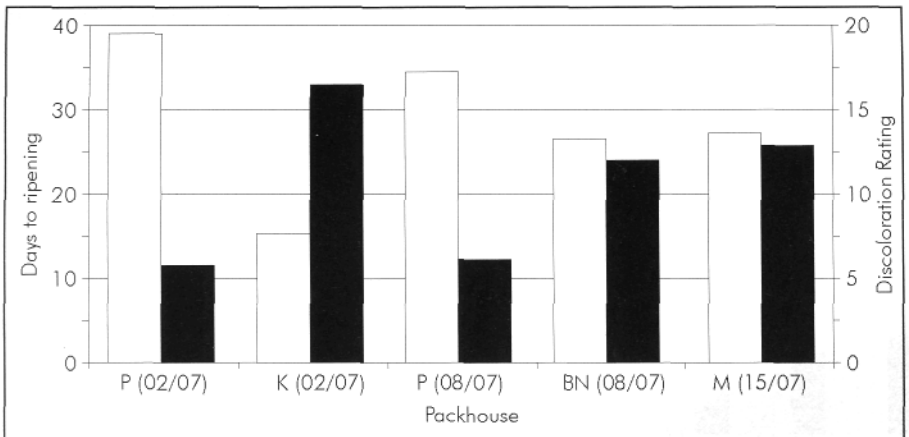

Figure 4 ***Days to ripening and mesocarp discoloration (cumulative for all treatments) within the respective packhouses: P = Pienaar, K = Kiepersol, BN = Bella Nova and M = Mayo. date of harvest indicated in brackets. □ Days to ripening, ■ Discoloration***

The metabolic activity should be reflected in the rate of fruit respiration. Figure 5 shows the mean respiration rate during softening, after each of the storage temperatures.

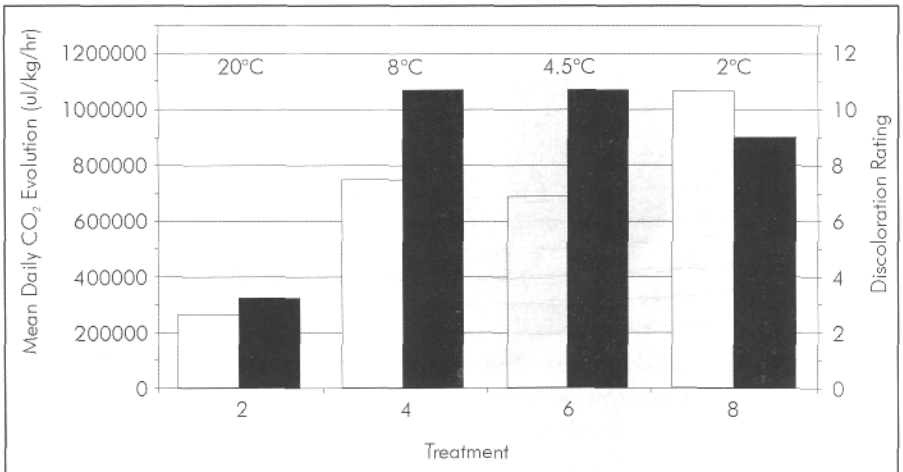

***Figure 5** Mean daily rate of CO_2 evolution and mesocarp discoloration (cumulative for all treatments) in fruit sampled on softening after storage at the designated temperatures.*
□ Rate of CO_2 evolution ■ Discoloration

There was no consistent trend between respiration rate and mesocarp discoloration. Similarly, individual packhouses (data not shown) did not produce clear trends. This does not, however, mean those metabolic activity or respiration rates are not involved in the development of the disorder. A lower than expected respiration rate may be an indication of earlier cellular damage, rendering the respiratory process less functional at the time of measurement. This damage may have occurred during the storage period (fruit will be respiring, albeit at a lower rate, than during the ripening period at ambient temperature). If sufficient damage has occurred at an early stage, mesocarp discoloration could be expected to have taken place during the storage period. In fruit from the Kiepersol area in particular (Figure 3a) this was indeed so, as fruit cut immediately after removal from storage did show considerable mesocarp discoloration. Further study of respiratory activity during storage will be necessary to confirm the link.

The reasons for the possible destruction of membranes and cellular disruption are at this stage speculative. It is possible, that more than one mechanism operates. Blanke (1991) points out that should anaerobic respiration occur, free radicals will be produced, which if not removed from the system, will result in damage to membranes. Kosiyachinda & Young (1977) found that in avocado, a phase change in lipids occurs at 9°C. This would likely change the activity of membrane bound enzymes involved in respiration. If aerobic respiration is replaced by anaerobic, free radicals will be produced. The number of free radicals and subsequent destruction could be dependent upon the rate of respiration (which should decrease with temperature), and the ability to remove free radicals by way of free radical scavengers. The damage done by free radicals will also depend upon membrane structure, which may be modified by numerous items, including calcium, boron and degree of lipid saturation. Dependent upon the combination of factors present, an optimal temperature range for storage exists, which is either above the critical temperature at which lipids undergo phase changes, or as low as possible such that it is just above that which extensive cell

disruption and subsequent death takes place.

Woolf, Bowen & Furguson (1999) found that avocados grown in warmer areas contained more saturated lipids.

The Kiepersol area is said to be warmer than the other areas from which fruit was obtained. This would imply a higher critical temperature at which anaerobic respiration will occur. In addition, the soils in the area contain high levels of potassium and nitrogen, said to depress tochopherol content (Avdonin & Kochubei, 1979). The avocado contains high levels of α-tochopherol in the chloroplasts and as it is known to be an efficient antioxidant to protect lipid peroxidation (Leipner, Fracheboud & Stamp, 1997), the concentration in the chloroplasts could be critical.

CONCLUSIONS

Work conducted thus far, indicates that mesocarp discoloration in Pinkerton cannot be attributed to chilling injury *per se* and that in fact shipping temperatures lower than presently used would be more desirable. There is also strong evidence that potential for the disorder is determined preharvest, by both environmental and cultural conditions.

Further work in relation to respiration, ethylene formation and anti-oxidants (α-tochopherol in particular), will be necessary to further define the factors pre-disposing fruit to the disorder. Thereafter, both predictive and remedial action can be taken. The major component to decreasing the incidence of the disorder will likely be orchard modification of the critical components, which could vary from site to site, hence the need to clearly identify them.

LITERATURE CITED

AVDONIN, N.S. & KOCHUBEI, I.V. 1979. The effect of derno-podzolic soil and fertilizers on spinach chemical composition. *Horticultural Abstracts* 49: 49-70.

BLANKE, M.M. 1991. Respiration of apple and avocado fruits. *Postharvest News and Information* 2(6): 429-436.

BOWER, J.P. & CUTTING, J.G.M. 1988. Avocado fruit development and ripening physiology. *Horticultural Reviews* 10: 229-271.

DeELL, J.R., VAN KOOTEN, O., PRANGE, R.K. & MURR, and D.R 1999. Applications of chlorophyll fluorescence techniques in postharvest physiology. *Horticultural Reviews* 23: 69-107.

EKSTEEN, G.J., BEZUIDENHOUT, J., KEEVY, C., NELSON, R., REAY, N. & ROBINSON, R. 1998. The effect of intransit and storage conditions on the quality of avocados - 1977 seasons. *South African Avocado Growers' Association Yearbook* 21: 100-108.

KAHN, V. 1977. Latency properties of polyphenol oxidase in two avocado cultivars differing in their rate of browning. *Journal of Science Food and Agriculture* 28: 233-239.

KOSIYACHINDA, S. & YOUNG, R.E. 1977. Succinoxidase activity of avocado fruit mitochondria in relation to temperature and chilling injury throughout the climacteric cycle. *Plant Physiology* 60: 470-474.

LEIPNER, J., FRACHEBOUD, Y. & STAMP, P. 1997. Acclimation by suboptimal growth temperature diminishes photo-oxidative damage in maize leaves. *Plant cell & environment* 20: 366-372.

ROGIERS, S.Y., KUMAR, G.N.M. & KNOWLES, N.R. 1998. Maturation and ripening of fruit of *Amelanchier alnifolia* Nutt. are accompanied by increasing oxidative stress. *Annals of Botany* 81: 203-211.

VAN RENSBERG, E. & ENGELBRECHT, A.M.P. 1986. Effect of calcium salts on susceptibility to browning of avocado fruit. *Journal of Food Science* 51: 1067-1070.

VAUGHAN, K.C. & DUKE, S.O. 1984. Function of polyphenol oxidase in higher plants. *Physiologica Plantarum* 60: 106-112.

VENKATARAYAPPA, T., FLETCHER, R.A. & THOMPSON, J.E. 1984. Retardation and reversal of senescence in bean leaves by benzyladenine and decapitation. *Plant and cell physiology* 25(3): 407-418.

WOOLF, A.B., BOWEN, J.H. & FERGUSON, I.B. 1999. Preharvest exposure to the sun influences postharvest responses of 'Hass' avocado fruit. *Postharvest Biology and Technology* 15: 143-153.

ZAUBERMAN, G. & JOBIN-DECOR, M.P. 1995. Avocado (*Persea americana* Mill.) quality changes in response to low-temperature storage. *Postharvest Biology and Technology* 5: 235-243.