CHAPTER 5

Postharvest treatments used to reduce external chilling injury in 'Pinkerton' avocado (*Persea americana* Mill.)

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SUMMARY

The results of previous research (Chapter 2) found that the poor internal quality of 'Pinkerton' avocados, which threatened the export of this cultivar, could be improved by storing fruit at 2°C. This posed a challenge to the industry as 'Pinkerton' avocado fruit are found to be sensitive to storage temperatures below 5.5°C, especially for extended periods (30 d), and fruit often develop external chilling injury. Thus, in order to market 'Pinkerton' fruit of an overall high quality a solution to the problem of external chilling injury development was needed, especially as South Africa is also constantly trying to enter new markets. Furthermore, export to some countries might require fruit to be subjected to a period of cold disinfestations at temperatures lower that 2°C in order to meet quarantine standards. In this study, it was hoped that techniques could be found to precondition fruit to low temperature storage. Preconditioning treatments consisted of fruit that were kept at either 10°C, 15°C or 20°C for 1 d or 2 d before being placed into storage for 30 d at 2°C or 5.5°C. All preconditioning treatments were compared to fruit that were placed directly into storage. The effect of fruit packaging on weight loss and chilling injury was also investigated using unwaxed fruit, commercially waxed and unwaxed fruit individually sealed in micro-perforated polypropylene bags with an anti-mist coating on the inside (polybags). While chilling injury was more severe in fruit stored at 2°C, keeping fruit at 10°C for 2 d significantly reduced the chilling injury severity, compared to control fruit placed directly into storage. The role of weight loss in chilling injury development was not always clear. Chilling injury was more severe in unconditioned fruit and fruit preconditioned for 1 d, however, fruit preconditioned at 15°C or 20°C prior to storage were found to lose the most weight, especially when

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preconditioned for 2 d. Fruit stored at 5.5°C also lost significantly more weight than fruit stored at 2°C. Unwaxed fruit lost the most weight during the preconditioning treatments and storage and polybag fruit the least, with chilling injury being more severe in waxed and then unwaxed fruit. In this study proline accumulation appeared to reflect the level of stress experienced by the fruit. Proline concentrations were the lowest in the polybag treatments, which lost the least weight during preconditioning and storage. However, while the waxed fruit did not lose the most weight during the preconditioning treatments, proline levels were higher in these fruit. As external chilling injury was also more severe in waxed fruit it is suspected that the waxed fruit may have been exposed to an additional stress, namely suffocation caused by the clogging of lenticels. The success of low temperature conditioning in reducing chilling injury may thus not be related directly to weight loss but rather to biochemical and physiological modifications induced by the treatments.

The desire to reach distant markets with avocado fruit often means that fruit are subjected to a storage period of up to 30 days. In order to control the ripening of these climacteric fruit and to ensure optimal fruit quality on arrival, these fruit are subjected to low temperatures during storage in an attempt to slow down all biological processes. Unfortunately, 'Pinkerton' fruit are susceptible to the development of certain physiological disorders during this storage period. While previous research (Chapter 2) found that the development of the disorder most threatening to the export of this cultivar (mesocarp discolouration) could be reduced by storing fruit at 2°C, thus below the industry standard of 5.5°C, the potential for damage to the fruits' exocarp was increased. Thus a solution was needed that would ensure both good internal and external quality. The ability to store fruit at very low temperatures would also increase the potential for South African fruit to be exported to new markets as some countries require cold disinfestation treatments in order to minimise the risk of insect pests entering their country. Fortunately, certain postharvest techniques have been found to alleviate low temperature injury in various chilling-sensitive commodities; these include preconditioning, heat treatments, intermittent warming, controlled atmosphere storage, waxing, film packaging, genetic modification, and applications of certain chemicals and plant growth regulators (Morris, 1982; Wang, 2001). This paper will concentrate on the effect of low temperature conditioning, waxing and fruit packaging on external chilling injury.

Low temperature conditioning involves holding cold-sensitive tissue at temperatures slightly above those at which injury occurs to induce tolerance to these normally damaging temperatures and thus delay the development of injury symptoms. This technique has been successful in reducing chilling injury in grapefruit (Hatton and Cubbedge, 1983; Chalutz *et al*., 1985), tomato seedlings (Wheaton and Morris, 1967), tomato fruit (Saltveit, 1991), papaya (Chen and Paull, 1986), zucchini squash (Wang, 1994), and more recently in avocados (Woolf *et al*., 2003; Hofman *et al*., 2003). Adaptation to lower temperatures is thought to be the result of various biochemical and physiological modifications induced by the conditioning treatment.

Chilling injury can also be prevented in many crops by reducing moisture loss from tissues (Ben-Yehoshua *et al*., 1983a; Wang, 1990), with conditions of high relative humidity being thought to inhibit the collapse of the epidermal and underlying cells. High relative humidity, in the atmosphere around commodities, can generally be achieved by waxing fruit or by using film packaging. Waxing and film packaging are also thought to increase the carbon dioxide (CO_2) and decreases the oxygen (O_2) concentrations of the internal atmosphere during storage (Durand *et al*., 1984). These factors are believed to contribute to the reduction in chilling injury in many crops (Wang, 1993). In avocados the use of either waxing (Lunt *et al*., 1981) or film packaging (Eksteen and Truter, 1985; Wang, 1993; Bower and Jackson, 2003) has been successful in reducing chilling injury, although, Bower and Magwaza (2004) found that polypropylene packaging in ‘Fuerte’ avocados was more effective in reducing external chilling injury than waxing.

The amino acid proline is thought to be a non-specific indicator of stress as it accumulates in plants after they have been exposed to different stresses (Aspinall and Paleg, 1981). The exact mechanism whereby proline accumulates under stress, and the precise role of proline accumulation has not been unequivocally determined to date. Proline accumulation is argued by some researchers to be advantageous to a plant as far as stress tolerance in concerned (Singh *et al*., 1973). For example, grapefruit, which have accumulated relatively high concentrations of carbohydrates and proline, have been found to be less likely to develop chilling injury caused by low, non-freezing temperatures (Purvis, 1981; Purvis and Grierson, 1982). Conversely, some researchers argue that proline accumulation is simply an indication of the damage suffered by the plant during stress conditions (Hanson *et al*., 1979).

Objectives of this study were (a) to identify postharvest treatments that would lead to the successful storage, in terms of overall fruit quality, of ‘Pinkerton’ avocados, with special emphasis on the reduction of external chilling injury; (b) to identify the degree and kind of stress (moisture *vs* temperature) placed on the fruit by the various postharvest treatments; and (c) to elucidate the role of proline concentrations in avocado stress physiology.

MATERIALS AND METHODS

Plant material and treatments

‘Pinkerton’ avocado fruit (*Persea americana* Mill.) were obtained from a grower near Wartburg in KwaZulu Natal (29°27’S, 30°40’E) on 03/08/04 and 23/08/04. One third of the fruit were commercially waxed at the packhouse (Canuaba Tropical, Sasol Waxes, RSA; 0.71 ℓ tonne^{-1} fruit), one third were left unwaxed, and the last third were left unwaxed and individually heat-sealed in 30 µm thick polypropylene bags with 9 µm perforations and an anti-mist coating on the interior (Polylam Packaging, Johannesburg, RSA) on arrival at the University of KwaZulu Natal (6-8 h after harvest). Prior to waxing, fruit were placed in a fungicide dip (Sporekill, Hygrotech, Pietermaritzburg, RSA; 0.25 ℓ 100 ℓ$^{-1}$ water). On arrival fruit were divided into the respective treatments, labelled, weighed and visually assessed for any external blemishes, with ten individual fruit replications per treatment (5 fruit being sampled immediately after a treatment and 5 allowed to ripen). Treatments consisted of fruit preconditioned at 10, 15 or 20°C for 1 or 2 d before being placed in storage at either 2°C or 5.5°C. Fruit in the control treatments were not preconditioned in any way and were placed either directly into storage at 2°C or 5.5°C, sampled immediately or left to ripen at 20°C. The maturity of the fruit, as determined by moisture content, was determined on arrival. The polybags were removed once the fruit were removed from storage, to allow for ripening. After each treatment stage (*viz*. preconditioning, storage or ripening) the fruit were weighed, fruit firmness was determined and fruit were visually assessed for any signs of external chilling injury or anthracnose (*Colletotrichum gloesosporioides* Penz.). After cold storage 5 fruit per treatment were removed for destructive analysis to see if any mesocarp discolouration was present, while the remaining fruit were allowed to reach "eating ripeness". The number of days taken to reach "eating ripeness" was recorded for all treatments. On sampling the exocarp of each fruit was cut into small pieces (1 cm^2) and flash frozen in liquid nitrogen before being placed in a freezer until further analysis could be conducted.

Maturity

The maturity of each consignment was ascertained on arrival by determining the moisture content (Kruger *et al*., 1995) of a sample of mesocarp tissue (20 g) from each of 5 fruit. The tissue was cut into small pieces (1 cm^3) and immersed in liquid nitrogen. Once frozen, the samples were placed on a freeze drier for 5 d. This was determined to be sufficient time to remove moisture and attain constant weight.

External chilling injury

Chilling injury was assessed by giving the external black discolouration (pitting) a visual rating using a scale of 0 to 10, with 0 = no visible discolouration and 10 = 100% of surface area black.

Anthracnose and mesocarp discolouration

The presence of anthracnose was given a score of 0 = no infection and 1 = some infection. For mesocarp discolouration ratings fruit were bisected longitudinally and immediately rated using a visual scale of 0 to 10, with 0 = no visible discolouration and 10 = 100% of cut surface area black.

Fruit firmness

"Eating ripeness" 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^o after each measurement. The fruit firmness tester measures firmness by means of a metal ball (diameter 5 mm) that is pressed onto the fruit. "Eating ripe" was considered to be at a reading of 50 – 55 units.

Proline analysis

Avocado exocarp tissue was analysed for proline concentration using a modified method of Bates *et al.* (1973). For each fruit sample (analysed in triplicate) 1 g frozen avocado exocarp was cut into very small pieces before being homogenised in 10 mℓ 3% sulphosalicylic acid using an Ultra-turrax T25 (Janke and Jackson, Staufen, Germany). Samples were then filtered through Whatman® No. 1 filter paper and the supernatant/filtrate collected. Two mℓ of the filtrate was then combined with 2 mℓ acid-ninhydrin and 2 mℓ glacial acetic acid. The samples were incubated for 1 h in a boiling waterbath, and the reaction terminated on ice. Four mℓ toluene was added to the reaction mixture and vortexed for 15 s. Time was allowed for the toluene to separate from the aqueous phase, before the toluene phase was extracted. The absorbance of the samples was determined at 520 nm using a Beckman Coulter DU 800 spectrophotometer (Fullerton, California, USA). The proline concentration was determined using a standard curve, using L-proline (Sigma) as standard. The µmole proline per g of fresh weight sample was determined using the formula [(µg proline/mℓ*mℓ toluene)/115.13 µg/µmole]/[g of sample/5] (Bates *et al*., 1973). All the fruit that were sampled came from treatments that were terminated immediately after storage at 2^{o}C and consisted of 5 single fruit replications per treatment.

Statistical Analysis

Data was subjected to analysis of variance (ANOVA) using the GenStat® statistical package (VSN International Ltd, Hemel Hempstead, UK). Least significant difference (LSD) was used to separate treatment means. Due to the existence of many significant interactions (between factors) all the results are displayed in tables indicating the various interactions and their significance. Proline data were subjected to multiple linear regressions.

RESULTS

Maturity

The mean moisture content of fruit harvested on 03/08/04 was 76.8% and those harvested on 23/08/04 was 75.5%.

Preconditioning weight loss

The unwaxed and waxed fruit lost significantly (P < 0.001) more weight, during the preconditioning treatments, than the fruit individually sealed in polybags, irrespective of temperature, length of conditioning or harvest date (Table I). Keeping the fruit at higher temperatures (*viz*. 15^{o}C or 20^{o}C) and for greater periods (2 d) resulted in increased weight loss. Preconditioning the fruit at 10^{o}C and/or placing the fruit in polybags was thus the most successful in terms of reducing weight loss prior to storage.

Storage weight loss

For both harvest dates the unwaxed fruit lost the most weight during storage followed by the waxed fruit (Table II). Fruit stored at 5.5^{o}C lost significantly (P < 0.001) more weight during storage than fruit stored at 2^{o}C. The relative humidity in the 2^{o}C container was 80-85% while that in 5.5^{o}C was at around 75-80%. As the polybags were only removed from the fruit after storage, the difference between storage and preconditioning weight loss could not be determined. Nevertheless, polybag fruit still lost less than 1% of their original fruit weight by the time they were removed from storage. This was attributed to the presence of some free water in the bags after storage, and to readings of a 100% relative humidity within the bags. Small differences in weight loss were seen between the preconditioning treatments and a significant interaction was found between fruit packaging, preconditioning temperature and storage temperature on the weight loss during storage. During the 30 d storage period the control fruit and fruit preconditioned at 10^{o}C lost more weight than fruit from other treatments (Table II).

Ripening weight loss

Fruit originally sealed in polybags, lost the most weight during the ripening period compared to the other fruit packaging treatments (Table III). The unconditioned/control fruit and fruit preconditioned for 1 d, irrespective of temperature, lost significantly (P < 0.001) more weight than fruit receiving 2 d preconditioning. Furthermore, fruit stored at 2^{o}C lost significantly (P < 0.001) more weight during ripening than fruit stored at 5.5^{o}C (Table III).

Total weight loss

For both harvest dates, the total weight loss of fruit sampled immediately after storage revealed that fruit packaging and storage temperature had a significant (P < 0.001) effect on weight loss (Table IV) with unwaxed fruit losing considerably more weight than polybag fruit, and fruit stored at 2°C losing less weight than those stored at 5.5°C. Prior to ripening, weight loss was also significantly affected (P < 0.001) by the length of preconditioning, with weight loss being greater in the 2 d treatments. More weight was lost during storage (Table II) than during the actual preconditioning treatments (Table I). After attaining "eating ripeness" the differences in weight loss, between the fruit packaging and preconditioning treatments, while being significant (P < 0.001) were less dramatic (Table V). The final weight loss figures were significantly affected (P = 0.05) by interactions between all the postharvest treatment factors.

Days to ripening

Fruit stored at 2°C, regardless of fruit packaging or harvest date, took significantly (P < 0.001) longer to ripen than fruit stored at 5.5°C (Table VI). Fruit packaging also significantly (P < 0.001) affected the number of days taken to ripen, with the waxed fruit, stored at 2°C, taking the longest to ripen, for both harvest dates, followed by fruit originally sealed in polybags (Table VI). However, in the 5.5°C storage treatments the fruit sealed in polybags took the longest to ripen after the first harvest date (03/08/04), although the waxed fruit appeared to take longer after the second harvest date (23/08/04) in some treatments. Harvest date was found to significantly affect (P < 0.001) on days taken to ripen with fruit harvested on 23/08/04 taking less time to ripen that fruit harvested on 03/08/04. The preconditioning treatments were also found to significantly affect the days taken to ripening (Table VI). In summary, however, the days taken to reach "eating ripeness" were significantly affected by a number of high order interactions between the postharvest treatments (Table VI). Nevertheless, it appeared that weight loss played a significant role in ripening time. The unwaxed fruit consistently lost the most weight during preconditioning and storage and also ripened the fastest, and similarly the fruit stored at 5.5°C lost more weight and subsequently took less time to ripen.

Fruit firmness

Fruit firmness, immediately after storage, was affected by significant (P < 0.001) interactions between the various treatment factors (Table VII). Nevertheless, unwaxed fruit appeared the least firm and polybag fruit the most firm. After storage at 5.5^{o}C all fruit (except the polybag fruit) were less firm than those stored at 2^{o}C.

External chilling injury

During both harvest dates, regardless of treatment, the external chilling injury severity immediately after storage (Table VIII) or after ripening (Table IX) was never found to exceed 4, out of a possible rating of 10. Nevertheless, the severity of external chilling injury was found to be higher in fruit stored at 2^{o}C than at 5.5^{o}C. Preconditioning treatments significantly affected chilling injury severity, with the lowest ratings being found in the 2 d preconditioning at 10^{o}C treatments, whether fruit were stored at 2^{o}C or 5.5^{o}C. For both harvest dates the waxed fruit appeared to be more severely affected by chilling injury and pitting was often observed around the lenticels of the fruit (Figure 1). Storing fruit in polybags significantly reduced chilling injury, however this was negated to a certain extent by a higher incidence of fungal infections.

Mesocarp discolouration

Very little mesocarp discolouration was observed in this study, and ratings out of 10 never exceeded 3, with an average below 1 (Table X). Mesocarp discolouration ratings were significantly (P = 0.05) affected by interactions between the various treatment factors (Table X). Fruit packaging had a significant affect (P < 0.001) on mesocarp discolouration, with waxed fruit showing the highest incidence of discolouration.

Anthracnose

Anthracnose scores were affected by significant interactions between treatment factors (Table XI). A higher incidence of anthracnose infection was found at 2^{o}C than in fruit stored at 5.5^{o}C. The waxed and polybag fruit were more severely affected than the unwaxed fruit. However, the fungal lesions in the polybag fruit did not initially resemble the “typical” anthracnose symptoms. Immediately after storage (i.e. while fruit were hard) the exocarp of fruit sealed in polybags had large dull black areas, with smooth boundaries, and these were generally concentrated on the lower half of the fruit (Figure 2). As the fruit ripened these areas on the exocarp started to collapse, resembling chilling injury/pitting (Figure 3). Removal of the exocarp immediately after storage showed that the mesocarp tissue beneath the “infected” exocarp was not always affected (Figure 4). As gases, such as carbon dioxide, were found to pass readily through the perforations in the bag, and a possible plasticiser effect was ruled out, we feel confident that these black areas were in fact the result of a fungal infection. This is also supported by microscopic studies, which revealed the presence of fungal hyphae on the exocarp of the fruit. Furthermore, the typical symptoms of a fungal infection (Figure 5) started to develop as the fruit ripened and the affected areas collapsed, with the infection extending into the mesocarp tissue beneath these areas. Thus, for the sake of simplification we have scored this fungal infection under anthracnose.

Proline analysis

Proline analysis of fruit exocarp, sampled immediately after storage at 2°C, revealed that preconditioning, harvest date, fruit packaging and interactions between these factors had significant effects ($P < 0.001$) on proline concentrations (Table XII). Proline concentrations were significantly higher ($P < 0.001$) in fruit harvested on 23/08/04. During both harvest dates waxed fruit, receiving no preconditioning treatments or 1 d preconditioning, had the highest proline concentrations and fruit sealed in polybags the lowest. However, when fruit were subjected to preconditioning for 2 d the waxed fruit had the lowest proline concentrations and the unwaxed fruit, for the most part, had the highest. Overall, the 2 d preconditioning treatment at 10°C rendered fruit with the lowest proline concentrations (Table XII). Regression analysis revealed that chilling injury and preconditioning weight loss contributed the most to proline concentrations ($P < 0.001$), followed by total weight loss (immediately after storage) ($P = 0.05$). However, together these factors only accounted for 21% of the variance. When the analysis was divided into fruit packaging treatments the significance of chilling injury, preconditioning and total weight loss remained significant for the unwaxed and waxed fruit, but only the presence of chilling injury was found to contribute to proline concentrations in the polybag fruit. The addition of preconditioning time, harvest date and fruit packaging treatment to the analysis accounted for 40% of the variance.

DISCUSSION

Both the preconditioning treatments and the fruit packaging treatments had a significant effect on the weight loss and external chilling injury severity of 'Pinkerton' avocado fruit. In terms of weight loss, the 2 d storage delay, with fruit held at either 15°C or 20°C resulted in the greater weight loss. This was to be expected, as was the greater weight loss of unwaxed fruit, and fruit stored at 5.5°C compared to 2°C. The lower relative humidity in the 5.5°C container was thought to create a greater water vapour pressure deficit between the fruit and air within this container, resulting in greater weight loss. Minimising weight loss prior to storage, and during storage, is thought to be crucial to sustaining membrane integrity and thus the optimal functioning of cells (Wang, 1993). Maintaining a low water vapour pressure deficit during storage is therefore crucial in minimising weight loss during this time, as supported by the insignificant weight loss of fruit sealed in polybags during storage. Throughout the study the external chilling injury severity was the lowest in fruit sealed in polybags during preconditioning and storage.

However, care should be taken when storing fruit at a relative humidity close to 100%, as this condition is favourable to the spread and growth of pathogens. The accumulation of free water in the polybags could have been limited to a certain extent by modifying the technique used to apply the polybags; for example, keeping the bags and fruit in the cold rooms for a set time period to equilibrate before applying the bags, and/or by placing an absorptive material at the bottom of the bag. Ensuring timely fungicide applications both during the season and after harvest can also decrease the presence of pathogenic fungi on the fruit. In this study, the unwaxed (and therefore polybag) fruit were not put through the normal packhouse treatment and did not, therefore, receive a fungicide dip prior to storage. The results do, however, indicate that this would not have solved the problem completely as the waxed fruit were also affected by anthracnose infections. The higher incidence of anthracnose infections in fruit stored at $2^{o}C$ was thought to be related to the increased number of days taken to reach "eating ripeness" after storage at $2^{o}C$ (Table VI), as found by Eksteen and Truter (1985).

Weight loss after storage appeared to have no effect on chilling injury severity as the polybag fruit lost significantly more weight during ripening (Table III), and at a greater rate, as reflected by the similar number of days, to waxed fruit, taken to reach "eating ripeness" (Table VI) while still developing very little chilling injury. Furthermore, no significant differences were found in chilling injury ratings between fruit sampled immediately after storage (Table VIII) and fruit allowed to ripen (Table IX). The higher weight loss during ripening, of the fruit originally sealed in polybags, also appeared to indicate that 'Pinkerton' avocado fruit need to lose a certain amount of weight in order to attain "eating ripeness".

Chilling injury could not be attributed solely to weight loss, as the waxed fruit in this study were the most significantly affected by chilling injury despite the fact that the unwaxed fruit lost more weight prior to ripening. The higher incidence of chilling injury in the waxed fruit could have been caused by either the thickness or type of the wax not being optimal for very low temperature storage (i.e., below the $5.5^{o}C$ standard) (Johnston and Banks, 1998). In fact, Bower *et al.* (2003) found that the type of fruit packaging used in avocados significantly affected the incidence of external chilling damage. Furthermore, some studies have indicated that waxing can affect the gaseous exchange of fruit either through the incorrect thickness of the wax or by the clogging of stomatal pores (Ben-Yehoshua *et al*., 1983b). The method of wax application can also be detrimental to fruit quality. In avocados lenticels may become damaged if the brushes used in the application of the wax are too hard. This could account for chilling injury symptoms often being more prevalent around the lenticels of the fruit (Figure 1). The high proline levels in the waxed fruit, which were placed either directly into storage or preconditioned for 1 d prior to storage at 2^{o}C, seem to suggest that these fruit were experiencing an additional stress other to that inflicted by weight loss. The unwaxed fruit consistently lost the most weight during preconditioning and storage and would thus be expected to exhibit the highest proline concentrations, which was not always the case (Table XII).

The low chilling injury ratings of the polybag treatments, accompanied by low proline concentrations, appear to support the theory that proline concentrations may well reflect the extent of damage caused by a stress (Hanson *et al*., 1979) rather than be used as a predictor of stress tolerance. Thus, the low stress levels experienced by the polybag fruit prior to storage may well have enabled the fruit to better withstand the low-temperature storage (2^{o}C). The degree of hydration in plant membranes is known to affect membrane fluidity. In general, it is found that when the cell water percentage falls below a certain level, membranes lose the ability to maintain homeostatic viscosity and this in turn may affect the thermodynamic stability of the membrane (Nilsen and Orcutt, 1996). However, the role of weight loss in chilling injury development was not always clear in this study, and it is possible that the reduced chilling injury might well have been the result of other biochemical and physiological modifications induced by the conditioning treatments. These changes could include increases in the degree of unsaturation of fatty acids in the membranes, in response to temperature conditioning, which would in turn affect membrane fluidity and permeability. Preconditioning treatments have also been found to affect the sugar content of plant tissues (Purvis, 1990). Bower and Jackson (2003) found that carbon dioxide evolution rates were lower in fruit sealed in polybags during storage, than in unwaxed and waxed fruit. Over time this was suspected to result in a decrease in the respiratory requirement for carbohydrates during storage thus possibly leading to a more controlled rate of energy consumption, which would in turn enable the fruit to tolerate the stress induced by low temperature storage.

CONCLUSION

The high level of interactions between the various postharvest treatments, fruit packaging treatments and harvest dates clearly illustrate that avocados are living organisms and as such cannot be handled uniformly throughout the season. In fact, postharvest care starts as soon as the fruit are harvested. An understanding of how postharvest conditions affect fruit quality, in terms of external chilling injury, will therefore enable the manipulation of these factors in order to ensure optimal fruit quality, after storage at $2^{o}C$, and possibly allow for fruit to be entered into new markets where a period of cold disinfestation is required to meet quarantine standards. Low temperature preconditioning treatments show great potential in allowing fruit to be stored at very low temperatures while maintaining high fruit quality, thus further studies should try to elucidate how preconditioning treatments acclimatise avocado fruit to these conditions. This would possibly enable the manipulation, or at least management, of these factors preharvest; for example, determining the effect of the fatty acid saturation of membranes on chilling development. Preharvest temperatures are thought to influence the degree of lipid saturation and might help in identifying which growing areas are more suitable to low storage temperatures of $2^{o}C$. In fact, storage temperatures lower than $2^{o}C$ might need to be attained in order to guarantee pest eradication and thus additional work needs to be done on establishing what this temperature is. The effect of waxing on chilling development also needs further investigation as the formulation and thickness of the wax application may be easier to manipulate in the short term. Furthermore, the method of application, in the packhouse, may need to be slightly modified in accordance with the type of wax used. The use of micro-perforated polypropylene pallet wraps during storage also needs to be considered as this may prove to be more practical in terms of dealing with large fruit numbers.

REFERENCES

(See final reference section, pg's 129-155)

TABLE I

Weight loss of unwaxed, waxed and fruit sealed in micro-perforated polypropylene bags, harvested on 03/08/04 or 23/08/04, during the respective preconditioning treatments

Preconditioning time (d)	Preconditioning temperature (°C)	Weight loss (%) 03/08/04 Unwaxed	Waxed	Polybag	23/08/04 Unwaxed	Waxed	Polybag
1	10	0.46	0.33	0.08	0.31	0.21	0.09
	15	0.99	0.76	0.12	1.26	0.81	0.08
	20	1.22	0.97	0.13	1.12	0.87	0.07
2	10	0.64	0.52	0.12	0.60	0.40	0.14
	15	2.07	1.83	0.20	2.08	1.71	0.22
	20	2.59	1.92	0.28	2.01	1.49	0.21

Date = 0.04**

Packaging = 0.05**

Preconditioning time = 0.04**

Preconditioning temperature = 0.05**

Packaging x Date = 0.05*

Packaging x Preconditioning time = 0.07**

Date x Preconditioning time = 0.06**

Date x Preconditioning temperature = 0.07**

Packaging x Preconditioning temperature = 0.09**

Preconditioning temperature x Preconditioning time = 0.07**

Packaging x Date x Preconditioning temperature = 0.12**

Packaging x Date x Preconditioning time = 0.07*

Packaging x Preconditioning temperature x Preconditioning time = 0.12**

Date x Preconditioning temperature x Preconditioning time = 0.10**

Packaging x Date x Preconditioning temperature x Preconditioning time = 0.13*

* = significant ($LSD_{0.05}$); ** = significant ($LSD_{0.001}$); n = 10

TABLE II

Weight loss of unwaxed, waxed and fruit sealed in micro-perforated polypropylene bags, harvested on 03/08/04 or 23/08/04, during storage at 2°C or 5.5°C (30 d) and after exposure to the various preconditioning treatments

Precon. time (d)	Precon. temperature (°C)	Storage temperature (°C)	Weight loss (%) 03/08/04 Unwaxed	03/08/04 Waxed	03/08/04 Polybag	23/08/04 Unwaxed	23/08/04 Waxed	23/08/04 Polybag
0	none	2	5.0	3.6	0.5	4.6	3.3	0.4
		5.5	7.6	5.9	0.4	7.1	5.9	0.3
1	10	2	4.7	3.6	0.4	4.3	3.1	0.4
	15		4.1	3.7	0.5	4.3	3.0	0.5
	20		4.6	3.3	1.0	4.0	3.1	0.4
	10	5.5	7.7	5.6	0.4	6.9	5.2	0.4
	15		7.1	5.6	0.5	6.5	5.0	0.5
	20		7.0	5.2	0.6	6.6	5.3	0.5
2	10	2	5.3	3.3	0.4	4.9	3.2	0.4
	15		4.4	3.3	0.8	4.0	3.4	0.5
	20		4.1	2.9	0.7	4.2	3.2	0.6
	10	5.5	7.6	5.7	0.8	7.2	5.5	0.4
	15		6.8	5.2	0.6	6.9	4.3	0.5
	20		6.6	5.0	0.8	6.6	4.3	0.6

Date = 0.1**

Packaging = 0.1**

Storage temperature = 0.1**

Preconditioning temperature = 0.2**

Packaging x Preconditioning time = 0.2**

Packaging x Storage temperature = 0.2**

Preconditioning temperature x Storage temperature = 0.2**

Preconditioning temperature x Preconditioning time = 0.2*

Preconditioning time x Storage temperature = 0.1*

Packaging x Preconditioning temperature x Storage temperature = 0.3*

* = significant ($LSD_{0.05}$); ** = significant ($LSD_{0.001}$); n = 10

TABLE III

Weight loss of unwaxed, waxed and fruit sealed in micro-perforated polypropylene bags, harvested on 03/08/04 or 23/08/04, during ripening (after preconditioning and storage at 2°C or 5.5°C (30 d))

Precon. time (d)	Precon. temperature (°C)	Storage temperature (°C)	Weight loss (%)					
			03/08/04			23/08/04		
			Unwaxed	Waxed	Polybag	Unwaxed	Waxed	Polybag
0	none	2	8.7	10.2	9.5	8.6	8.0	9.0
		5.5	5.4	4.8	7.5	6.9	6.7	7.7
1	10	2	7.3	8.8	8.4	7.0	7.3	9.9
	15		7.2	7.9	9.8	9.6	7.8	10.2
	20		8.2	8.8	11.3	8.2	7.7	9.0
	10	5.5	5.1	4.9	8.6	5.6	4.6	6.8
	15		5.4	6.1	9.0	5.5	4.7	7.9
	20		4.9	4.2	9.8	6.0	5.8	8.0
2	10	2	5.7	6.3	8.5	7.2	6.2	8.0
	15		6.6	9.2	9.3	5.4	6.6	8.9
	20		6.7	7.6	9.0	7.5	6.8	9.2
	10	5.5	4.5	4.7	6.7	5.0	5.3	7.7
	15		4.3	4.7	7.3	4.7	4.0	6.8
	20		4.7	5.1	6.4	5.1	4.8	8.0

Packaging = 0.3**
Storage temperature = 0.3**
Preconditioning time = 0.3**
Preconditioning temperature = 0.4**
Packaging x Date = 0.6**
Packaging x Storage temperature = 0.5**
Date x Storage temperature = 0.4**
Preconditioning temperature x Storage temperature = 0.4*
Date x Preconditioning temperature x Preconditioning time = 0.7*
Packaging x Date x Storage temperature = 0.5*
Date x Preconditioning temperature x Storage temperature = 0.6*
Date x Packaging x Preconditioning temperature x Preconditioning time = 1.3*
Packaging x Preconditioning temperature x Preconditioning time x Storage temperature = 1.3*
Date x Preconditioning temperature x Preconditioning time x Storage temperature = 1.0*
Packaging x Date x Precon temperature x Precon time x Storage temperature = 1.8*
* = significant ($LSD_{0.05}$); ** = significant ($LSD_{0.001}$); n = 5

TABLE IV

Total weight loss of unwaxed, waxed and fruit sealed in micro-perforated polypropylene bags, harvested on 03/08/04 or 23/08/04, after exposure to various preconditioning treatments, and storage at 2°C or 5.5°C (30 d)

Precon. time (d)	Precon. temperature (°C)	Storage temperature (°C)	Weight loss (%)					
			03/08/04			23/08/04		
			Unwaxed	Waxed	Polybag	Unwaxed	Waxed	Polybag
0	none	2	5.3	3.4	0.5	4.5	3.1	0.4
		5.5	7.7	5.7	0.4	7.2	4.7	0.4
1	10	2	5.5	4.1	0.4	4.5	3.5	0.4
	15		5.0	5.0	0.5	5.8	3.9	0.5
	20		5.7	3.9	1.4	5.5	3.9	0.5
	10	5.5	7.6	6.1	0.5	7.1	6.0	0.4
	15		8.5	7.0	0.5	7.9	6.9	0.5
	20		7.4	6.6	0.6	7.2	6.4	0.5
2	10	2	6.1	4.4	0.4	5.7	4.1	0.4
	15		6.9	4.5	0.7	6.1	5.0	0.6
	20		6.4	4.9	0.7	5.9	4.9	0.6
	10	5.5	8.3	5.8	0.6	8.4	6.4	0.5
	15		9.6	6.3	0.6	9.0	5.8	0.5
	20		9.1	6.5	0.8	8.1	5.6	0.6

Date = 0.2**

Packaging = 0.2**

Storage temperature = 0.2**

Preconditioning time = 0.2**

Preconditioning temperature = 0.2**

Packaging x Preconditioning temperature = 0.4**

Packaging x Preconditioning time = 0.3**

Packaging x Storage temperature = 0.3**

Preconditioning temperature x Storage temperature = 0.2*

Packaging x Preconditioning time x Storage temperature = 0.4*

* = significant ($LSD_{0.05}$); ** = significant ($LSD_{0.001}$); n = 5

TABLE V

Total weight loss of unwaxed, waxed and fruit sealed in micro-perforated polypropylene bags, harvested on 03/08/04 or 23/08/04, after preconditioning, storage at 2^{o}C or 5.5^{o}C (30d) and ripening

Precon. time (d)	Precon. temperature (^{o}C)	Storage temperature (^{o}C)	Weight loss (%)					
			03/08/04			23/08/04		
			Unwaxed	Waxed	Polybag	Unwaxed	Waxed	Polybag
0	none	2	13.4	14.0	9.9	13.3	11.5	9.3
		5.5	13.0	11.0	8.0	13.8	13.9	8.1
1	10	2	12.3	12.8	8.8	11.8	10.7	10.2
	15		12.5	12.2	10.3	15.2	11.5	10.7
	20		13.7	13.3	12.0	13.1	11.7	9.4
	10	5.5	13.8	11.1	9.0	13.6	10.1	7.2
	15		14.7	12.1	9.4	13.6	9.5	8.3
	20		13.7	10.0	10.5	14.4	11.9	8.4
2	10	2	12.2	10.2	8.9	12.7	9.9	8.4
	15		13.3	15.1	10.1	11.2	11.9	9.4
	20		13.6	12.5	9.8	14.0	11.7	9.8
	10	5.5	13.0	11.9	7.6	12.7	11.0	8.1
	15		13.0	12.1	7.9	14.2	9.7	7.3
	20		13.1	12.6	7.3	14.2	11.0	8.5

Date = 0.3*

Packaging = 0.4**

Storage temperature = 0.4**

Preconditioning temperature = 0.5**

Packaging x Date = 0.5*

Packaging x Storage temperature = 0.6**

Packaging x Preconditioning time = 0.6*

Date x Storage temperature = 0.4*

Date x Preconditioning temperature x Storage temperature = 0.8*

Date x Preconditioning temperature x Preconditioning time x Storage temperature = 1.4*

Packaging x Preconditioning temperature x Preconditioning time x Storage temperature = 1.7*

* = significant ($LSD_{0.05}$); ** = significant ($LSD_{0.001}$); n = 5

TABLE VI

Days taken to reach "eating ripeness" of unwaxed, waxed and fruit sealed in micro-perforated polypropylene bags, harvested on 03/08/04 or 23/08/04, after preconditioning and storage at 2^{o}C or 5.5^{o}C (30 d)

Precon. time (d)	Precon. temp (^{o}C)	Storage temp (^{o}C)	Days taken to ripen 03/08/04 Unwaxed	Waxed	Polybag	23/08/04 Unwaxed	Waxed	Polybag
0	none	2	11.0	13.6	10.2	7.6	10.8	9.0
		5.5	5.0	5.2	6.8	6.2	7.0	6.4
1	10	2	8.2	12.4	9.6	6.4	10.0	9.0
	15		8.4	11.4	10.0	9.8	10.8	9.2
	20		9.2	12.4	12.4	7.8	10.8	8.8
	10	5.5	4.8	6.4	9.0	5.0	6.0	6.0
	15		5.2	9.6	11.0	5.0	7.4	6.8
	20		4.6	6.6	9.4	5.0	7.0	6.2
2	10	2	6.0	9.8	8.8	7.4	8.8	7.6
	15		7.2	10.0	9.8	6.0	8.6	9.6
	20		7.2	11.8	9.2	6.6	9.8	8.8
	10	5.5	4.4	6.0	7.6	4.8	7.0	7.4
	15		4.6	6.6	7.4	4.0	5.8	7.2
	20		5.4	7.4	6.6	4.4	7.0	6.6

Date = 0.3**
Packaging = 0.4**
Storage temperature = 0.3**
Preconditioning time = 0.4**
Preconditioning temperature = 0.4*
Date x Storage temperature = 0.5**
Packaging x Preconditioning time = 0.5*
Packaging x Storage temperature = 0.6**
Packaging x Preconditioning temperature = 0.5*
Preconditioning temperature x Storage temperature = 0.7**
Preconditioning temperature x Preconditioning time = 0.6*
Date x Preconditioning temperature x Storage temperature = 1.0**
Packaging x Date x Storage temperature = 0.6*
Packaging x Date x Preconditioning temperature x Preconditioning time = 1.6*
Date x Preconditioning temperature x Preconditioning time x Storage temperature = 1.3*
* = significant ($LSD_{0.05}$); ** = significant ($LSD_{0.001}$); n = 5

TABLE VII

Fruit firmness of preconditioned unwaxed, waxed and fruit sealed in micro-perforated polypropylene bags, harvested on 03/08/04 or 23/08/04, immediately after storage at 2^{o}C or 5.5^{o}C (30 d)

Precon. Time (d)	Precon. Temp (^{o}C)	Storage temp (^{o}C)	Firmness					
			03/08/04			23/08/04		
			Unwaxed	Waxed	Polybag	Unwaxed	Waxed	Polybag
0	none	2	85.7	86.9	88.9	83.4	84.7	88.9
		5.5	79.5	82.9	90.0	78.3	80.7	85.4
1	10	2	85.7	87.1	88.8	83.2	85.5	88.8
	15		84.7	85.1	87.9	83.2	85.3	86.4
	20		87.2	86.4	89.6	83.2	86.3	86.3
	10	5.5	79.1	84.9	88.8	81.4	82.8	85.9
	15		80.7	83.1	86.1	78.8	81.7	85.5
	20		80.8	84.9	87.2	78.6	81.5	86.0
2	10	2	83.2	84.6	87.7	85.1	84.5	86.2
	15		82.6	83.7	87.9	85.7	84.8	86.5
	20		82.6	86.3	86.9	82.8	85.3	87.9
	10	5.5	82.4	83.7	88.3	79.6	84.2	87.7
	15		80.8	83.0	87.9	78.6	82.9	87.9
	20		81.3	81.9	87.3	78.8	82.9	87.4

Date = 0.4**

Packaging = 0.5**

Storage temperature = 0.4**

Preconditioning temperature = 0.4*

Date x Preconditioning temperature = 0.6*

Date x Preconditioning time = 0.7**

Date x Storage temperature = 0.4*

Packaging x Storage temperature = 0.7**

Packaging x Preconditioning temperature = 0.9**

Preconditioning temperature x Storage temperature = 0.8**

Preconditioning time x Storage temperature = 0.7**

Packaging x Date x Preconditioning time x Storage temperature = 1.6**

Packaging x Date x Precon temperature x Precon time x Storage temperature = 2.5*

* = significant ($LSD_{0.05}$); ** = significant ($LSD_{0.001}$); n = 10

TABLE VIII

External chilling injury of preconditioned unwaxed, waxed and fruit sealed in micro-perforated polypropylene bags, harvested on 03/08/04 or 23/08/04, rated immediately after storage at 2°C or 5.5°C (30 d)

Precon. time (d)	Precon. temp (°C)	Storage temp (°C)	External chilling injury rating (0..10)‡					
			03/08/04			23/08/04		
			Unwaxed	Waxed	Polybag	Unwaxed	Waxed	Polybag
0	none	2	1.2	1.6	0.4	1.0	2.6	0.2
		5.5	0.2	0.2	0	1.0	2.2	0.2
1	10	2	0.8	0.6	0	0.4	0.4	0.2
	15		0	0.6	0	1.6	2.6	0.2
	20		1.8	2.8	0.4	1.6	2.6	0.2
	10	5.5	0.8	0	0	0	0.4	0
	15		0	0.6	0	0	0	0
	20		0.8	1.6	0	0.2	0.2	0
2	10	2	0	0	0	0.2	0.4	0.2
	15		0.6	0.4	0	0	0.4	0
	20		1.4	3.2	0	1.8	0.8	0.2
	10	5.5	0.4	0	0	0	0	0
	15		0	0	0	0	0	0
	20		0	0	0	0	0.6	0

Date = 0.1*
Packaging = 0.2**
Storage temperature = 0.2**
Preconditioning time = 0.2**
Preconditioning temperature = 0.2**
Date x Preconditioning temperature = 0.3*
Packaging x Storage temperature = 0.3**
Packaging x Preconditioning temperature = 0.4**
Preconditioning time x Storage temperature = 0.3**
Date x Preconditioning temperature x Preconditioning time = 0.4*
Date x Preconditioning temperature x Storage temperature = 0.4*
Date x Preconditioning time x Storage temperature = 0.3*
Packaging x Date x Preconditioning temperature = 0.4*
Date x Preconditioning temperature x Preconditioning time x Storage temperature = 0.6*
Packaging x Date x Precon temperature x Precon time x Storage temperature = 1.1*
* = significant ($LSD_{0.05}$); ** = significant ($LSD_{0.001}$); n = 5

‡ *0 = no injury, 10 = 100% surface area of fruit affected*

TABLE IX

Chilling injury severity of preconditioned unwaxed, waxed and fruit sealed in micro-perforated polypropylene bags, harvested on 03/08/04 or 23/08/04, which were stored at 2^{o}C or 5.5^{o}C (30 d) and rated after ripening

Precon. Time (d)	Precon. Temp (^{o}C)	Storage temp (^{o}C)	External chilling injury rating (0..10)‡					
			03/08/04			23/08/04		
			Unwaxed	Waxed	Polybag	Unwaxed	Waxed	Polybag
0	none	2	1.4	1.4	0.2	1.4	3.8	0.2
		5.5	0.2	1.0	0	0.2	0.6	0.4
1	10	2	0	0.2	0	0.8	1.4	0.4
	15		1.2	0.4	0	1.2	2.0	0.4
	20		1.6	2.2	0	2.0	2.2	0.8
	10	5.5	0	0.2	0	0	0.2	0
	15		0.2	0.2	0	0.2	0	0
	20		0	0.6	0	0	2.0	0.2
2	10	2	0	0	0	0	0.2	0
	15		0	0	0	0	0.2	0
	20		0.6	0.8	0.2	1.2	1.0	0.4
	10	5.5	0	0	0	0	0.4	0
	15		0	0	0	0.2	0	0
	20		0.4	0	0	0.6	0.4	0

Date = 0.1**
Packaging = 0.2**
Storage temperature = 0.1**
Preconditioning time = 0.2**
Preconditioning temperature = 0.2**
Date x Storage temperature = 0.2**
Packaging x Date = 0.3**
Packaging x Preconditioning time = 0.2*
Packaging x Storage temperature = 0.3**
Packaging x Preconditioning temperature = 0.4**
Preconditioning temperature x Storage temperature = 0.3**
Preconditioning time x Storage temperature = 0.3**
Packaging x Date x Storage temperature = 0.3*
Packaging x Date x Preconditioning temperature x Storage temperature = 0.6*
* = significant ($LSD_{0.05}$); ** = significant ($LSD_{0.001}$); n = 5

‡ *0 = no injury, 10 = 100% surface area of fruit affected*

TABLE X

Mesocarp discolouration ratings of unwaxed, waxed and fruit sealed in micro-perforated polypropylene bags, harvested on 03/08/04 or 23/08/04, after preconditioning, storage at either 2^oC or 5.5^oC (30 d), and ripening

Precon. Time (d)	Precon. Temp (^oC)	Storage temp (^oC)	Mesocarp discolouration (0..10)‡					
			03/08/04			23/08/04		
			Unwaxed	Waxed	Polybag	Unwaxed	Waxed	Polybag
0	none	2	0.8	0.6	0.6	0.2	0.4	0.2
		5.5	0	2.8	0	0	0	0.2
1	10	2	0.2	0.8	0.2	0.2	0.8	0
	15		0.4	0.4	0.6	0.6	0.6	0.2
	20		0.2	0.6	0.2	0.6	0.8	0
	10	5.5	0	1.0	0.4	0	0.2	0
	15		0	0.2	0	0	0	0
	20		0	0.4	0.4	0.4	1.4	0.2
2	10	2	0.2	0.4	0.4	0	0	0
	15		0.2	0.2	0.6	0	0	0.6
	20		0	0.2	0	0.4	0.2	0.6
	10	5.5	1.4	0	0	0	0.6	0.2
	15		0.2	0	0	0.4	0.2	0.2
	20		1.4	0	0	0.8	0.4	0

Date = 0.1*

Packaging = 0.2**

Storage temperature = n.s.

Preconditioning time = n.s.

Preconditioning temperature = n.s.

Date x Preconditioning temperature = 0.3*

Packaging x Storage temperature = 0.2*

Packaging x Preconditioning time = 0.3*

Packaging x Date x Storage temperature = 0.3*

Packaging x Preconditioning time x Storage temperature = 0.4*

Packaging x Date x Preconditioning temperature x Storage temperature = 0.6*

Packaging x Date x Preconditioning time x Storage temperature = 0.4*

* = significant ($LSD_{0.05}$); ** = significant ($LSD_{0.001}$); n.s. = non-significant; n = 5

‡ *0 = no discolouration, 10 = 100% of cut surface area black*

TABLE XI

Anthracnose score of unwaxed, waxed and fruit sealed in polybags, harvested on 03/08/04 or 23/08/04, after preconditioning, storage at either 2°C or 5.5°C (30 d), and ripening

Precon. Time (d)	Precon. Temp (°C)	Storage temp (°C)	Anthracnose score (0/1)‡ 03/08/04			23/08/04		
			Unwaxed	Waxed	Polybag	Unwaxed	Waxed	Polybag
0	none	2	0.2	0.6	0.6	0.2	0.6	0.4
		5.5	0	0.2	0.2	0	0.2	0
1	10	2	0	0.4	0.2	0.2	0.6	0
	15		0.4	0.2	0.6	0.4	0.6	0.2
	20		0	0.2	1.0	0.2	0.4	0.6
	10	5.5	0	0.2	0.2	0	0	0
	15		0	0.4	0.2	0	0	0
	20		0	0.2	0.2	0	0.4	0.2
2	10	2	0	0.2	0.2	0	0	0
	15		0	0.4	0.6	0	0	0.2
	20		0	0	0.2	0	0.2	0.4
	10	5.5	0.2	0	0	0	0.2	0
	15		0	0	0	0	0	0.2
	20		0.2	0	0	0	0.2	0

Date = n.s.
Packaging = 0.1**
Storage temperature = 0.1**
Preconditioning time = 0.1**
Preconditioning temperature = 0.1*
Packaging x Date = 0.1*
Packaging x Storage temperature = 0.1*
Packaging x Preconditioning temperature = 0.1*
Preconditioning temperature x Storage temperature = 0.1*
Preconditioning time x Storage temperature = 0.1*
Packaging x Preconditioning time x Preconditioning temperature = 0.2*
Date x Preconditioning temperature x Preconditioning time x Storage temperature = 0.3*
* = significant ($LSD_{0.05}$); ** = significant ($LSD_{0.001}$); n.s. = non-significant; n = 5

‡ *0 = no pathogen detected; 1 = pathogen detected*

TABLE XII

Proline concentration of unwaxed, waxed and fruit sealed in polybags, harvested on 03/08/04 or 23/08/04, after preconditioning, and storage at either 2°C or 5.5°C (30 d)

Preconditioning time (d)	Preconditioning temperature (°C)	Proline concentration (μmole proline/ g fresh weight)					
		03/08/04			23/08/04		
		Unwaxed	Waxed	Polybag	Unwaxed	Waxed	Polybag
0	none	0.019	0.025	0.016	0.039	0.059	0.028
1	10	0.018	0.037	0.012	0.034	0.033	0.027
	15	0.020	0.022	0.017	0.044	0.056	0.025
	20	0.027	0.023	0.022	0.039	0.047	0.026
2	10	0.017	0.008	0.009	0.021	0.024	0.026
	15	0.021	0.014	0.014	0.025	0.021	0.027
	20	0.024	0.017	0.019	0.041	0.026	0.028

Date = 0.003**

Packaging = 0.004**

Preconditioning time = 0.004**

Preconditioning temperature = 0.004**

Packaging x Date = 0.004*

Packaging x Preconditioning time = 0.007**

Date x Preconditioning temperature = 0.005*

Date x Preconditioning time x Preconditioning temperature = 0.008*

Packaging x Date x Preconditioning time x Preconditioning temperature = 0.014*

* = significant ($LSD_{0.05}$); ** = significant ($LSD_{0.001}$); n = 5

Figure 1. Photo illustrating chilling injury damage/pitting around the lenticels of a waxed ‘Pinkerton’ fruit after cold storage at 2^{o}C.

Figure 2. Photo illustrating the symptoms of the unidentified fungal infection in unwaxed fruit preconditioned and stored in micro-perforated polypropylene bags.

Figure 3. Photo illustrating the typical symptoms of external chilling injury/pitting in ‘Pinkerton’ avocado fruit (unwaxed).

Figure 4. Photo illustrating the symptoms of the unidentified fungal infection on the exocarp tissue, and immediately underneath.

Figure 5. Photo illustrating the typical symptoms of an anthracnose infection in ripening avocado fruit.