Ultra-low temperature shipping and cold chain management of 'Hass' avocados: Investigation into reducing shipping costs

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ABSTRACT
Alternate treatments that could be used to possibly extend the shelf life of avocado fruit as effectively as the use of 1-MCP were evaluated, as the use of 1-MCP is costly. Previous work indicates that cold storage at 1ºC might be able to replace the use of 1-MCP in 'Hass' avocado fruit. Fruit harvested at early (72% moisture content), mid (66% moisture content) and late (60% moisture content) season were stored at 1°C or 5.5°C for 28 and 56 days. Additional treatments were 1-MCP treated and untreated, waxed and non-waxed as well as cold chain breaks (24 hour delay, break at day 14). Fruit weight, softness, ethylene evolution, days to ripening, external and internal quality were determined. It was found that fruit stored at 1°C softened more slowly and lost less mass, thereby ultimately extending the shelf life to the same extent as the 1-MCP treated fruit at 5.5°C. There were also indications that the 1°C negated the effects of cold chain breaks to a certain extent, while a delay of 24 hours before placing the fruit into cold storage resulted in significant water loss, which was detrimental to final fruit quality and shelf life. It was found that the early season fruit had the most desirable attributes with respect to shelf life and least affected by cold chain breaks, however, the immaturity of the fruit lead to chilling injury. The mid season fruit proved to be the best in terms of quality. The 56 day storage trial showed that if 1°C as well as 1-MCP are used in conjunction, it can be a credible option, however not for early season fruit due to excessive chilling injury. Overall, it may indeed be possible to replace the use of 1-MCP with low temperature shipping at 1°C for 'Hass' avocados, with respect to extending shelf life and negating effects of cold chain breaks. Further investigations are required in terms of the physiological aspects, and are currently being conducted.

INTRODUCTION
Fruit quality is of utmost importance for the export market, and all possible measures should be used in order to ensure the fruit that is exported will arrive at its market with the desired quality. In the case of the South African avocado industry, unique challenges are present with respect to the logistical effort required to export avocados to the European market (Dodd *et al*., 2007). With a total transit time of up to 30 days, there is an obvious need to slow the natural ripening processes of the avocado fruit so that export can be possible to Europe. The current technologies used are controlled- and modified atmosphere (CA and MA) or the use of an ethylene receptor blocker 1-methylcyclopropene (1-MCP), but it has been found that problems are still associated with the use of these protocols. Examples of such problems include 1-MCP fruit having uneven ripening (Mare *et al*., 2002), extreme CA conditions with high CO_2 levels (>5%) leads to carbon dioxide poisoning, inhibiting ripening, body rots, hypoxia and anoxia (Kader, 2003; Arpaia *et al*., 1990; Burdon *et al*., 2008), and not least of which are the costs involved to implement these technologies. An opportunity has arisen in trying to achieve the same, or even better, export standards without the use of current protocols (*i.e*. 1-MCP and 5.5°C) by instead using ultra-lower temperatures such as 1°C for 'Hass'. If it is found that shipping at lower temperatures is comparable to the use of CA or 1-MCP, then costs would be reduced. There has also been indication that cold chain breaks associated with the logistics have severe effects on the final quality of the fruit (Undurraga *et al*., 2007). There has been very little work done on this issue and the actual losses that are involved due to this problem are unknown. Work conducted by Blakey and Bower (2009) as well as Kok *et al.* (2010) illustrated that the use of 1°C significantly improved the quality of the fruit whereas fruit at 5.5°C which incurred the same cold chain break treatment had a figure of less than 30% being of acceptable quality. To date there has been no physiological work done with respect to the effect cold chain breaks have on the fruit in terms of enzyme activity. It is clear therefore that this could

be an opportunity to determine what actually is involved within the fruit on a physiological level when a cold chain break occurs, and link these results to the final quality of the fruit and the expected shelf life. Better understanding of the fruit physiological changes under these conditions will help optimise logistics and enhance fruit arrival quality.

Marketing flexibility is also possible if the length of storage can be extended. It would also be useful if delays in shipping occur.

The research objectives were thus to ascertain whether storage at 1°C as opposed to current protocol of 5.5°C is comparable to the use of 1-MCP during simulated shipping, as well as to ascertain the effects of cold chain breaks on the final fruit quality for 'Hass'. Furthermore an investigation into an extended storage time of 56 days was conducted to ascertain whether it can be a credible option for export.

MATERIALS AND METHODS

Fruit

'Hass' avocado fruit were obtained from a designated pack house in Wartburg, KwaZulu-Natal. The fruit were harvested throughout the season at significantly different maturity levels from one block in the orchard. Early season fruit were harvested on 28 July 2010 with a moisture content of 72%. The mid season fruit were harvested on 2 September 2010 with a moisture content of 66%, and the late season fruit were harvested on 16 September 2010 with a moisture content of 60%. Post-harvest operations of waxing, 1-MCP, pre-cooling, grading, sizing and packing to 'count 20' were all conducted at the pack house. Half the fruit samples were collected off the packline before waxing, while the other half remained on the packline to be waxed. The fruit treated with 1-MCP were to standard export protocols for sixteen hours at a temperature of 5.5°C, whilst the untreated fruit were stored under the same temperature for the same period but without 1-MCP. After the initial pack house treatment, all fruit were transported to the laboratories of the Horticultural Science Department at the University of KwaZulu-Natal, and immediately prepared for simulated shipping for a period of 28 days. Half the fruit were stored at 1°C (±0.5°C) and the other half at 5.5°C (±0.5°C). Cold chain break treatments were applied throughout the cold storage period where there was a control (no break for 28 days and 56 days), 24 hour delay before cold storage, and a break at day 14 where the break would be regulated to eight hours. To monitor the internal temperature and relative humidity of the storage containers, HOBO® H8 data loggers were used.

Each of the treatment combinations consisted of ten fruit replicates.

Data collection

Visual observations of fruit condition were made at start, during each break period, when removed from cold storage and during ripening. Before storage, fruit mass, ethylene evolution and fruit softness were also measured. Fruit were visually assessed after storage and warming to room temperature, for external damage, including chilling injury and lenticel damage. After ripening at room temperature (20-25°C), fruit were cut and assessed for anthracnose, stem-end rot, vascular browning and mesocarp discolouration. Ethylene evolution was also determined during the ripening period on a daily basis.

Ethylene measurement

Ethylene was measured using a gas chromatograph (DANI 1000, DANI Instruments S.p.A., Monzese, Italy). Measurements were taken prior to treatment as well as through the ripening period until fruit reached the 'ripe' stage. To measure ethylene production, a 20 ml glass vial was placed in a sealed 1 L jar for 30 min, thereafter sealed and transferred to the GC autosampler (HT250D, HTA S.r.L., Brescia, Italy). The GC was equipped with a flame ionization detector (FID), stainless steel packed column with an alumina-F1 stationary phase. The injector, column and detector temperatures were 160, 80 and 180°C, respectively (Blakey & Bower, 2009).

Fruit softness

Ripening time was calculated as the number of days from harvest until 'eating soft' stage. Fruit were deemed ripe when the average reading on a densimeter was approximately 60. A 5 mm densimeter was used to measure fruit softness (ripeness) on a scale of 85-90 (hard) to 55-60 (soft). Four equally spaced readings were taken around the circumference of each fruit and the average reading recorded.

Statistical analysis

The data was analysed in the form of a factorial design, where each treatment combination consisted of ten fruit, each constituting a single replication. A general analysis of variance was run, using Genstat12, where the ANOVA, table of means, and LSD was computed to identify significantly different treatment combinations.

RESULTS AND DISCUSSION

Fruit softening

In terms of the seasonal maturity variances it was found that the 1°C treatment significantly suppressed the amount of softening occurring within cold storage in comparison to the 5.5°C treatment (**Figure 1**). It was unexpectedly found that the mid season fruit experienced the highest percentage softening, as one may expect the late season fruit to incur the highest softening. A similar trend was shown for the 1-MCP interaction (**Figure 2**). However, the significant differences were not as great as the temperature interaction shown previously. With respect to the cold chain break interaction it was found that the early season fruit were least affected by the cold chain breaks, whereas the mid and late season fruit followed the expected trend where the 24 hour delay causes the highest amount of softening followed by

the break within storage at day 14 and then the least softening resulting from no break occurring (**Figure 3**).

More importantly, as shown in previous findings by Kok *et al.* (2010), it was shown that fruit stored at 1°C, regardless of whether 1-MCP treatment was used, had substantially less softening than fruit at 5.5°C (**Figure 4**). It highlighted that even fruit treated with 1-MCP at 5.5°C still experience significantly higher softening throughout storage as compared to fruit at 1°C without the use of 1-MCP.

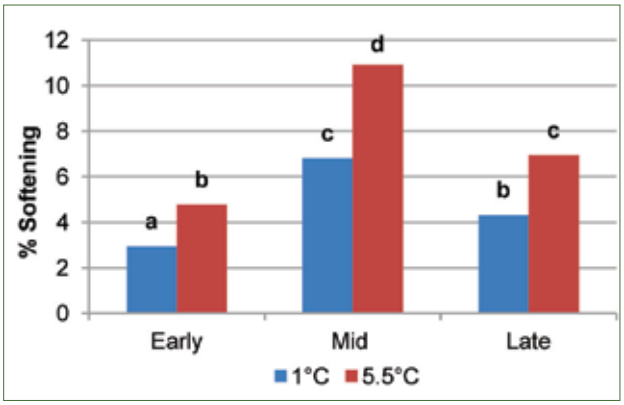

Figure 1. Percentage softening after storage period of 28 days as influenced by maturity stages and temperature treatments.

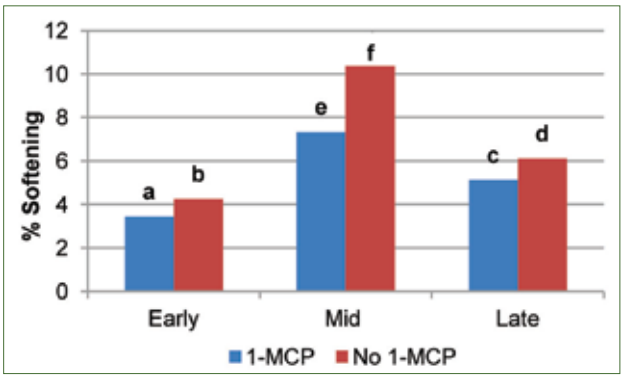

Figure 2. Percentage softening after storage period of 28 days as influenced by maturity stages and 1-MCP treatments.

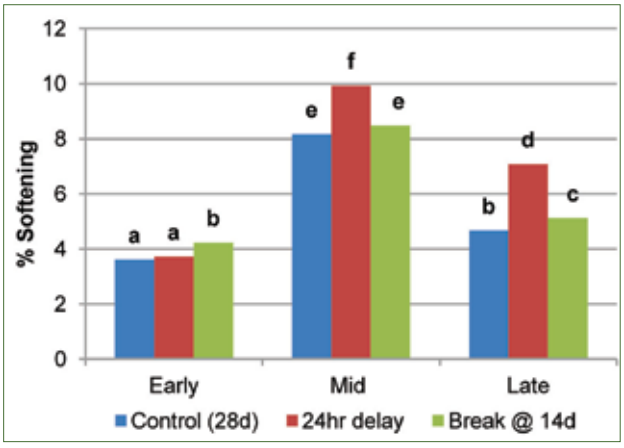

Figure 3. Percentage softening after storage period of 28 days as influenced by maturity stages and cold chain break treatments.

Treatment interactions of 1-MCP as well as 1°C were found to be not significant in terms of negating cold chain breaks. It was indicated, however, that if a cold chain break occurs within storage (*i.e.* break at day 14) then both the applied treatments successfully negated the effect of cold chain breaks in terms of fruit softening which correlated to findings by Kok *et al.* (2010), as well as the findings of Blakey and Bower (2009). The 24 hour delay could not be rectified, which is to be expected to a certain extent due to this occurring before any of the treatments were implemented.

Mass loss

The mass loss, measured as a percentage, can be assumed to be predominantly due to the loss of water. It was found that the main effects of temperature, waxing and cold chain breaks were significant in differences experienced amongst their respective treatments. In **Figure 5** it can be seen that storage at 5.5°C resulted in a higher fruit mass loss than 1°C. These findings correlate to those found by Kok *et al.* (2010) as well as Blakey and Bower (2009). The waxing treatment indicated that the unwaxed fruit lost a significantly higher amount of water when compared to the waxed fruit, as found by Bower and Jackson (2003), however, this did not translate to any subsequent softening as there was no significant difference

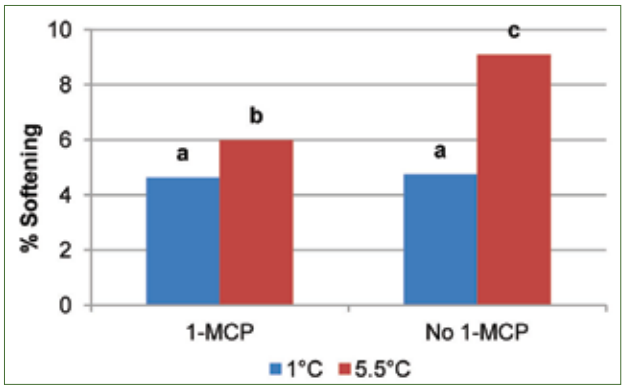

Figure 4. Percentage softening after storage period of 28 days as influenced by treatment with 1-MCP and temperature.

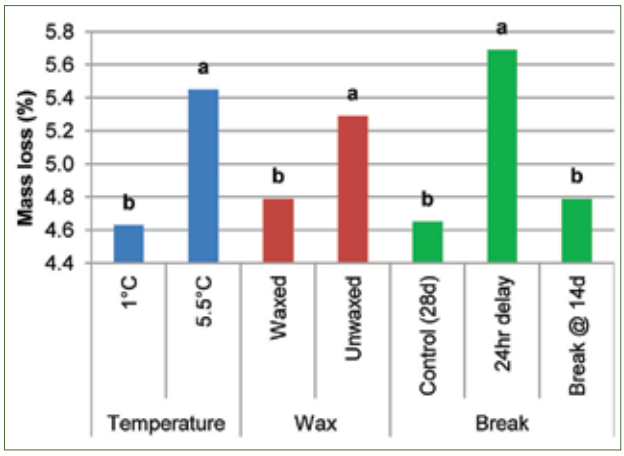

Figure 5. Main effects of temperature, waxing and cold chain breaks on the percentage mass loss of fruit after 28 days cold storage.

found for the waxing treatments in terms of softening. The results for the cold chain breaks illustrated that the 24 hour delay causes significant mass loss, which is to be expected due to the vapour pressure deficit (VPD) between the fruit and the surrounding atmosphere being higher for warm fruit compared to cold fruit. This clearly illustrates the importance of cooling the fruit as fast and soon as possible after being harvested in order to reduce the VPD between the fruit and atmosphere, and therefore the subsequent water loss (Mitchell, 1992).

External quality

The main focus was that of seasonal maturity variances with respect to chilling injury at the two temperature treatments. It was found that the 1°C early season fruit experienced significantly higher chilling injury when compared to the higher temperature of 5.5°C. There was, however, an interesting trend shown in that the 1°C improved as the season progressed and fruit matured, whereas the 5.5°C got progressively worse in performance as the season progressed (**Figure 6**). It can be noted that by the late season there is no significant difference between the two temperature treatments with respect to chilling injury. The results also need to be placed in perspective. On a scale of 0-10 the early season fruit averaged at approximately 1.7 and by the late season just under 1. There were individual fruit which did get extensive chilling injury, but overall the majority of the fruit within this trial were of good external quality at 1°C. Chilling injury may not be a significant factor for the 'Hass' cultivar as it does change colour when ripened, so the external chilling injury is easily masked. A semi-commercial trial conducted on 'Hass' at 1°C showed that minimal severe incidences of chilling injury were found (Van Rooyen, 2009). In terms of waxing it was found that the waxed fruit experienced significantly higher external damage, the same result as found by Kok *et al*. (2010). This was an unexpected trend due to previous findings by Bower and Jackson (2003) as well as Bower and Magwaza (2004), indicating that waxing reduced the incidence of chilling injury.

Internal quality

The important parameters with respect to the internal quality include mesocarp discolouration, vascular browning, anthracnose and stem-end rot. These parameters were recorded on a scale of 0-5 when the fruit reached their optimal ripeness and were then cut for observation.

Only 13 of 720 fruit within the trial showed vascular browning, hence no significant differences were found.

For mesocarp discolouration the main effects of temperature as well as waxing were found to show significant differences. The 5.5°C treatment induced a significantly higher incidence of mesocarp discolouration, which is expected due the higher softening involved with the increased temperature leading to the decrease of cell wall and membrane integrity, which allows for an increased probability of rupturing and solute leakage. Once this occurs in either the mesocarp or vascular bundles, PPO is released from the thylakoids, where it is latently held, and spills into the cytoplasm where browning occurs (Bower & Cutting, 1988). The study conducted by Van Rooyen (2009) confirms that the internal quality for 'Hass' is excellent at 1°C. With respect to the waxing treatment, it was found that the waxed fruit had a significantly higher incidence of mesocarp discolouration. These results correlate to those of Bower and Jackson (2003).

It was found that for both anthracnose and stem-end rot the 1-MCP treated fruit experienced a significantly higher incidence. The same trends were found in the work conducted by Kok *et al*. (2010). The other trend shown for stem-end rot was that the season maturity variances of the fruit proved to be significant when interacting with the 1-MCP treatments (**Figure 7**).

Days to ripening

An expected trend was shown for the days to ripening in terms of the season maturity variances where the early season fruit had the highest number of days to ripening at an average of approximately 8, followed by the mid season fruit at 7.5 days and the late sea-

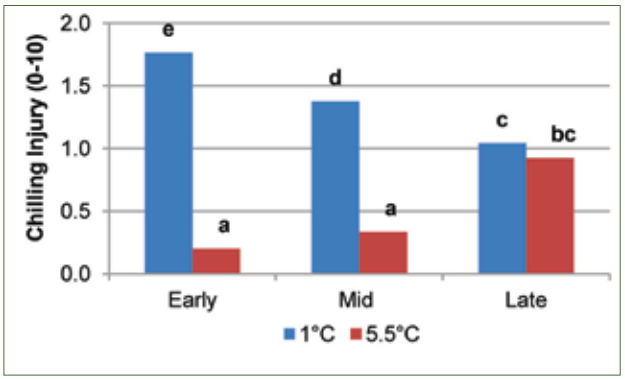

Figure 6. Effect of storage temperature and season maturity variances on external chilling injury at 28 days (scale of 0-10).

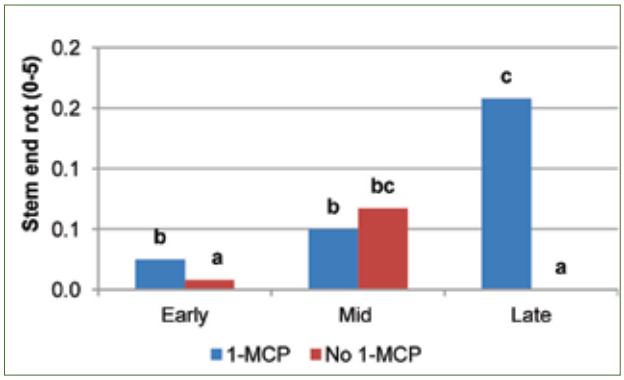

Figure 7. Effect of season maturity variances and 1-MCP treatments on stem-end rot (scale 0-5).

son fruit at 7 days. This shows that even though the mid season fruit experienced the highest softening during storage, the days to ripening trend was not influenced. One may normally expect a correlation between fruit softening in storage and the days to ripen.

An interesting trend was shown in **Figure 8** where the 1°C fruit maintained consistency in the number of days taken to ripen throughout the season, whereas the 5.5°C was not only significantly worse (fewer days to ripen) in comparison to the 1°C, but also seemed to significantly decline in performance as the season progressed. A similar effect was shown for the 1-MCP treatments (**Figure 9**). Overall it is evident that 1°C extended the ripening of the fruit, which is to be expected as there is a lowering of enzyme activity, respiration and ethylene production (Brady, 1987).

56 day storage

Days to ripening

There was a substantial difference shown between the two temperature treatments throughout the season in terms of the days to ripening for the 56 day storage trial. In the early season there was up to a 6.5 day ripening difference. For the 5.5°C treatment it was found that the shelf life of the fruit would be minimal at best and in fact the majority of the fruit at the 5.5°C temperature had nearly fully ripened within storage. Even the use of 1-MCP could not significantly extend the ripening period at this temperature. However, the 1°C treatment significantly increased the ripening period to such an extent it was comparable to the shelf life experienced by fruit under the 28 days storage trial. While there was a decline in days to ripen for the 1°C treatment, it still allowed for an acceptable shelf life even in the late season (**Figure 10**). This indicates that if 1°C is used as the storage temperature, a storage time of 56 days may be a credible option in terms of the shelf life of the fruit.

External quality

In terms of chilling injury, 56 days storage at 1°C resulted in the early season fruit incurring the highest amount of chilling injury at approximately 3.7 on a scale of 10, where as the 5.5°C was well below 0.5 (**Figure 11**). The mid and late season fruit did, however, have significantly less chilling injury.

Internal quality

Approximately 12% of the fruit within the 56 day storage trial experienced some form of internal disorder. The main findings were those of mesocarp discolouration, as well as vascular browning (**Figure 12**). The results indicated that the use of 1-MCP significantly suppressed the occurrence and severity of these disorders. The reason for this could be linked to the ability of 1-MCP to reduce the amount of softening within storage, hence maintaining the cell

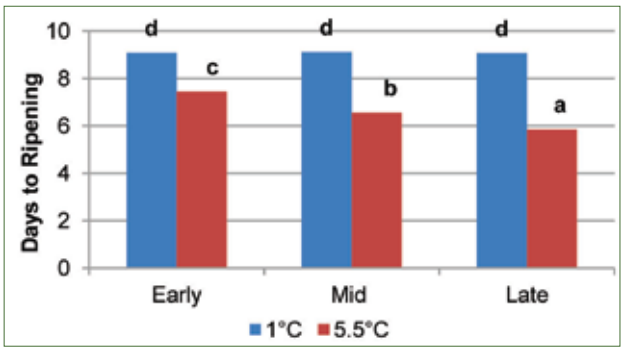

Figure 8. Effect of temperature and seasonal maturity variances on the days to ripening after 28 days storage.

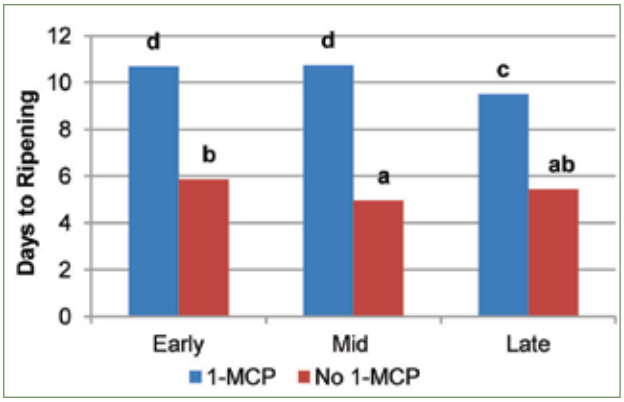

Figure 9. Effect of 1-MCP and seasonal maturity variances on the days to ripening after 28 days storage.

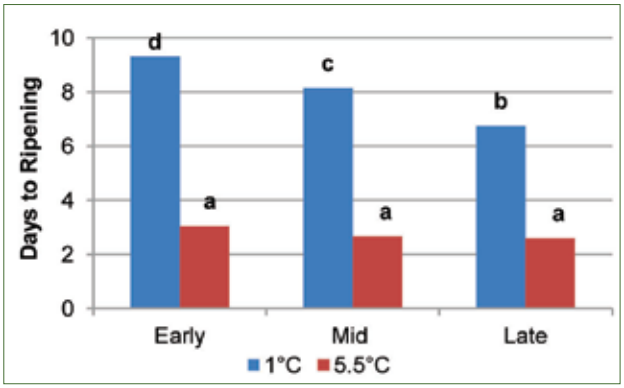

Figure 10. Effect of temperature and seasonal maturity variances on the days to ripening after 56 days storage.

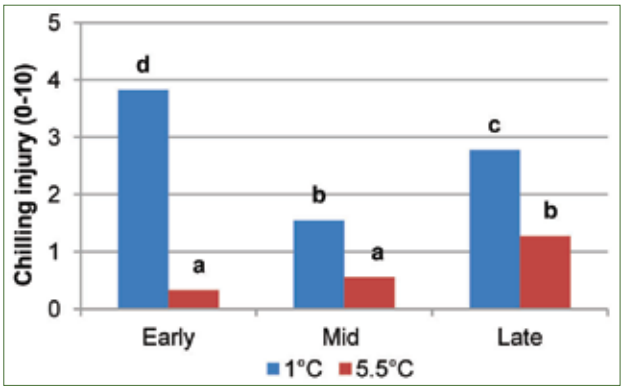

Figure 11. Effect of storage temperature and season maturity variances on external chilling injury at 56 days (scale of 0-10).

wall and membrane integrity over the extended time period. The 1°C treatment also managed to suppress the occurrence of mesocarp discolouration to a certain extent due to the same effect in reducing the softening within storage (**Figure 13**).

CONCLUSION

It was found that shipping at 1°C in the absence of 1-MCP was comparable, and in most cases even better, than the use of the protocol temperature of 5.5°C in conjunction with the use of 1-MCP. There is a strong possibility that the use of 1°C could be a credible alternative to the use of 1-MCP as this study, as well as previous studies in recent years, showed positive results towards this outcome. The positive aspects not only include the elimination of significant costs involved with 1-MCP, but there is the advantage of significantly reducing softening leading to enhanced shelf life and internal quality, as well as negating the detrimental effects of in-storage cold chain breaks. This is probably due to the core temperature of the fruit not being able to rise sufficiently within an 8 hour break to reactivate the enzymes involved with softening and break down of cell walls. However, if a delay of 24 hours occurs before the fruit are placed into cold storage, a significant amount of water loss from the fruit occurred, which it is suggested made them more susceptible to chilling injury as well as ripening after storage occurring at a much faster rate.

In terms of the fruit maturity, it was found that the early season fruit had the longest days to ripen as well as being the least affected by cold chain breaks. However, the relative immaturity of the fruit resulted in internal disorders as well as uneven ripening. It was indicated that the mid season fruit were most sound in terms of ripening and quality aspects.

Shipping at 1°C may cause concern of substantial chilling injury. However, it has been shown in this study that there is minimal chilling injury present. The early season fruit resulted in a higher rating of chilling injury, yet the level recorded was not particularly high and may be acceptable when one considers that the 'Hass' cultivar does have the advantage of a colour change which will mask the trace amounts of chilling injury. The mid and late season fruit proved to be acceptable in terms of the chilling injury shown.

The 56 day storage trial produced data which may be useful to growers and suppliers required to delay marketing. It was shown that the 1°C is needed for this storage time period in order to ensure a suitable shelf life. The use of 1-MCP is also essential for this extended storage period, not only to help extend the shelf life but also to maintain the internal quality and integrity of the fruit. If 1-MCP is not used there are issues of excessive softening and subsequent cell rupture leading to browning and discolouration of the mesocarp. Unfortunately the use of 1°C can inflict significant external chilling injury. It was found that this storage temperature would not be a viable option for early season fruit as there was excessive chilling injury. However, the mid and late season fruit were affected significantly less. The best option for this storage period would be to place the fruit in a 'ready ripe' programme after storage so that the chilling injury would be masked in the mid and late season fruit.

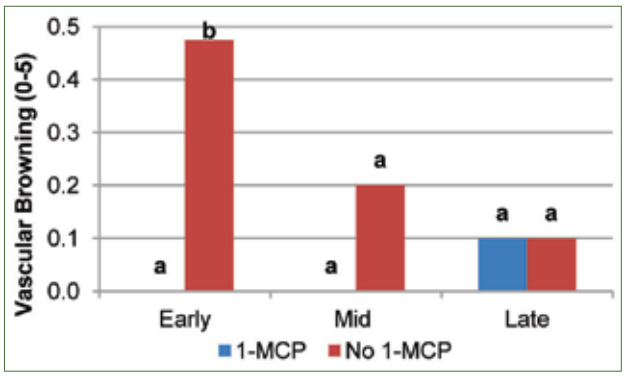

Figure 12. Effect of 1-MCP treatments and season maturity variances on vascular browning at 56 days (scale of 0-5).

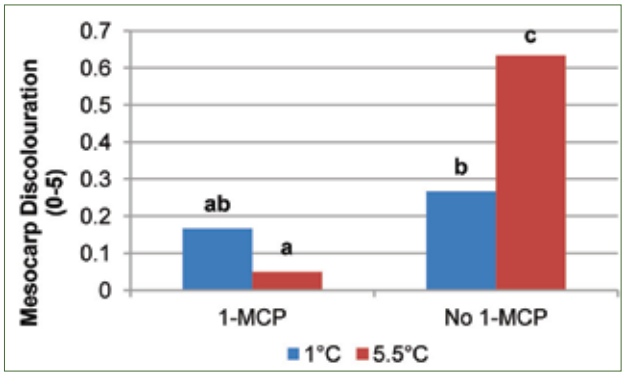

Figure 13. Effect of 1-MCP treatments and storage temperatures on mesocarp discolouration at 56 days (scale of 0-5).

Overall, the data resulting from this study implies that 1°C can be used as an alternative method to extend shelf life, improve internal quality and negate in-storage cold chain breaks of the 'Hass' avocado fruit.

Therefore, low temperature shipping may, under the correct circumstances, replace other technologies used to export avocado fruit.

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