CHAPTER 6

GENERAL DISCUSSION AND CONCLUSIONS

Initially the aim of this research was to find ways of reducing the development of mesocarp discolouration in ‘Pinkerton’ avocado, while at the same time trying to identify factors that increase the potential for disorder development, so that the future of the cultivar could be ensured. As mesocarp discolouration was not a new or unique disorder to avocado fruit, the study started out by testing various hypotheses made during past studies. One such hypothesis was that mesocarp discolouration was the result of “chilling injury”.

Throughout the study there was unequivocal evidence to suggest that postharvest storage temperature does, in fact, have a significant effect on the severity of mesocarp discolouration development in ‘Pinkerton’ avocado fruit. However, contrary to common beliefs the disorder was not found to be the result of too low, but rather too high a storage temperature. Storage at 2°C proved to be highly beneficial in maintaining internal fruit quality. Fruit remained hard during the 4-week storage period, which is a highly desirable trait for export consideration. Membrane integrity was also retained at this temperature. It appears that storage at 2°C slows down the metabolic activity of the fruit, possibly enough to prevent imbalances in metabolism, and subsequently the accumulation of toxic intermediates. Fruit stored at intermediate temperatures have often been found to develop more “chilling disorders” than fruit stored at higher or lower temperatures (Ulrich, 1958). In the case of ‘Pinkerton’ it would appear that temperatures between 5.5°C and 8°C fall in the “intermediate” range. The improved membrane integrity, found in fruit stored at 2°C, becomes even more important when considering the fact that the total phenolic content of fruit, while lower at 2°C, was found to increase during the harvest season, thereby increasing the potential for mesocarp discolouration later in the season. Furthermore, fruit from historically poor quality/ “high risk” areas had higher PPO activities to start with and would therefore benefit greatly from the improved membrane integrity found in fruit stored at 2°C.

Nevertheless, while postharvest storage conditions may be beneficial in reducing the severity of mesocarp discolouration identifying and treating the cause of the disorder is the preferred mode of action. The differences in mesocarp discolouration severity between fruit origins and between seasons indicate that preharvest conditions play a significant role. This presents a more complex interaction of variables, and furthermore these may differ between areas. However, determining the mineral status of fruit appears to be a valid starting point. From the results of the study excessive nitrogen concentrations (>1%) at harvest proved to be detrimental to fruit quality. This is supported by the work of Snijder *et al.* (2002). The high nitrogen content of the fruit implied that the soil and tree might contain very high nitrogen levels and subsequently excessive tree vigour may have been a problem. Vegetative/shoot growth is known to be a stronger sink for minerals, such as calcium (Witney *et al.,* 1990), as well as other metabolites (carbohydrates) and water. Bower and Van Lelyveld (1985) previously found that preharvest water stress could have an adverse effect on PPO activity and consequently fruit quality. Thus irrigation schedules would have to be adapted to the vegetative state of the tree. The carbohydrate status of the fruit would also be important to postharvest fruit quality as the fruit depends on this for maintaining the energy supply during respiration and ripening, with membrane integrity also being affected. While 'Pinkerton' is not severely affected by an alternate bearing pattern there is circumstantial evidence to suggest that prevalence of the disorder is. Furthermore, the soluble C7 sugar, mannoheptulose, and a related C7 sugar alcohol, perseitol, have been found to play a role in fruit ripening. These sugars display a distinct seasonal fluctuation in response to vegetative growth flushes in 'Hass' (Liu *et al.*, 1999). Further studies could, therefore, investigate how excessive vigour and crop load affects the carbohydrate status of 'Pinkerton' fruit, especially as this cultivar has an extended flowering period.

The condition of the tree will also affect the fruit mineral content and thus fruit from different areas will be expected to differ. The mineral status of a tree is affected by many factors (which may interact), such as light, temperature, water, and plant growth regulators. The relationship between calcium and auxin may be a good example of this, with auxin concentrations being high in metabolically active organs, such as developing shoots, and thus being stronger sinks for calcium. Calcium is also thought to move in the transpiration stream of the plant and the new vegetative flush would have a high transpiration rate. In this study, however, calcium was not found to contribute significantly to disorder development as previous studies have indicated (Chaplin and Scott, 1980; Cutting *et al*., 1992). Furthermore, while the ratio of nitrogen:calcium was found to play a significant role, many of the other elements were found to contribute more significantly. However, it is difficult to eliminate the roles of certain elements altogether, as it may be that their concentrations play a significant role at certain periods of fruit growth and not necessarily during postharvest storage.

Furthermore, the carbohydrate status of a fruit may play a deciding role in whether a certain element is deficient or not. There are, thus, dynamic sets of interactions that can affect the tree at any one time and this will require intelligent and adaptive management practices throughout the changing conditions of the season.

In summary, the factors contributing to mesocarp discolouration development are complex. However, there do appear to be some factors, which can be managed to a certain extent, such as preharvest water stress and mineral status. Studies are currently being undertaken in South Africa to identify critical periods at which certain mineral concentrations play a role. These studies include regularly sampling fruit for mineral content, from about two weeks post petal fall until harvest, and should help with establishing benchmark concentrations so that management practices can be adapted. In the meantime ways of managing nitrogen need to be found to reduce vigour and improve source:sink relationships. Management practices might include choosing more suitable rootstocks for the prevailing conditions. Rootstocks have been known to affect mineral uptake of trees (Marques, 2002) as well as tree vigour (Wolstenholme, 2003). Unfortunately the resistance of the rootstock to *Phytophthora cinnamomi* will also have to be considered under South African conditions. Applying suitable mulches to soils that contain high nitrogen concentrations may also be beneficial in decreasing the available soil nitrogen (Wolstenholme, 2004). Mulches with a high carbon:nitrogen ratio (>30) would result in micro-organisms using the soil nitrogen to break down the carbon/mulch thus withdrawing excess nitrogen from the soil.

Unfortunately the chlorophyll fluorescence technique used in this study did not prove to be useful in predicting internal fruit quality. However, improvements in this field are being made daily and the equipment used to determine chlorophyll fluorescence is getting more advanced, thus this technology may become useful in the future.

In order to finalise a postharvest protocol suitable for commercial application, when handling ‘Pinkerton’ fruit, certain factors had to be addressed. This included studying the effects of a 3 d storage delay, experienced during 2000 and 2001, on fruit quality, as well as the affect of 2^{o}C storage on external chilling injury development. Past studies conducted by other researchers (Kruger *et al*., 2000) using fruit from the same risk areas were unsuccessful at storing fruit at temperatures below 5.5^{o}C from both an internal and external point of view. It was suspected that fruit used in the other studies were placed into storage a lot sooner than fruit in this study as fruit were received on the same day as the fruit were harvested. The optimal procedure after harvest is thought to entail getting fruit into the packhouse as soon as possible, with cooling following shortly thereafter. This technique was thought to reduce fruit moisture loss, which has been correlated to fruit quality (Bower *et al*., 1989), and to reduce the rate of metabolism of the stored fruit in order to prolong its postharvest life. While many fruit types benefit from rapid cooling after harvest, some fruit are more resistant to "chilling injury" if held at temperatures slightly above those at which injury normally occurs before storage.

Studies, conducted in 2004, appeared to support the fact that a storage delay could be beneficial in terms of reducing both mesocarp discolouration and external chilling injury in 'Pinkerton' avocados. However, the temperature at which the fruit are held prior to storage plays a significant role. In this study holding fruit at 10°C for 2 d consistently rendered fruit with the best internal and external quality regardless of how fruit were packaged. Low temperature conditioning has also been successful in reducing external chilling injury in 'Hass' avocados (Woolf *et al*., 2003; Hofman *et al*., 2003). It appears, therefore, that this form of preconditioning shows considerable potential in terms of being able to store fruit at very low temperatures without damage. The ability of fruit to undergo a cold disinfestation treatment therefore becomes an attainable goal, in terms of meeting quarantine standards while maintaining high fruit quality, and opens up the possibility of exporting fruit to new lucrative markets.

The role of fruit packaging in external chilling injury development still needs further investigation. Previous research had indicated that decreasing moisture loss (as determined by weight loss) during storage could possibly overcome the development of external chilling injury (Bower and Jackson, 2003). Fruit sealed in micro-perforated polypropylene bags (polybags) lost the least weight during preconditioning and storage, developed the least external chilling injury and consistently had the lowest proline concentrations. Unwaxed fruit, while losing the most weight during preconditioning and storage did not always display the most external chilling injury, nor were proline concentrations consistently higher in these fruit. If we accept the theory that proline concentrations reflect the level of stress within a plant tissue then it is safe to assume that the waxed fruit were exposed to a stress other than that inflicted by moisture loss. Waxing can affect the gaseous exchange of fruit and further studies might include observing the fruit exocarp under a scanning electron microscope to see whether lenticels do become clogged and whether these areas become the primary sites for membrane collapse and “pitting’. Measuring the carbon dioxide or ethylene production of fruit subjected to different packaging treatments, and different wax thicknesses may also prove to be enlightening. However, the interaction between temperature and the various packaging treatments will also have to be taken into account, as this may account for the differences found between studies using similar materials.

Kosiyachinda and Young (1976) showed that fruit susceptibility to disorders was affected by the stage of climacteric development. Fruit were found to be more susceptible to chilling disorders as they approached the climacteric peak. In practice, Zauberman and Jobin-Décor (1995) found that the storage temperature of Australian ‘Hass’ could be reduced to 2^{o}C for preclimacteric fruit. Increasing maturity as the season progresses would thus be expected to reduce the time taken to reach the climacteric peak and thus care would have to be taken not to delay storage for too long later in the season. Packaging treatments that will result in some kind of modified atmosphere around the fruit, either by reducing weight loss or by affecting gaseous exchange, will possibly affect the period of preconditioning needed to acclimatise fruit to low temperature storage. As weight loss is affected by harvest date studies will have to be undertaken over the whole harvest season to determine the best handling procedure. Furthermore, as preharvest conditions, such as temperature, are known to affect the sensitivity of fruit to subsequent postharvest chilling, these studies will also have to be conducted with fruit from different origins.

In terms of more advanced studies the determination of lipid saturation in ‘Pinkerton’ fruit from different origins may also help in predicting how fruit will respond to various storage temperatures. It is reported that as temperature increases the degree of lipid unsaturation is reduced, while as temperature decreases, unsaturation increases. Insertion and removal of sterols from membranes is suspected to be part of the mechanism by which membranes adjust to temperature changes (Nilsen and Orcutt, 1996). As membrane permeability increases a change in ionic leakage occurs, as well as a possible increases in the activation energy of membrane-bound enzyme systems. Ultimately this can lead to imbalances in metabolism, which eventually lead to cell death. Determining the physiological and biochemical makeup of avocado fruit may, thus, enable horticulturalists to predict how fruit will respond to postharvest chilling.

FINAL RECOMMENDATIONS

Better ways than presently used to manipulate tree cropping in 'Pinkerton' may be essential. The influence of water stress, the role of minerals and perhaps of special note, carbohydrate relationships, need to be investigated further. It is necessary to ensure that during fruit development membrane systems, sufficiently robust to cope with pre- and postharvest stress, are laid down. The fruit must also contain sufficient carbohydrates to sustain the rapid energy requirement of the large fruit, which 'Pinkerton' normally has, without resorting to potentially destructive alternative energy forms. Coupled to this, a new postharvest strategy including low temperature storage (2-4^{o}C) imposed after suitable acclimation, plus packaging to eliminate external chilling injury, is likely to ensure that the poor quality image of 'Pinkerton' is changed.

While further work is still needed, as outlined in the previous section, the relative roles of numerous factors relating to the 'Pinkerton' problem have been elucidated, and even without complete knowledge of the complex interaction of systems causing the problems, it is believed that a protocol for an acceptable risk in exporting the cultivar has been achieved. The technique may also improve the quality of the less risky cultivars. In South Africa the 'Hass' cultivar makes up a very large portion (49%) of the export crop and, unlike the greenskin cultivars, fruit quality in 'Hass' is not hindered as much by the development of external chilling injury, as these symptoms become masked as the fruit ripen and the exocarp darkens. However, if the risk of external chilling can be decreased in this cultivar, then the potential for secondary fungal infections also decreases and fruit of a better quality can be expected thus potentially enhancing returns. Furthermore, fruit of consistent and predictable quality will be more competitive in the market.