

# Contribution of the seed to fruit development: A tool to understand avocado tree management and fruit maturity parameters

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## ABSTRACT

Mesocarp percentage moisture of 'Fuerte' and 'Hass' avocado (*Persea Americana Mill.*) fruits harvested during 1999-2000 and 2001-2002 growing seasons was determined at different stages of development and correlated to seed water potential. The two relations declined with increasing fruit ontogeny, and were linearly and significantly influenced by the number of days after full bloom (stage of development) and the cultivar ( $P < 0.001$ ). Immature 'Fuerte' fruit had lower seed water potential than 'Hass' ( $-0.86 \pm 0.09$  MPa and  $-1.18 \pm 0.11$  MPa respectively). At maturity, the early harvested 'Fuerte' had higher seed water potential ( $-2.13 \pm 0.13$ ) than the late harvested 'Hass' ( $-1.35 \pm 0.06$  MPa). Seed water potential of developing 'Fuerte' and 'Hass', for which a stoichiometric relationship change to the cell sap content was observed, was measured to negatively correlate with mesocarp percentage moisture ( $r^2 = 0.991$ ). Postharvest analysis showed that the effect of the storage temperature (5.5°C) on 'Fuerte' seed water potential significantly depended on the fruit size rather than the time in storage. We proposed that in addition to being a reliable element for 'Fuerte' and 'Hass' tree management, seed water potential may be used as an index for physiological maturity determination.

## INTRODUCTION

Avocado fruit maturity has been extensively studied and progress achieved in solving postharvest related injuries (Cutting and Wolstenholme, 1992; Chen *et al.*, 1993; Mizrach *et al.*, 1999). However, set-back in the quality of fruits exported by the South African avocado industry have been reported from time to time (Nelson *et al.*, 2001) despite identifications of risk factors necessary to formulate maturity cut-off points for all major cultivars (Kruger *et al.*, 2001, 2004; Snijder *et al.*, 2002). In addition, several authors have expressed concern on the disparity in the quality of fruit from the same cultivar picked up at different times in the same growing season (Cutting *et al.*, 1988; Cutting *et al.*, 1992; Kruger *et al.*, 2001, 2003, 2004). These observations were recently echoed by Hofman *et al.* (2000) who reported that for late harvested 'Hass', currently set maturity standards, mesocarp moisture content and percentage oil are not reliable.

Indications are that the degree of postharvest disorders in developing avocado fruit such as mesocarp discoloration is closely related to the amount of moisture available to the fruit, especially at harvest (Bower and Van Lelyveld, 1986; Bower *et al.*, 1989; Blanke and Whiley, 1995). A better fruit quality, based on incidence of rots and physiological disorders of the flesh, was obtained with 'Fuerte' avocado by decreasing water stress on the fruit during storage. Furthermore, fruit from humidified storage had lower immediate mesocarp browning as estimated by the activity of PPO, than fruit from 'dry' cold room (Bower *et al.*, 1989). These studies suggested that the amount of water present in the mesocarp at harvest has an important role in determining the extent of mesocarp discoloration disorders. Cutting and Wolstenholme (1992) also reported that passive water infusion in the

mesocarp of picked 'Fuerte' fruit resulted in total inhibition of the manifestation of postharvest browning disorders. It is therefore plausible to hypothesize that inadequate fruit moisture content at harvest is the major controlling factor that influences postharvest quality of avocado fruit.

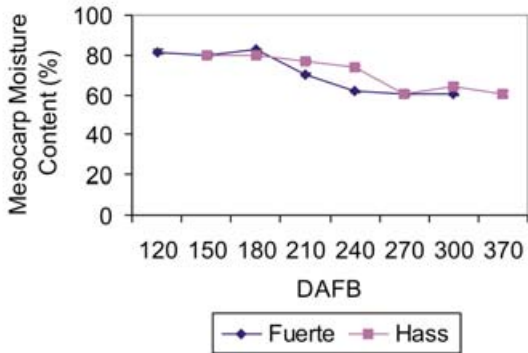
The seed of avocado fruit behaves like a recalcitrant seed (Egüi, 1990; Wolstenholme and Whiley 1999) and acts as the potential 'water reservoir' during fruit development (Jones, 1985). The influence of the seed on the developing avocado fruit is well documented (Wolstenholme *et al.*, 1985; Cannel, 1985; Cowan *et al.*, 2001) and indications are that during development, it assumes dominance over the mesocarp for available water and solutes (Moore-Gordon *et al.*, 1998; Cowan *et al.*, 2001). The presence of a 'healthy' pachychalazal seed coat, which acts as the trafficking route through plasmodesmata for solutes between the mesocarp and seed (Moore-Gordon *et al.*, 1998; Crawford and Zambryski, 1999; Botha *et al.*, 2000; Van Bel, 2003), and the ability of the seed to be non-endospermic and the major storage organ at maturity (Bewley and Black, 1994), indicate that this organ regulates physiological and biochemical processes in the avocado fruit during development and maturation (Gillapsy *et al.*, 1993). Under these conditions, an imbalance between lipids and water in the mesocarp may ensue. The result will be the collapse of the single layer encapsulating the oil bodies (oleosomes) followed by membrane lipification, cell plasmolysis and the onset of many other physiological disorders. Despite these characteristics, little or no work has addressed developmental issues of the avocado fruit using the seed. This avenue was used to re-assess maturity parameters of 'Fuerte' and 'Hass' avocado. We investigated changes in water relations of the seed of 'Fuerte' and 'Hass'

avocado fruit during growth and storage, and related them to fruit maturity. A novel approach in determining physiological maturity of avocado fruit is suggested.

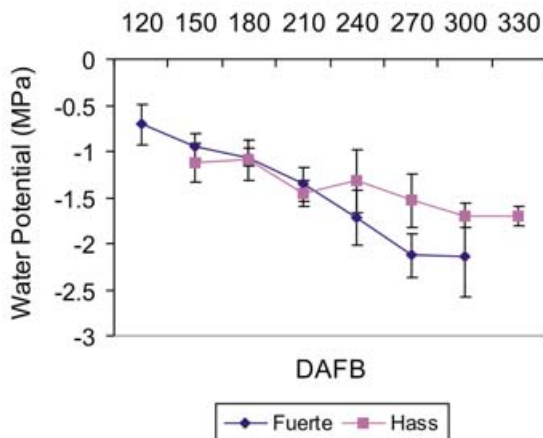
## MATERIALS AND METHODS

### Plant material

Six fruits of avocado (*Persea americana* Mill.) were collected from Everdon Estate, (Howick, KwaZulu-Natal, South Africa) at monthly intervals from March to November during the 1999-2000 and 2001-2002 growing seasons separately from 'Hass' and 'Fuerte' trees grafted on the clonal Duke 7 rootstock. Fruits attached to their fruit stems were randomly harvested using a



**Figure 1. Mesocarp percentage moisture content of seed tissue of 'Fuerte' and 'Hass' avocados during fruit ontogeny. A thin mesocarp section (ca 30 g) was dried at 75°C in an oven for 48 to 72 h, to a constant mass. The ratio of wet mass minus dry mass to wet mass, expressed as a percentage, was considered to be mesocarp percentage moisture. Data are means of  $\pm$  SE of 6 replicates per analysis during 1999-2000 and 2001-2002 growing seasons. Error bars not visible are smaller than data points. DAFB = number of days after full bloom.**



**Figure 2. Change in  $\Psi_w$  of the cotyledon of seed tissue of 'Fuerte' and 'Hass' avocados during fruit development and maturation. Cotyledonary seed tissue from 'Fuerte' and 'Hass' fruit (ca 1 g) was incubated in graded-solution of mannitol of known concentrations for 2 hours, and the  $\Psi_w$  of incubates was calculated using the equation  $\Psi_w = miRT$  (1) (Nobel, 1991), where m = molarity of the solute, i = ionization constant of the solute, R = gas constant (0.083 L bars/mole degree) and T = absolute temperature ( $^{\circ}C + 273$ ). There were six replicates per experiment per growing. Data points are means of 6 replicates  $\pm$  SE per analysis during 1999-2000 and 2001-2002 growing seasons. DAFB = number of days after full bloom.**

knife, wrapped in chamois leather to prevent moisture loss, and transported on ice in a cooler box to the laboratory for analysis.

Fruits were cut open with a knife, checked that they had a fresh, non-degenerated and functional seed coat, and the mesocarp separated from the seed for the analyses. Where necessary, fruits were graded into export counts as recommended by the South African Avocado Growers' Association (Nelson *et al.*, 2000).

### Determination of mesocarp percentage moisture

Mesocarp percentage moisture was determined as described by Kaiser (1994). A thin mesocarp section (ca 30 g) was dried at 75°C in an oven (Lasec, Scientific Equipment, Cape Town, RSA) for 48 to 72 hours, to a constant mass. The ratio of wet mass minus dry mass to wet mass, expressed as a percentage, was considered to be mesocarp percentage moisture. There were six replicates per experiment during each growing season.

### Determination of water potential

Water potential ( $\Psi_w$ ) was determined according to Lamfermeijer (1997) using tissue volume method. Cotyledonary seed tissue (ca 1 g) was obtained by transverse perforation of the cotyledon with a cork borer and this was immediately incubated in a graded-solution of mannitol (MAN) (purchased from Associated Chemicals Enterprises, RSA) of known concentrations. The incubation time required creating osmotic balance between incubated tissues and the osmoticum was determined to be 2 hours (results not shown). Following monitoring of changes in fresh weight of incubates; the extrapolated MAN concentration at which no change in fresh weight occurred was recorded. Assuming equal turgour pressure between the incubate and the osmolite, the  $\Psi_w$  of incubates was calculated using the equation  $\Psi_w = miRT$  (Nobel, 1991), where m = molarity of the solute, i = ionization constant of the solute, R = gas constant (0.083 L bars/mole degree) and T = absolute temperature ( $^{\circ}C + 273$ ) and the osmolality of the apoplasmic solution was estimated according to Lamfermeijer (1997). There were six replicates per experiment per growing season.

### Statistical analysis

Analysis of variance (ANOVA) and regression analysis on data generated from seed and mesocarp % moisture studies were performed using GENSTAT 4.1.

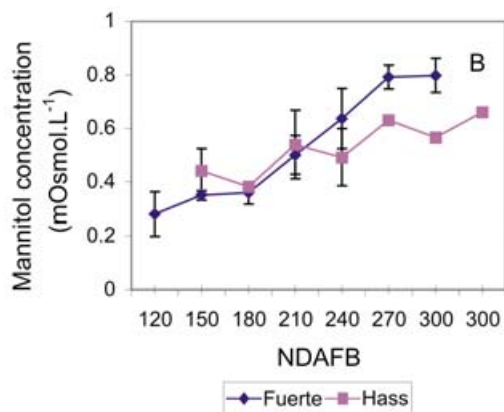
## RESULTS AND DISCUSSION

### Mesocarp moisture content

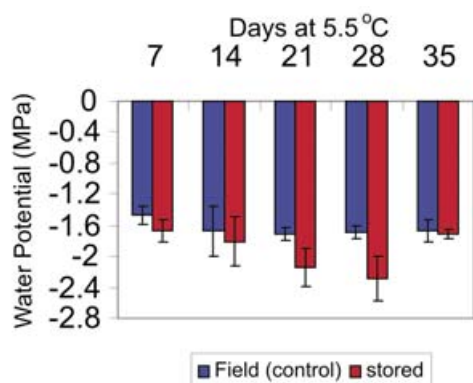
Mesocarp tissue obtained from fruits used to study seed  $\Psi_w$  served as samples for moisture content analysis. Percentage mesocarp moisture declined with increasing fruit ontogeny, a trend previously reported (Kruger *et al.*, 1995) (Fig.1). A loss of approximately 40% in mesocarp moisture content was measured in both cultivars in over mature fruit.

### Seed water potential

There was a decline in 'Fuerte' and 'Hass' seed tissue  $\Psi_w$  with increasing fruit maturity. Water potential of seed tissue from immature 'Fuerte' fruits was initially lower (higher negative value) than immature 'Hass' avocados ( $-0.86 \pm 0.09$  MPa at 120 DAFB and  $-1.18 \pm 0.11$  MPa at 150 DAFB respectively) (Fig. 2). As fruit development progressed,  $\Psi_w$  of the seed of the early harvested 'Fuerte' became less negative, thus higher, than the late harvested 'Hass'. At maturity (approximately 210 DAFB) 'Fuerte' seed  $\Psi_w$  reached  $-1.35 \pm 0.06$  MPa and declined to  $-2.13 \pm 0.13$  MPa. 'Hass' seed at the same stage of development (approximately 240 DAFB) displayed the  $\Psi_w$  of between  $-1.45 \pm 0.08$  and  $-1.76 \pm 0.09$  MPa. It was interesting to notice that there were no further significant changes in the seed  $\Psi_w$  of over-mature 'Fuerte' and 'Hass' avocados (Fig. 2). Because MAN concentration required to create an equilibrium between incubates from the immature cotyledon and the osmoticum for 'Fuerte' and 'Hass' were 0.281



**Figure 3.** Change in the concentration of the cell sap solution in the seed tissue of 'Fuerte' and 'Hass' during avocado fruit development and maturation. Data are means of  $\pm$  SE of 6 replicates per analysis during 1999-2000 and 2001-2002 growing seasons. DAFB = number of days after full bloom.



**Figure 4.** Water potential of cotyledonary seed tissue of mature 'Fuerte' avocado. Fruits (count 18) were harvested and stored at 5.5°C and  $\Psi_w$  of the cotyledon measured simultaneously on seeds of fruits from the field (control) and kept at 5.5°C. Cotyledonary seed tissue was incubated in graded-solution of mannitol of known concentrations for 2 hours, and the  $\Psi_w$  of incubates was calculated using the equation  $\Psi_w = miRT$  (Nobel, 1991), where  $m$  = molarity of the solute,  $i$  = ionization constant of the solute,  $R$  = gas constant (0.083 L bars/mole degree) and  $T$  = absolute temperature ( $^{\circ}\text{C} + 273$ ). Data are means of 6 fruits  $\pm$  SE collected during the 1999-2000 and 2000-2001 season.

**Table 1.** Mean squares from analysis of variance for effects of number of days after full bloom (DAFB) and cultivar and their interactions on seed  $\Psi_w$  and mesocarp moisture content of 'Fuerte' and 'Hass' ontogeny during 1999-2000 and 2001-2002 seasons.

Source of variation	d.f.	Seed $\Psi_w$		Mesocarp moisture content	
		1999-2000	2001-2002	1999-2000	2001-2002
DAFB	71	561.22***	517.61***	1.149***	1.563***
Cultivar	1	158.72***	143.61***	0.338**	0.402**
Linear	1	3273.16***	3045.6***	7.305***	6.952***
Quadratic	1	13.01 <sup>NS</sup>	12.9 <sup>NS</sup>	0.150 <sup>NS</sup>	0.141 <sup>NS</sup>
Cubic	1	1.83 <sup>NS</sup>	1.63 <sup>NS</sup>	0.204*	0.205*
<b>Interaction</b>					
Cultivar. DAFB	5 (2)	100.65 <sup>NS</sup>	164.45 <sup>NS</sup>	0.169**	0.179**
Cultivar. Linear	1	5.15 <sup>NS</sup>	4.73 <sup>NS</sup>	0.313*	0.416*
Cultivar. Quadratic	1	0.23 <sup>NS</sup>	0.22 <sup>NS</sup>	0.001 <sup>NS</sup>	0.001 <sup>NS</sup>
Cultivar. Cubic	1	34.31 <sup>NS</sup>	36.12 <sup>NS</sup>	0.402**	0.389**

\*, \*\*, \*\*\* Significant at  $P < 0.5$ ,  $P < 0.05$ ,  $P < 0.01$  levels respectively; NS – not significant; d.f. – Degree of freedom.

M and 0.441 M, the osmolality of the cell sap content in the seed tissue of fruits at this stage of development should be equal to  $281 \pm 0.08$  and  $441 \pm 0.08$  mOsmol.L<sup>-1</sup> (Fig. 3). Over-mature 'Fuerte' and 'Hass' (fruits harvested after 270 and 300 DAFB respectively) showed an increase to  $798 \pm 0.06$  and  $661 \pm 0.04$  mOsmol.L<sup>-1</sup> in sap osmolality (Fig. 3). These results, when compared to changes in seed  $\Psi_w$ , revealed the existence of a stoichiometric relation between the two measurements.

#### Correlation between seed water potential and mesocarp percentage moisture of 'Fuerte' and 'Hass' fruit during ontogeny

The DAFB and the cultivar significantly influenced the  $\Psi_w$  of the seed and mesocarp moisture content (Table 1). While the two relations were strongly influenced by DAFB ( $P < 0.001$ ), cultivar effects on seed  $\Psi_w$  and mesocarp percentage moisture were found to be significant at 5% level (Table 1). Additionally, the effects of DAFB and cultivar on changes in seed  $\Psi_w$  and mesocarp percentage moisture in 'Fuerte' and 'Hass' seed occurred in linear way (Table 1).

Furthermore, the amount of moisture present in the mesocarp, not the seed  $\Psi_w$ , depended on an interaction between DAFB and cultivar. The interaction between DAFB and cultivar in influencing mesocarp moisture content was initially linear ( $P < 0.5$ ) and then cubic ( $P < 0.05$ ) (Table 1). Regression analysis of the seed  $\Psi_w$  on mesocarp % moisture revealed the existence of a strong linear relationship ( $P = 0.001$ ) in 'Fuerte' and 'Hass' during fruit development and maturation. These relations were, however, negatively correlated ( $r^2 = -0.991$ ).

These results clearly indicate that during the development and maturation of 'Fuerte' and 'Hass' avocado, an increase in seed  $\Psi_w$  (values become more negative), which is an indication of increase in vacuolar solute content, takes place simultaneously with a decline in mesocarp moisture content.

#### Avocado water relations during the postharvest period

Comparison between seed  $\Psi_w$  of fruits left in the orchard and stored at 5.5°C

'Fuerte' and 'Hass' fruits were collected from 5 marked trees, graded into export counts (10, 12, 14, 16, 18, 20, 22 and 24) and stored at 5.5°C for 35 days. Significant changes were observed in  $\Psi_w$  of seed of 'Fuerte' left in the orchard compared to those stored at 5.5°C (Fig. 4). Increasing fruit maturity resulted in higher seed  $\Psi_w$  for 'Fuerte' under storage. These results suggest that while the  $\Psi_w$  of seed mature 'Fuerte' left in the orchard does not vary significantly, certain changes are associated with postharvest storage.

Similar studies undertaken on 'Hass' seeds revealed no

significant changes in seed tissue  $\Psi_w$  of fruits left in the orchard compared with those stored at 5.5°C (Fig. 5).

### Change in mesocarp % moisture and seed $\Psi_w$ of 'Fuerte' avocado during storage

While the time of storage did not have a significant effect on neither the seed  $\Psi_w$  ( $P \geq 0.05$ ) nor the mesocarp moisture content, the former was, however, significantly affected by the size (Table 2). There was no interaction between the time of storage and fruit size in influencing the  $\Psi_w$  seed tissue of 'Fuerte' stored at 5.5°C (Table 2). In corroboration with observations described above on changes in seed  $\Psi_w$  of count 18 during storage, it is probable that the size of the fruit rather than the time of storage plays an important role in influencing  $\Psi_w$  relation.

Mesocarp moisture content (or its reciprocal, oil content and percentage dry moisture, Lee and Young, 1983(a&b); Ranny, 1991) is the accepted maturity index. For many cultivars, including 'Fuerte' and 'Hass', maturity markers / indices based on mesocarp moisture content or percentage dry matter have been established (Hofman *et al.*, 2000 and references within). These authors have suggested the existence of an interval, rather than specific values as maturity markers or indices. Our results are in agreements with these suggestions.

Analysis of the change in mesocarp moisture content in 'Fuerte' and 'Hass' during fruit ontogeny (Fig. 1) showed its decline with increasing fruit development (Cutting *et al.*, 1992), and ranged between 80% and 76% at maturity. Kaiser and Wolstenholme (1994) proposed at least 75% and 70% mesocarp moisture content as maturity indices for 'Fuerte' and 'Hass' respectively. The difference between their values and ours stems from the vulnerability of mesocarp moisture (and any currently used maturity indices) at harvest to several factors (Lee and Young, 1983(b); Lahav and Kalmar, 1977; Hofman and Jobin-Décor, 1999; Vuthapanich, 2001).

In an orchard, indications are that mesocarp moisture content of matured fruits may vary between and/or within a cultivar largely due to difference in fruit set period (Van den Dool and Wolstenholme, 1983; Kaiser, 1993; Milne, 1994), albeit other actors such as cultural practices and geographical locations could play a role (Coggins, 1984; Kruger, 1995, 2001, 2004; Hofman *et al.*, 2000). These suggestions are confirmed by results presented in Table 1. We observed that in addition to the cultivar, the number of days after full bloom significantly affected mesocarp moisture content. Interestingly, we observed that these parameters significantly constituted the source of variation for seed  $\Psi_w$ , an indication of certain level of communication between the mesocarp and the

seed during 'Fuerte' and 'Hass' development (Moore-Gordon *et al.*, 1998; Richings *et al.*, 2000; Cowan *et al.*, 2001). Furthermore, indication that changes in mesocarp moisture and seed  $\Psi_w$  (and therefore solute concentration, Fig. 2) occurred linearly, is in agreement with reports that cell division in developing avocado fruit is a continual process, albeit at reduced rate as fruits approach maturity (Schroeder, 1953), not only in the mesocarp but also the seed. These results suggest that the mesocarp and the seed interact during 'Fuerte' and 'Hass' fruit development, opening new avenue for considering the seed in different developmental aspects these cultivars.

The interaction between the mesocarp and the seed in controlling avocado fruit development is well documented (Moore-Gordon *et al.*, 1998; Richings *et al.*, 2000; Cowan *et al.*, 2001; Taylor and Cowan, 2001). These authors attempted to further elucidate physiological causes of the existence of small variants in 'Hass' by showing that biosynthesis of key metabolites such as ABA and sugars in the seed was inextricably linked to the final fruit size. Our results support their hypothesis and confirm the existence of "a cross-talk" between the seed and the mesocarp during avocado fruit development.

Statistical studies of 'Fuerte' and 'Hass' seed  $\Psi_w$  and mesocarp moisture content during fruit ontogeny showed that these two relations were negatively correlated and, linearly and significantly influenced by cultivar and the time of growth (Table 1). These results clearly confirm the hypothesis proposed by Moore-Gordon *et al.* (1998) that the trafficking route for solution and solutes occur along the pathway mesocarp vasculature  $\rightarrow$  seed coat vasculature  $\rightarrow$  seed  $\rightarrow$  mesocarp. In addition, our findings indicate the existence of two-way movement between the seed and the mesocarp and lend physiological explanations to observations by Davenport and Ellis (1959). In this regard we propose that mesocarp vacuolar water that is gradually replaced by lipids during avocado fruit development (Davenport and Ellis, 1959) is preferentially re-directed to the seed to sustain biological processes, in addition to leaving the fruit through transpiration stream (Whiley *et al.*, 1995).

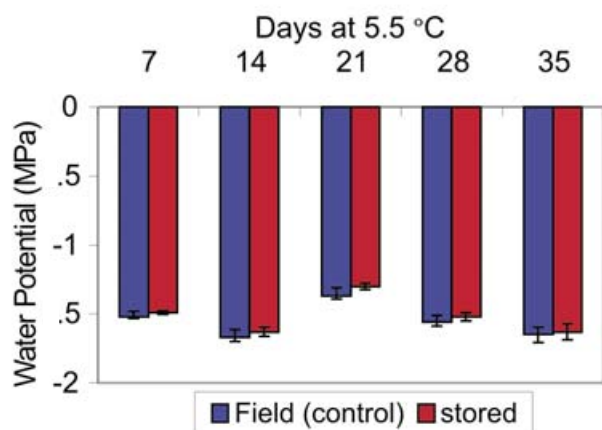
The decline in seed  $\Psi_w$  (as shown by the numerical values) is matched by an increase in cell sap osmolality (Fig. 2), indicates the ability of the seed to withdraw water from the mesocarp. Our investigations unambiguously provide the first evidence to support a two-way route trafficking of water and solutes between the mesocarp and the seed in 'Fuerte' and 'Hass' during fruit ontogeny (Moore-Gordon *et al.*, 1998; Richings *et al.*, 2000; Taylor and Cowan, 2001).

The seed status has been related to the maturity stage of many fruits. In apple and grape, phenotypic (seed colour) and genotypic (polyphenol content and composition) changes of the seed have been used to ascertain the maturity stage of the fruit respectively (Brookfield *et al.*, 1996; Kennedy *et al.*, 2000, 2001; Oberholster, 2003). The possibility of exploiting this avenue for avocado fruit has been mooted by Steyn *et al.* (1993); Moore-Gordon *et al.* (1998); Richings *et al.* (2000); and Cowan *et al.*, (2001), whose analyses inextricably linked seed development to fruit. Interestingly, we observed that between 180–210 DAFB, which corresponds to the attainment of maturity for 'Fuerte' and 'Hass' at the experimental site, seeds from the two cultivars had very close  $\Psi_w$  values (Fig. 2). Concurrent studies to compare seed  $\Psi_w$  of mature fruit of 'Fuerte' and 'Hass' left on the orchard or stored at 5.5°C, showed

**Table 2. Mean squares from analysis of variance for effects of storage time and fruit size, and their interactions on seed  $\Psi_w$  and mesocarp moisture content of 'Fuerte' during 1999-2000 and 2001-2002 seasons.**

Source of variation	d.f.	Seed $\Psi_w$		Mesocarp moisture content	
		1999-2000	2001-2002	1999-2000	2001-2002
Storage time	3	7.14 <sup>NS</sup>	6.98 <sup>NS</sup>	9.75 <sup>NS</sup>	9.56 <sup>NS</sup>
Size	7	5.84*	7.85*	3.43 <sup>NS</sup>	4.01 <sup>NS</sup>
Linear	1	2.27 <sup>NS</sup>	2.56 <sup>NS</sup>	2.37 <sup>NS</sup>	3.21 <sup>NS</sup>
Quadratic	1	0.42 <sup>NS</sup>	1.09 <sup>NS</sup>	6.11 <sup>NS</sup>	5.98 <sup>NS</sup>
Cubic	1	1.00 <sup>NS</sup>	1.52 <sup>NS</sup>	1.67 <sup>NS</sup>	0.92 <sup>NS</sup>
Interaction					
Time. Size	14	3.48 <sup>NS</sup>	2.87 <sup>NS</sup>	8.97 <sup>NS</sup>	9.27 <sup>NS</sup>
Time. Linear	2	8.52 <sup>NS</sup>	9.65 <sup>NS</sup>	35.25 <sup>NS</sup>	35.51 <sup>NS</sup>
Time. Quadratic	2	3.15 <sup>NS</sup>	3.78 <sup>NS</sup>	14.21 <sup>NS</sup>	12.98 <sup>NS</sup>
Time. Cubic	2	3.09 <sup>NS</sup>	3.09 <sup>NS</sup>	4.56 <sup>NS</sup>	5.12 <sup>NS</sup>

\* Significant at  $P < 0.5$  level; NS – Not significant; d.f. – Degree of freedom.



**Figure 5. Water potential of cotyledonary seed tissue of mature 'Hass' avocado. Fruits (count 18) were harvested and stored at 5.5°C and  $\Psi_w$  of the cotyledon measured simultaneously on seeds of fruits from the field (control) and kept at 5.5°C. Cotyledonary seed tissue (ca 1 g) was incubated in graded-solution of mannitol of known concentrations for 2 hours, and the  $\Psi_w$  of incubates was calculated using the equation  $\Psi_w = m_iRT$  (Nobel, 1991), where  $m$  = molarity of the solute,  $i$  = ionization constant of the solute,  $R$  = gas constant (0.083 L bars/mole degree) and  $T$  = absolute temperature (°C + 273). Data are means of 6 fruits  $\pm$  SE collected during the 1999-2000 and 2000-2001 season. Error bars not visible are smaller than data points.**

little variation in this relation, particularly for 'Hass' (Fig. 4 and 5). We have therefore tentatively established this relation as the maturity marker for 'Fuerte' and 'Hass'. In fact, observations of the existence of the cross-talk between the seed and mesocarp described previously, coupled to indication of a relationship between susceptibility of avocado fruit to postharvest injuries and amount of water present in the mesocarp at harvest (Bower and Van Lelyveld, 1986; Bower *et al.*, 1989; Blanke and Whiley, 1995; Cutting and Wolstenholme, 1992; Milne, 1994), make seed  $\Psi_w$  an ideal maturity marker for this crop. The rationale behind this is the ability of seed  $\Psi_w$  to represent the chemical energy status of the fruit (Boyer, 1995) and ipso facto the better indicator of maturity for avocado fruit. Our studies have determined these values to be  $-1.35 \pm 0.06$  MPa for 'Fuerte' and  $-1.45 \pm 0.08$  for 'Hass', respectively.

The importance of adequate moisture to the developing avocado fruit, particularly during the early stage, is well documented (Coggins, 1984; Ranny, 1991; Kruger, 1995). Extreme water stress has been shown to lead to solute accumulation deficiency in developing seed, causing cell bursting, increased plasma membrane permeability and sensitivity to solute accumulation turgour (Oliver *et al.*, 1998; Bryant *et al.*, 2001). This emphasises the importance of irrigation during development of 'Fuerte' and 'Hass' fruit, which has been investigated before. Blanke and Whiley (1995) measured the  $\Psi_w$  of the entire immature fruit of 'Fuerte' and 'Hass', and found the former to have a more negative  $\Psi_w$ . Our results indicated rather a rapid solute (more negative  $\Psi_w$ ) accumulation in immature 'Hass' compared to 'Fuerte' (Fig. 3). In addition to explaining some of the differences between 'Fuerte' and 'Hass' to susceptibility to postharvest disorders linked to vascular traces (Bangerth, 1989; Bower and Lelyveld, 1986; Blanke and Lovatt, 1993), our results provide further physiological evidence for the necessity of managing 'Fuerte' and 'Hass' irrigation practices differently. More negative  $\Psi_w$  observed in the seed of young 'Hass' fruit than 'Fuerte' (Fig. 2) clarifies the observations by Blanke and Whiley (1995) that young 'Hass' requires more water to compensate for higher transpiration rate and maximize fruit size.

The reverse situation in  $\Psi_w$  observed in mature 'Fuerte' (Fig. 2) justifies the susceptibility of 'Fuerte' to postharvest moisture content and vascular system related physiological disorders (Bower *et al.*, 1989; Cutting and Wolstenholme 1992; Blanke and Lovatt, 1993; Blanke and Whiley, 1995). These obiter dicta, further confirmed by postharvest studies (Fig. 3), not only provide physiological explanations to the propensity of 'Fuerte' to mesocarp membrane lipification during storage, but may also indicate the necessity to re-assess irrigation management practices of 'Fuerte', any other cultivar (for example 'Pinkerton') subjected to severe postharvest conditions, and late harvested 'Hass' (Hofman *et al.*, 2000).

## CONCLUSIONS

Physiological observations presented in this paper underline the importance of irrigation schedules based on the time of development and cultivar rather than locations (Coggins, 1984; Ranny, 1991; Kruger, 1995; Wolstenholme and Kaiser, 1994). Perhaps, irrigation scheduling of avocado should be revised based on soil and seed water relations determined for each cultivar, in addition to any other measurements taken on tree organs. Irrigation based on plant parameters, particularly the fruit, will not only help determine the needs of water status of the tree, but also provide reliable data on crop water use. Water status of avocado fruit at harvest is undoubtedly the major factor in determining its storage potential because of its involvement in the integrity of cell, particularly its membrane (Bower and Lelyveld, 1986; Bower *et al.*, 1989; Cutting and Wolstenholme, 1992; Blanke and Whiley, 1995). We suggest that irrigation schedules should be based on seed  $\Psi_w$  and tensiometer readings, the former giving a better indication of fruit water content.

Water potential can be measured using method described in this paper or as reported by Jobling *et al.* (1997) and Jones *et al.* (2002). Physiological studies undertaken in this research also underline the importance of selective harvesting based on fruit size, a proposition recently suggested by Hofman *et al.* (2000). More results related to postharvest physiology of 'Fuerte' and 'Hass' based on ripening parameters such as days to soften, incidence of mesocarp discoloration, respiration, ethylene, and enzymes, are however required to establish the link between seed water relations and the incidence mesocarp disorders at harvest, and thus unequivocally confirm our attempt to establish seed  $\Psi_w$  as the new maturity index system.

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