INTRODUCTION

The South African avocado industry is highly export driven and during 2005 an estimated total of 11.5 million, 4 kg cartons (46 000 tons), were expected to be exported to France, Germany, the U.K. and the rest of Europe. Of the exported crop almost 51% is made up of greenskins and 49% of 'Hass'. During 2002 the 'Pinkerton' cultivar only made up 3% of the total value, however current figures reflect an estimated value of up to 7.1% of the final total (3 272 tons).

The avocado (*Persea americana* Mill.) belongs to the family Lauraceae and has been classified into three botanical races viz. *Persea americana* var. *americana*, the West Indian types; *Persea americana* var. *drymifolia*, of Mexican origin; and *Persea americana* var. *guatemalensis*, of Guatemalan origin (Bergh and Ellstrand, 1986). The 'Pinkerton' cultivar originated in 1959 as a 'Hass' X 'Rincon' hybrid, showing mostly Guatemalan characteristics (Wood, 1984). The tree has an irregular, moderately spreading growth habit and is a very precocious and consistent bearer.

Transport to European markets is mainly done by sea, which necessitates storage of up to 28 days. Due to the highly climacteric and rapidly softening nature of the fruit the only way to ensure that fruit arrive at their market with a reasonable shelf life is to ship the fruit at low temperatures. Unfortunately this low temperature storage may result in the development of certain physiological disorders (Eaks, 1976; Chaplin *et al.*, 1982; Swarts, 1984). Physiological disorders refer to the breakdown of tissue that is not caused by either the invasion of pathogens or by mechanical damage (Wills *et al.*, 1989).

Mesocarp discolouration

The export of the 'Pinkerton' cultivar, in particular, has been seriously threatened by the development of a disorder that is known as mesocarp discolouration, grey pulp or chilling injury, with the last name reflecting what is thought to be the cause of the disorder (Chaplin *et al.*, 1982; Couey, 1982). The disorder is characterized by a discolouration of the mesocarp, visible on cutting the fruit in half, with exposure to the atmosphere causing further blackening. This disorder is not unique to the 'Pinkerton' cultivar, however, and studies to ascertain the

cause have been conducted over many years, and in fact for similar symptoms in many other fruit types as well (Ferguson and Watkins, 1989; Crisosto *et al.*, 1997).

Chilling injury may manifest itself in many ways; for example, surface lesions on fruits, water soaking of tissues, internal discolouration (including vascular browning), increased susceptibility to decay, and failure to ripen normally (Saltveit and Morris, 1990). Studies on avocados have, however, found that the mesocarp discolouration may also develop in unstored fruit (Vakis, 1982). This has resulted in some confusion as to the role of storage temperature in disorder development. Studies, done specifically on 'Pinkerton', have shown inconsistent results with some research showing that this cultivar should be shipped at 7°C to reduce mesocarp discolouration (Sippel *et al.*, 1994; Kruger *et al.*, 2000), while yet other research showed that 5.5°C rendered better quality (Schutte, 1994). Zauberman and Jobin-Décor (1995) attributed mesocarp discolouration in 'Hass' to being the result of ripening occurring during cold storage, and suggested fruit could be stored at 2°C. However, Kritzinger *et al.* (1998) found that storage at 2°C in 'Pinkerton' resulted in a significantly higher incidence of mesocarp discolouration than fruit stored at 5°C. Shipping 'Pinkerton' in controlled atmosphere (CA) storage has also not alleviated the problem, with mesocarp discolouration even reported as being higher in CA than regular atmosphere (RA) (Eksteen *et al.*, 1998).

Matters are further complicated by the fact that the severity of mesocarp discolouration differs between fruit origins (Kruger *et al.*, 2000) and also between seasons, which has led to preharvest factors being thought to play a role (Rowell and Durand, 1982; Bezuidenhout and Kuschke, 1982; Bezuidenhout, 1983; Bower, 1984). Mineral nutrition is known to play a large role in tree health and also in fruit quality. Calcium has been associated with more physiological disorders than any other mineral (Bangerth, 1979; Wills *et al.*, 1989) and many studies have investigated the role of calcium in mesocarp discolouration. In addition, fruit firmness, after storage, was thought to be related to disorder potential (Eksteen *et al.*, 1998) and calcium is known to be important for membrane integrity (Kremer-Köhne *et al.*, 1993). While ensuring high calcium concentrations appeared to solve the problem in many of the other avocado cultivars (Chaplin and Scott, 1980; Cutting *et al.*, 1992; Hofman *et al.*, 2002; Thorp *et al.*, 1997), calcium applications to 'Pinkerton' rendered inconsistent results (Penter *et al.*, 2001).

Fruit maturity, prior to cold storage, has also been found to play an important role in the severity of mesocarp discolouration. In South Africa an attempt was made to identify which growing areas showed a higher potential for mesocarp discolouration development in 'Pinkerton' fruit. The effect of fruit maturity was then investigated and it was found that the severity of the disorder could be reduced by assigning various "cut-off" maturities for fruit from the respective areas based on their potential for disorder development (Kruger *et al.*, 2000). The determination of fruit maturity is, however, complicated by the fact that the 'Pinkerton' cultivar has an extended flowering period resulting in differences in fruit maturity at the harvesting stage. Late set fruit have a much faster growth rate than early set fruit, resulting in fruit being picked prematurely if fruit size is taken as a maturity index (Sippel *et al.*, 1994). Tree yield can also affect final fruit quality with high yielding trees being reported to produce fruit with a shorter shelf life and more internal browning (Cutting and Vorster, 1991). However, the percentage of fruit with internal disorders can also be higher in fruit from low yielding trees (Köhne *et al.*, 1992). Internal disorders and external skin discolouration due to chilling injury may also be greater in fruit from more vigorous tress (Vorster *et al.*, 1989).

In summary, the factors that contribute to mesocarp discolouration development in 'Pinkerton' avocados appear to be complex, and the lack of clear guidelines to both grower and packer, have resulted in guality so poor that by 1999, exporters were warning producers that they were finding it difficult to market the cultivar. In fact, the situation has become so serious that the future of 'Pinkerton' is threatened. The main purpose of this research was, thus, to identify possible pre- and postharvest factors, or interactions, which would contribute to mesocarp discolouration development. Postharvest studies would concentrate on elucidating the role of shipment temperature on the incidence of the internal disorder, and would include evaluations of membrane integrity (DeEll et al., 1999; Thompson, 1988; Stanley, 1991) and fruit respiration (Bain and Mercer, 1964). Fruit from different origins, varying in mesocarp discolouration histories, would be obtained, throughout the harvest season, to determine the effect of these variables on temperature response. Fruit weight loss during storage, and fruit firmness after storage would also be evaluated as they are thought to be related to membrane integrity. Furthermore, the effects of temperature and fruit origin on internal browning potential would also be investigated by determining the total phenolics concentration and the activity of the enzyme polyphenol oxidase (PPO) in fruit. The quantity of either total phenolics and/or the PPO enzyme in the avocado fruit flesh has been found to have an effect on the severity of discolouration (Golan et al., 1977).

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To determine possible preharvest differences between fruit from various origins, fruit mineral concentrations would be determined to evaluate what role they play in postharvest fruit quality. Previous studies raised some questions as to the role of calcium in disorder development and thus other elements would be included in the study. Elements with known roles in avocado fruit quality include nitrogen (Arpaia *et al.*, 1996), boron (Smith *et al.*, 1997), magnesium, potassium (Koen *et al.*, 1990; Witney *et al.*, 1990), and zinc (Vorster and Bezuidenhout, 1988).

External chilling injury

Horticulturists are faced with many challenges when trying to reduce the development of a certain disorder, as the factors contributing to a decrease in the severity of that disorder may aggravate and/or lead to the development of another. Furthermore, the search for new export markets requires that fruit from certain areas be subjected to periods of cold disinfestations before fruit are allowed to enter the country. While the main aim is to eliminate the potential for insect infestations in the fruit, the mandatory cold temperature treatment gives rise to the development of skin damage (external chilling injury). In South Africa external chilling injury is also known as cold damage, and typical symptoms may include the formation of black areas on the fruit, which become slightly sunken, and is thus called "pitting".

While external chilling injury has not been the limiting factor in exporting 'Pinkerton' fruit in the past it still affects overall fruit quality, and symptoms may become aggravated if cold disinfestation proves to be a successful way of eliminating quarantine pests. As with mesocarp discolouration, external chilling severity can also be affected by preharvest orchard conditions (Bower, 1988; Kremer-Köhne *et al.*, 1993; Kruger *et al.*, 2004). However, the use of certain postharvest techniques, that have proven to be successful in alleviating the severity of the disorder, may prove to be preferable in terms of logistics as it may enable fruit from different areas to receive the same postharvest handling. Many studies have demonstrated that low temperature conditioning can alleviate chilling injury symptoms of fruit and seedlings (Wang, 1993), and more specifically in avocados (Woolf *et al.*, 2003). Fruit packaging, for example the use of waxes and film packaging, have also shown varying degrees of success in reducing chilling severity (Ben-Yehoshua *et al.*, 1981; Forney and Lipton, 1990; Wang,

1993). The packaging treatments were thought to modify gaseous exchange and reduce moisture loss (as determined by weight loss), which is thought to prevent the collapse of membranes (Forney and Lipton, 1990; Wang, 1993).

In an attempt to improve overall fruit quality in 'Pinkerton' avocados the effect of low temperature conditioning and the application of various packaging treatments would be investigated in the second half of this study, as these treatments are fairly simple to implement and don't necessitate major modifications to the current cold chain system. To determine the role of moisture loss on external chilling injury development three packaging treatments would be used in the study, i.e., unwaxed, waxed and fruit sealed in polypropylene bags. Throughout the preconditioning and storage treatments the weight loss of fruit would be recorded and compared to external chilling injury ratings, as well as other fruit quality determinants (such as days to ripening, mesocarp discolouration severity and the presence of pathogenic infections). To establish the level of stress experienced by the fruit, during the preconditioning treatments, the proline concentrations of the fruit exocarp would be determined.

It was hoped that the results of the study would give a more holistic understanding of which factors, or interactions, play a significant role in mesocarp discolouration severity in 'Pinkerton', and possibly the mechanisms involved, so that corrective measures could be taken to avoid, or at least minimize, mesocarp discolouration development. This, together with determining a successful postharvest technique of reducing external chilling injury, will enable the South African avocado industry to produce fruit of a high quality and make the goal of entering new markets a reality.