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The quest for the function of 'Hass' avocado carbohydrates: clues from fruit and seed development as well as seed germination

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Abstract. *D*-Mannoheptulose (*D*-manno-2-heptulose) and perseitol (*D*-glycero-*D*-manno-heptose) are unusual seven-carbon (C7) sugars that have been postulated to act as storage and transport sugars in avocado. However, thus far, there is no published evidence that satisfactorily explains the physiological functions of these carbohydrates. Various tissues at different stages of the avocado life-cycle were therefore analysed for C7 carbohydrates to derive clues on the function of the six-carbon (C6) and C7 sugars. Adult reproductive tissues (flower buds and fruit) contained significantly greater concentrations of C7 than C6 sugars, whereas in juvenile avocado tissue, the classic C6 storage carbohydrate, starch, was found only in developing and mature seeds, predominatly in the cotyledons. The dramatic increase in shoot and cotyledon C7-sugar concentrations, together with a reduction of starch reserves in the cotyledons as a result of lack of light during germination, suggests that the C6 storage carbohydrate (CHO) starch can be converted to fuel the C7 metabolism. Therefore, a changeover from C6 carbohydrate to C7 sugar metabolism characterises the progression from juvenility to maturity in avocado. Avocado seems to mirror its evolutionary development characterised by a switch-over from the common C6- to a specialised C7-sugar metabolism in the juvenile *versus* adult tissue. The dominance of C7 carbohydrates over C6 in adult tissues is, therefore, due to the fact that C7 sugars have multifunctional roles as a source of energy and anti-oxidants.

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Introduction

Heptoses are C7 sugars, including the common Calvin cycle sugar sedoheptulose as well as the uncommon sugar aldose mannoheptulose and coriose (Häfliger *et al*. 1999) and the sugar alcohols perseitol, volemitol and β-sedoheptitol (Richtmyer 1970). Of these C7 sugars, mannoheptulose, perseitol and volemitol have been reported in *Persea americana* (Cowan 2004). Mannoheptulose and its alcohol form perseitol have been found in all major tissues of the avocado plant, which is able to synthesise and translocate significant amounts of these C7 sugars (Liu *et al*. 1999), indicating an important function of these compounds in avocado growth and development. The functions proposed for the C7 carbohydrates existing in plants are similar to known functions of C6 carbohydrates (Zimmermann and Ziegler 1975; Nadwodnik and Lohaus 2008) and include carbohydrate transport (Noiraud *et al*. 2001; Liu *et al*. 2002), carbohydrate reserves (Oliveira and Priestley 1988) and osmoprotection (Morgan 1984).

Given that specific carbohydrates are often synthesised at distinct developmental stages of a species, comparing the carbohydrate (CHO) profiles of various tissues of 'Hass' avocado at different ontogenic stages should provide insight into the avocado fruit ripening pattern, unique with the physiologically mature fruit not softening unless it is detached from the tree (Schroeder 1953). The sugar profile, not only during the development on the tree but also post-harvest of mature soft ('eat-ripe') fruit, was investigated the carbohydrate composition. Various juvenile and mature avocado tissues were analysed to address the questions whether avocado tissues differ in carbohydrate composition at specific developmental stages and whether these differences are reflected in the leaves. The experimental hypotheses were based on two plant developmental phases, at fruit maturity and juvenile stage. Therefore, the study aimed to investigate avocado carbohydrate-profile fluctuations of fruit tissues at fruit maturity and during ripening (softening), which occurs only after the mature avocado fruit is removed from the tree, and of tissues at various ontogenic stages (mature seed, germinating embryo, seedling, flowers and fruitlets). The result from the present study will assist in elucidating the role of C7 sugars in avocado and increase our knowledge of the function of these rare carbohydrates.

Material and methods

Chemicals

Chemicals were obtained either from Sigma-Aldrich/Fluka (St Louis, MO, USA), Saarchem (Gauteng, RSA), Glycoteam GmbH (Hamburg, Germany).

Plant material

Physiologically mature 'Hass' avocado (*Persea americana* Mill.) seeds, dark- and light-grown seedlings, flower buds (closed pistillate stage) (Ish-Am and Eisikowitch 1991), as well as fruit (mesocarp, exocarp and seed) and leaf tissues were analysed. Fruit and leaves were sampled from January to June, which represented progressive stages of fruit development and tree phenology in orchards in the KwaZulu–Natal Midlands, South Africa (29°28′S, 30°16′E). Additionally, commercially mature fruit were allowed to soften and their carbohydrate concentration was analysed during the softening period.

Pre-harvest leaf and fruit sampling

Leaves and fruit were collected first 112 days after full bloom (DAFB) (January) on a monthly basis, till June, when fruit had reached commercial maturity (62–67% moisture and 32% oil content of the mesocarp) (McOnie and Wolstenholme 1982; Tesfay *et al*. 2010). Each month, the youngest, but fully mature leaves from 10 trees (eight leaves per tree) and 20 fruit (two fruit per tree) at specific stages of tree phenology (early fruit set to commercial maturity) were collected. Fruit were dissected into exocarp, mesocarp and seed. All tissues were snap-frozen in liquid nitrogen, freeze-dried, ground and subsequently stored at −20°C until further analysis.

Post-harvest fruit sampling

Five ($n = 5$) mature 'Hass' fruit (32% w/w), which were assigned in to four replications, ripened at room temperature until they achieved the 'ready-to-eat' softness stage (fruit firmness <6 N). Fruit tissues (exocarp, mesocarp and seed) were separated, snap-frozen in liquid nitrogen, subsequently freeze-dried, ground and stored at −20°C until further analysis.

Seed germination and seedling developmental stages

'Hass' avocado seeds were excised from soft, mature fruit and submerged in tap water, which was exchanged every 5 days until seed-coat cracking, a sign of germination preceding radicle emergence occurred, about 6–8 weeks later. Seeds were then placed into heated beds (25−28°C) filled with perlite (Chemserve Perlite, RSA) for further development. After ~8 weeks of radicle emergence, shoots emerged, seedlings transplanted at the one-leaf stage into 200-mm-diameter pots filled with a mixture of composted pine bark, peat moss and sand (ratio 1 : 1 : 1) were placed into growth rooms maintained at 24°C (12-h day) and 16°C (night) with a 12-h light and a light intensity of 314 µmol m^{-2} s^{-1} photosynthetically active radiation (PAR). Etiolated seedlings were produced by transferring seeds containing a visible radicle to a dark growth-room set to 24°C for 12 h. followed by 16°C for 12 h. Seedlings were maintained under these conditions for 60 days, until sufficient leaf and shoot biomass had developed. The two treatments were applied to investigate the stage partitioning and profile of avocado seedlings under light exclusion to inhibit photosynthetic carbon assimilation. Plant sampling stages were (Fig. 1*a–d*) as follows: (1) mature seed, before imbibition; (2) embryo after 15 days of imbibition; (3) seedlings grown under artificial light; and (4) etiolated seedlings grown in the dark. The experiment was set up into four blocks, each consisting of five seedlings. Five ($n = 5$) samples were taken from each block. Samples were freeze-dried, ground and stored at −75°C for further analysis.

Phloem exudation

Phloem exudates were obtained by the ethylene–diamine–tetraacetic acid (EDTA) method described previously by Bachmann *et al*. (1994). Briefly, the buffer was prepared using 2% starch agarose in 5-mM phosphate buffer and 5-mM EDTA adjusted to pH 7.5. Agarose was used to avoid occurrence of oxidation and to improve survival of cut plant sections. The agarose-buffer solution was prepared by boiling for 10 min. An amount of 2 mL was poured into small plastic containers and left until solidified. One fresh-cut leaf petiole or shoot was placed into each plastic container filled with agarose buffer for exudate collection. The plant material was left in the buffer for 12 h under controlled environmental conditions (314 µmol m^{-2} s^{-1} light intensity, 25°C temperature, 70–75% relative humidity). After the plant material was removed from the agarose buffer, sugars were extracted from the agarose and immediately analysed by HPLC according to Liu *et al*. (1999). Five ($n = 5$) trees of the same age were used for exudates collection.

Determination of soluble sugar concentration

A sample (0.05–0.10 g dry weight, DW) of freeze-dried ground material was mixed with 10 mL of 80% (v/v) ethanol and homogenised for 60 s. Thereafter, the mixture was incubated

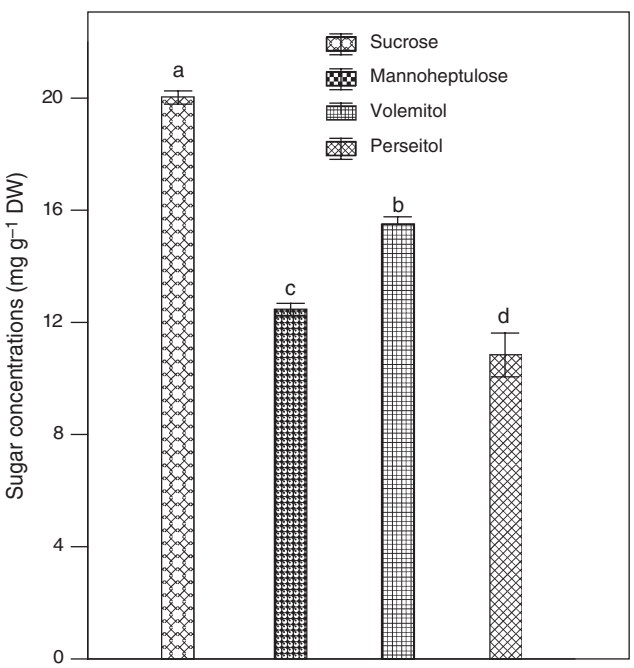

Fig. 1. Sugar profiles of avocado flower buds. l.s.d.$_{0.05}$ = 1.5. Vertical bars represent s.e. of the mean value ($n = 5$).

in an 80°C water bath for 60 min and kept at 4°C overnight. After centrifugation at 12 000*g* for 15 min at 4°C, the supernatant was filtered through glass wool and taken to dryness in a Savant vacuum concentrator (SpeedVac, Savant, Holbrook, NY, USA). Dried samples were resuspended in 2 mL of ultra-pure water, filtered through 0.45-μm nylon filters and sugars were analysed according to Liu *et al*. (1999), using a HPLC (LC – 20 AT, Shimadzu Corporation, Kyoto, Japan) equipped with a refractive index detector (RID-10 A, Shimadzu Corporation, Kyoto, Japan) and a Rezex RCM–monosaccharide column (300 mm × 7.8 mm) (8-μm pore size; Phenomenex, Torrance, CA, USA). The concentration of individual sugars was determined by comparison with authentic standards.

Starch determinations

Starch was determined according to Sluiter and Sluiter (2005). The dried pellet (0.1 g DW) obtained from the soluble sugar extracts was mixed with 2 mL of dimethyl sulfoxide (DMSO) and vortexed for 20 s; the test tube was tightly capped and placed in a boiling water bath for 5 min. After adding 2.9 mL of 3-(*N*-morpholino) propanesulfonic acid (MOPS) buffer (pH 7.0) and 0.1 mL thermostable α-amylase (equivalent to 300 units), tubes were vortexed vigorously and incubated in a boiling water bath for 6 min with mixing at 2-min intervals. After addition of 4 mL of sodium acetate buffer (1 M, pH 4.5) and 0.1 mL (equivalent to 20 units) of amyloglucosidase, samples were incubated at 50°C for 30 min. The samples were filtered through a 0.45-μm nylon filter and glucose was analysed using the isocratic HPLC–RID system described above.

Statistical analysis

Analyses of variance were performed using GENSTAT (version 12.1; VSN International, Hemel Hempstead, UK). Standard deviations (s.d.) were calculated and differences among treatments were separated by a significant difference (l.s.d.) at $P = 0.05$.

Results

Sugar profiles of flower, fruit and leaf tissues from adult avocado trees

Sucrose was the dominant carbohydrate in flower buds, followed by volemitol, *D*-mannoheptulose and perseitol (Fig. 1). Analysis of fruit tissues revealed that carbohydrates fluctuate substantially during fruit development and differ significantly between tissues (Fig. 2, Table 1). At the stage of early fruit development (January sampling, fruit size 87 mm × 61 mm), the C7 sugar *D*-mannoheptulose was the dominant mesocarp carbohydrate, followed by its alcohol equivalent, perseitol. Although mesocarp *D*-mannoheptulose concentrations dropped to lower levels during fruit development, concentrations of this sugar increased in the exocarp (Fig. 2*a*). Overall, mesocarp and exocarp tissues had the highest *D*-mannoheptulose concentrations and mesocarp and seed had the highest perseitol concentrations (Table 1). With the exception of the March sampling, the latter sugar was consistently higher in mesocarp and seed tissue than in exocarp and leaf tissue (Fig. 2*a*). Comparing total amounts of C6 sugars (sucrose, fructose and glucose) in fruit tissues with C7 sugars (*D*-mannoheptulose and perseitol), C6 sugars were present in lesser amounts than were C7 sugars. In leaves, sucrose was dominant over glucose, fructose and perseitol, with only a tendency to higher amounts than *D*-mannoheptulose (Table 1). The C6 polysaccharide starch was detectable only in seed tissue, accumulating in the cotyledons with seed maturation (Fig. 2*d*). *D*-mannoheptulose was the dominant sugar in fruit exocarp and mesocarp throughout the observed fruit-development period (Table 1).

Sugar profile of mature, ripe (soft) fruit

All three fruit tissues (mesocarp, exocarp and seed) contained sucrose and perseitol when the fruit were soft ('eat-ripe', 6N), with the highest perseitol concentration found in the seed (Fig. 3). At this stage of fruit development, the seed consisted mainly of cotyledons. Perseitol was also the dominant sugar in mesocarp tissue at this developmental stage, whereas *D*-mannoheptulose was the dominant sugar in the exocarp. The seed did not contain the latter sugar in detectable amounts.

Sugar profile of cotyledons during germination

In cotyledons of germinating avocado seeds, perseitol and sucrose concentrations declined rapidly, even before radicle emergence (Figs 4, 5), whereas *D*-mannoheptulose concentrations peaked on Days 3 and 4 after the first visibility of the embryo. Additionally, a decline in the C6 polysaccharide starch was observed in cotyledon tissue over the germination period. Starch and glucose concentrations were negatively correlated ($r = –0.61$) (data not presented).

Effect of light on carbohydrate profile of juvenile plant tissues

The bulk of the biomass of etiolated seedlings was made up by shoots, containing significantly higher concentrations of C7 sugars, as well as glucose and fructose (Fig. 6*b*), than for shoots of light-grown seedlings (Fig. 6*a*). Fructose was not detectable in light-grown seedlings, but was present in shoots, cotyledons and roots of dark-grown seedlings. Sucrose and starch were not detectable in leaves of such seedlings. Cotyledons of light-grown seedlings, however, contained a significantly higher concentration of starch than those of dark-grown ones, whereas such cotyledons contained higher amounts of C7 sugars than did the light-grown counterparts. Although leaves from seedlings in both treatments contained similar concentrations of C7 sugars per g DW, per plant the amount of C7 sugars produced in leaves of light-grown seedlings was much higher than the amount produced in leaves of dark-grown seedlings. Additionally, sucrose was not detected in leaves and roots of dark-grown seedlings. All seedling organs contained the hexoses glucose and sucrose as well as the two major C7 sugars (*D*-mannoheptulose and perseitol) (Fig. 6*a*, *b*).

Transported sugars in seedlings

Carbohydrate exudates collected from the primary (seedling) leaves differed from exudates of the 5th true (seedling) leaves. Whereas both the first and fifth leaf contained sucrose in similar amounts, the fifth leaf exuded predominantly the C7 sugar *D*-mannoheptulose, with its sugar-alcohol equivalent,

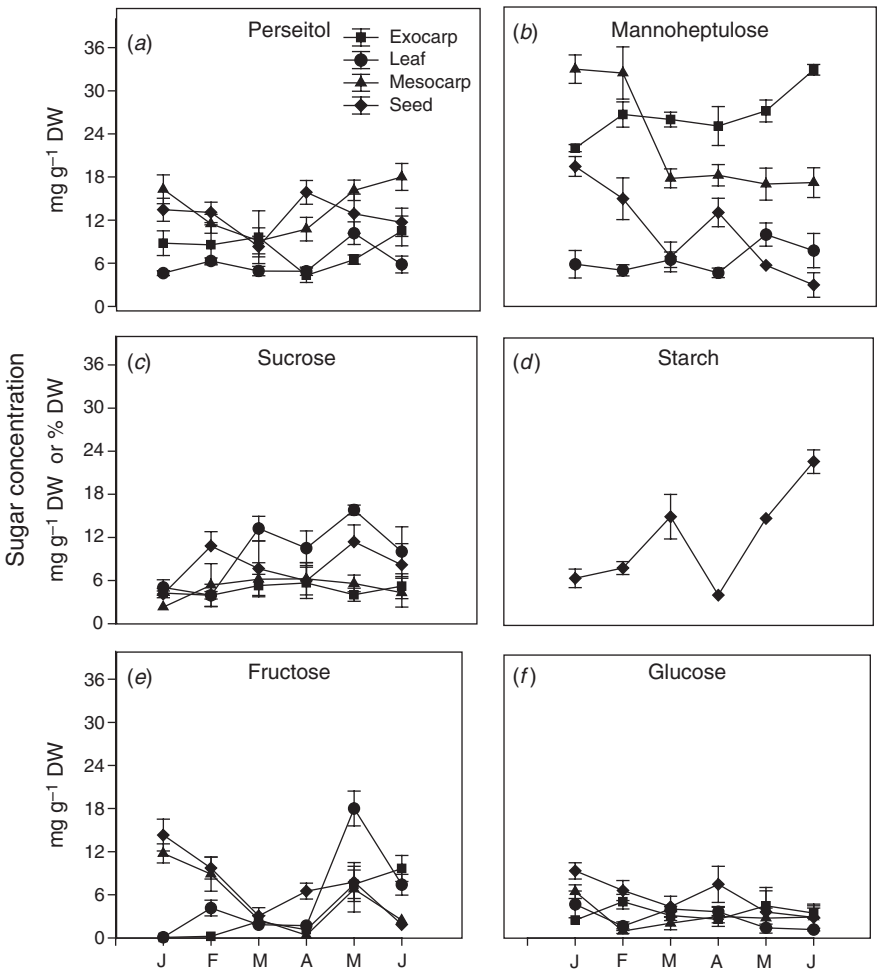

Fig. 2. Seasonal changes in non-structural soluble- and insoluble-carbohydrate concentrations in fruit mesocarp, exocarp, seed tissue and leaf of 'Hass' avocado during the fruit development period. l.s.d.$_{0.05}$ = 3.79 (soluble sugars), l.s.d. $_{0.05}$ = 5.40 (starch). Vertical bars represent s.e. of the mean (n = 5).

Table 1. Sugar concentrations (mean ± s.d., n = 5) of fruit tissues (mesocarp, exocarp and seed) and leaves of avocado over the observed 6-month developmental period (from early fruit set to physiological maturity)

Values followed by the same letters are not significantly different at P = 0.05

Tissue type	Sugar concentration (mg g^{-1} DW)					
	Fructose	Glucose	Sucrose	*D*-Mannoheptulose	Perseitol	Mean
Mesocarp	[1]6.5 ± 6.27fgh	3.03 ± 2.47i	4.88 ± 3.4ghi	22.63 ± 11.19b	13.63 ± 7.6c	[3]10.13a
Exocarp	3.77 ± 5.16hi	3.52 ± 2.01hi	4.73 ± 2.14ghi	26.65 ± 4.66a	8.05 ± 3.9efg	9.34a
Seed	12.93 ± 18.2cd	5.62 ± 4.44ghi	7.86 ± 4.7efg	10.51 ± 7.4cde	12.54 ± 4.6cd	9.68a
Leaf	5.71 ± 6.3ghi	2.75 ± 1.91i	9.76 ± 5.2def	6.63 ± 2.91fgh	6.13 ± 2.7ghi	6.2b
Mean	[2]7.23c	3.73d	6.81c	16.61a	10.09b	
[1]Tissue × sugar	l.s.d.$_{(0.05)}$ = 3.462					
[2]Sugar	l.s.d.$_{(0.05)}$ = 1.731					
[3]Tissue	l.s.d.$_{(0.05)}$ = 1.548					

perseitol, present in the exudates in amounts equal to sucrose (Fig. 7). Glucose and fructose were not detectable in leaf exudates. The seedling shoot exuded predominantly C7 sugars (*D*-mannoheptulose and perseitol), whereas sucrose and its components glucose and fructose were present in stem exudates in similar amounts.

Discussion

The C7 sugars *D*-mannoheptulose and perseitol are the dominant sugars in adult 'Hass' avocado tissues (Fig. 2*a–f*), whereas juvenile tissues, such as the primary leaves and seedling shoots additionally contain large amounts of C6 sugars (Figs 5, 6). The two C7 sugars, *D*-mannoheptulose and perseitol, were found

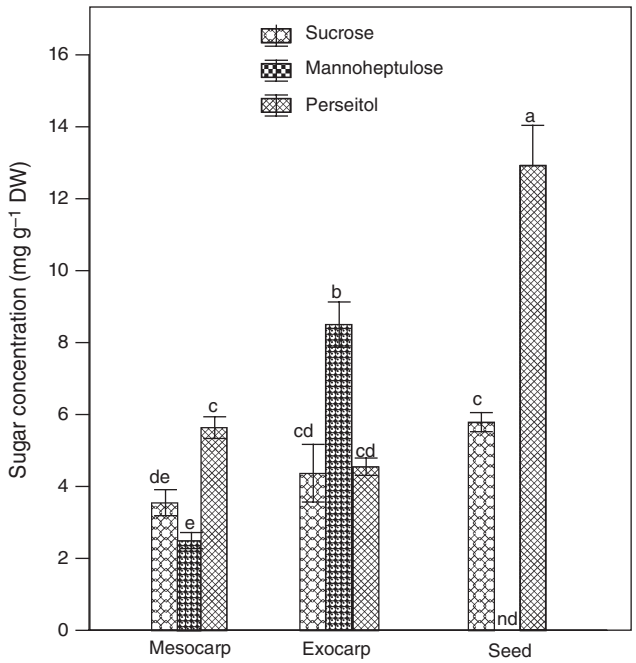

Fig. 3. Carbohydrates of avocado fruit tissues at the soft, 'eat-ripe' stage. Physiologically mature fruit were picked and stored for 28 days in cold storage (5.5°C), followed by ripening at room temperature (fruit firmness was <6 N); nd (not detectable). l.s.d.$_{0.05}$ = 1.564. Vertical bars represent s.e. of the mean (n = 5).

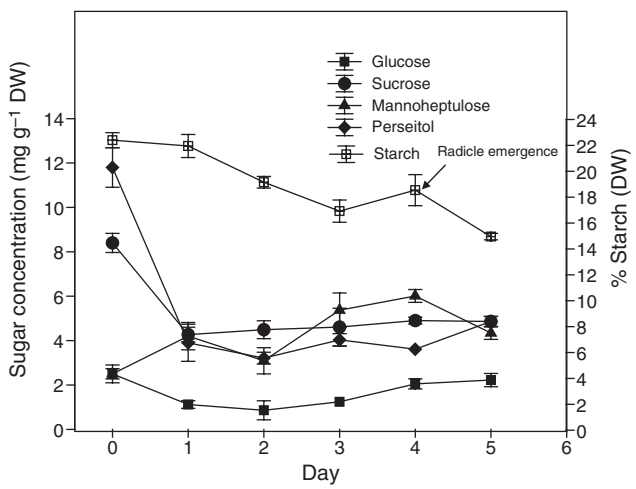

Fig. 4. Soluble and non-soluble carbohydrates of cotyledon tissue during the early germination period. l.s.d.$_{0.05}$ = 2.454 (soluble sugars); l.s.d.$_{0.05}$ = 4.039 (starch).

to be present in various mature avocado tissues (exocarp, mesocarp and seed) (Liu *et al.* 2002; Landahl *et al*. 2009). Flower buds were found to contain another rare C7 sugar, volemitol, which, like the presence of the other two C7 sugars, confirmed results by Cowan (2004) who also detected these C7 sugars in leaf petioles and in trunk sap of mature avocado trees.

The dominance of the C7 sugars *D*-mannoheptulose and perseitol in adult avocado tissue indicates their importance in

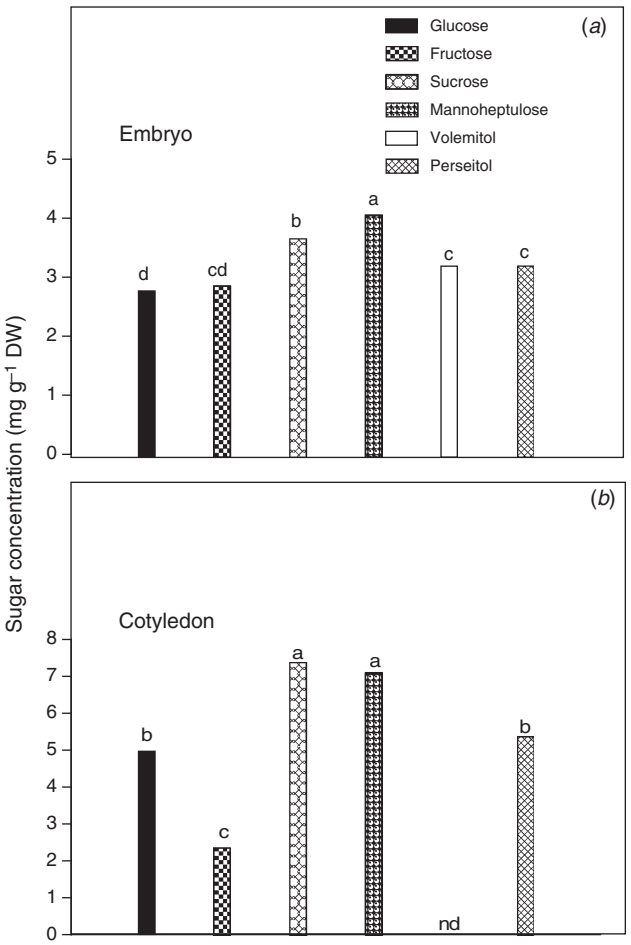

Fig. 5. Carbohydrates (% of total soluble sugars) in (*a*) a germinating avocado embryo and (*b*) cotyledon after 7 days of imbibition (mg g^{-1}). l.s.d.$_{0.05}$ = 1.805 (*a*); l.s.d.$_{0.05}$ = 1.324 (*b*).

these tissues. These sugars might perform major functions during fruit growth and development. Being transported in vascular tissue (Liu *et al*. 2002), both C7 sugars could play a role in carbon allocation and, hence, sink establishment. The dominance of perseitol among the soluble sugars in the avocado cotyledons, at physiological maturity (Fig. 3), points towards a function of this C7 alcohol as a C7, or possibly a general, carbon-storage compound (Tesfay *et al*. 2011).

Landahl *et al*. (2009) also reported considerable spatial variation in *D*-mannoheptulose concentration in avocado fruit tissue from stem end to base, with the highest concentration occurring in the apical region. It was also reported that mannoheptulose is metabolised in avocado leaves and that the C7 sugar is translocated via phloem during fruit growth (Liu *et al*. 1999). A possible explanation for the finding of firmer mesocarp tissue in the apical region is the suggested role of the C7 sugar as a ripening inhibiting factor. This is also in agreement with Liu *et al*. (2002), in that mannoheptulose could function as fruit-ripening inhibitor.

Once the avocado fruit is mature, detachment from the tree triggers post-harvest ripening of the fruit, a process initiated by a

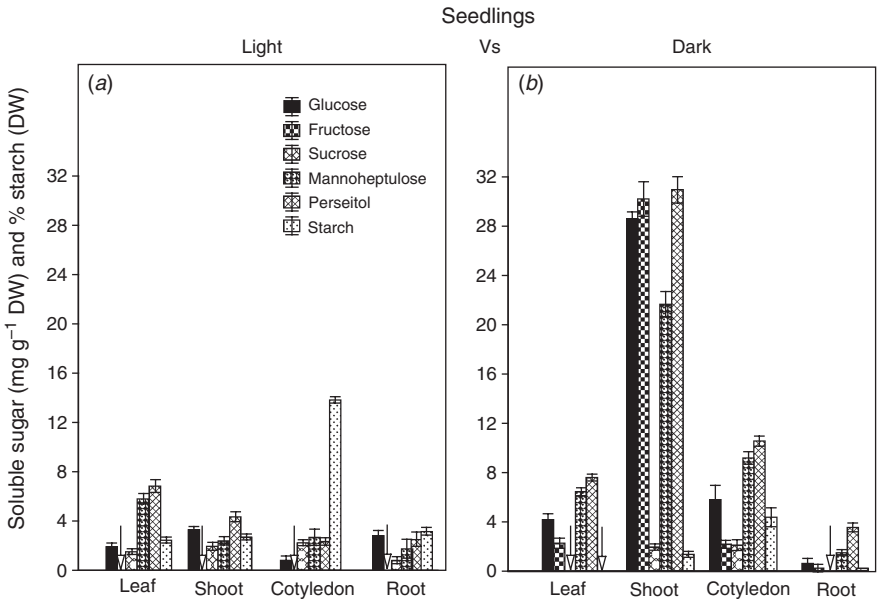

Fig. 6. Partitioning of carbohydrates in different organs of seedlings grown (*a*) under light and (*b*) darkness (etiolated); '↓' denotes not detectable. Vertical bars represent s.e. of the mean ($n = 5$).

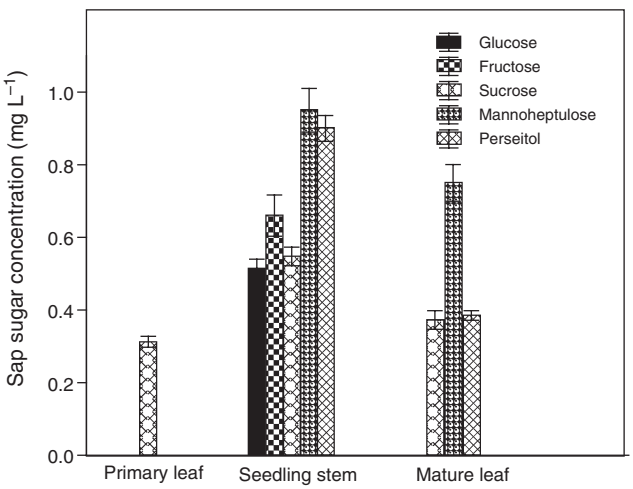

Fig. 7. Sugars exuded from petioles of primary leaf (13 L × 6 W (cm)), stem (28–30 cm height) and the fifth fully expanded leaf (18 L × 7.5 W (cm)) of avocado seedlings. Vertical bars represent s.e. of the mean ($n = 3$).

large CO_2 peak (Bower and Cutting 1988). Together with changes in certain enzyme activities (Cutting and Bower 1987), other ripening processes occur in 'Hass' avocado, such as the change in fruit colour from green to black, as well as fruit softening, ultimately resulting in the fruit becoming eat-ripe. The energy necessary to drive these processes is not derived from oils present in avocado, because little decrease in the mesocarp oil content is observed post-harvest (Liu *et al*. 1999; Meyer and Terry 2010). Therefore, a likely function of the C7 sugars is their use as energy sources for post-harvest fruit respiration. Liu *et al*. (2002) suggested *D*-mannoheptulose as a respiratory substrate of the ripening fruit, an assumption confirmed by the reduction in *D*-mannoheptulose in the mesocarp from 18 mg g^{-1} DW tissue pre-harvest (Fig. 2*a*) to 3 mg g^{-1} DW when the fruit has softened (Fig. 3). Liu *et al*. (2002), furthermore, postulated that the mesocarp *D*-mannoheptulose concentration must drop below ~20 mg g^{-1} DW to allow fruit to soften. Perseitol is convertible to *D*-mannoheptulose (Tesfay *et al*. 2011); the pool of perseitol in the seed (Fig. 3) could have sustained the *D*-mannoheptulose production for its use in respiration. However, because of the senescing of the seed coat at this stage, the transport of solutes from the seed through the seed coat into the mesocarp was probably interrupted (Cowan *et al*. 1997), and, hence, the *D*-mannoheptulose pool could not to be refilled by the perseitol in the cotyledon. Therefore, the mesocarp *D*-mannoheptulose concentration could also act as a commercial 'maturity indicator', as suggested by Tesfay (2009).

Besides morphologically unique features, the avocado fruit is relatively large and contains a large seed (Barlow 2000). The avocado seed affects fruit ripening by regulating the responsiveness to ethylene (Landahl *et al*. 2009; Hershkovitz *et al*. 2010). The provision of solutes from the seed to other fruit tissues depends on the ability of them to be transported through the seed coat (Steyn *et al*. 1993). This seed coat can, therefore, channel solutes, including C7 carbohydrates, mineral nutrients and hormones from the seed to other fruit via the plasma membrane and plasmodesmata. Via these structures, the avocado seed is able to supply the required energy compounds as well as possible building blocks of carbohydrates to the mesocarp. Therefore, early senescence of the seed coat results in small-sized 'Hass' avocado fruit, particularly because of the lack of transporting channels for growth-stimulating compounds such as cytokinins (Moore-Gordon *et al*. 1998).

Post-harvest, when avocado fruit finally softens and becomes eat-ripe, the seed prepares for germination. This seems to be the stage when the C7-sugar metabolism of the adult plant reverts to

the C6-sugar metabolism characterising juvenile growth in avocado. Large starch reserves accumulate in the seed during fruit development up to maturity (Fig. 2*d*), forming a pool of CHO reserves which are sequentially broken down to the C6-sugar sucrose, and eventually to glucose and fructose (Fig. 4). These monosaccharides are then used for initial embryo and seedling growth. Therefore, the avocado embryo contains higher monosaccharide concentrations than do the cotyledons (Fig. 5*a*), being a pattern similar to that described by Borisjuk *et al*. (2002) for pea (*Pisum sativum*). Although C7 sugars are already produced in the embryo (Fig. 4), they become dominant in leaf tissue and occur in other seedling parts only once several leaves are established (Fig. 5*a*). This C6–C7 sugar switch-over must provide some advantage for the avocado seedling because it is energy-expensive to run the C6 and C7 metabolism simultaneously. Hence, clues can be derived from observing alternations in the carbohydrate concentration during early seedling development under light exposure, and from comparing these seedlings with those grown under light exclusion, because the switch-over could be initiated only by light.

The C6 carbohydrate-reserve starch is depleted much quicker when the seedling develops under light exclusion (cf. Fig. 6*a*, *b*), indicating that starch is broken down to provide glucose and fructose to the shoot and not produced *de novo* via photosynthesis in the light-grown seedling. The carbon element, probably released from a storage-compound starch, could also be fed into the Calvin cycle to ultimately provide *D*-mannoheptulose and perseitol because the amount of perseitol present in the cotyledons is unlikely to have been sufficient to provide the amounts of C7 sugars present in the shoots (Fig. 6*a*, *b*). In light-grown seedlings, C7 sugars could have also been produced in the leaves and transported into the shoots, and etiolated seedlings, with a very limited leaf mass, also contained high amounts of C7 sugars. Therefore, the C7 sugars found in the stems of etiolated seedlings are more likely to have arisen from the starch reserves in the cotyledons, because these reserves were depleted quicker in dark- than light-grown seedlings. Starch must, therefore, also have acted as a carbon-storage form of C7 sugars. Ecologically, such carbon-allocation pattern would allow for fast growth of understorey species, being able to use the C6 as well as the C7 sugar metabolism to fast-forward growth to reach higher light areas quicker. The C7 metabolism must, however, provide also other advantages, besides the increase in antioxidant capacity of the fruit (Tesfay *et al*. 2010), because the switch-over from C6 to C7 metabolism happens early in the development, when no fruit are found on the tree yet. Sugars in the seedling and the primary leaf are indicating a dominance of the C6 metabolism (Fig. 7), whereas the fifth fully expanded leaf already predominantly transports C7 sugars. In its natural habitat, the seedling is still in the understorey at this stage, and is, therefore, unlikely to already require protection from stress through antioxidants. The switch from C6 to C7 metabolism must, therefore, provide additional advantages, such as possibly the ability to quicker transport carbohydrates, because the C7 storage sugar (perseitol) as well as the C7 sugar *D*-mannoheptulose were both found to be transported sugars (Fig. 7). These results also confirm findings by Liu *et al*. (2002). It is, hence, evident that the C7 sugars and, in particular, *D*-mannoheptulose, are multifunctional sugars. Consequently, their biosynthesis and metabolism warrant further investigation.

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References

Bachmann M, Matile P, Keller F (1994) Metabolism of the raffinose family oligosaccharides in leaves of *Ajuga reptans* L. Cold acclimation, translocation, and sink to source transition: discovery of chain elongation enzyme. *Plant Physiology* **105**, 1335–1345.

Barlow C (2000) Fruit development. In 'The avocado: botany, production and uses'. (Eds A Whiley, B Schaffer, B Wolstenholme) pp. 15–37. (CABI Publishing: Oxon, UK)

Borisjuk L, Wang TL, Rolletscheck H, Wobus U, Weber H (2002) A pea seed mutant affected in the differentiation of the embryonic epidermis is impaired in embryo growth and seed maturation. *Development* **129**, 1595–1607.

Bower JP, Cutting JG (1988) Avocado fruit development and ripening physiology. *Horticultural Reviews* **10**, 229–271.

Cowan AK (2004) Metabolic control of avocado fruit growth: 3-hydroxy-3-methylglutaryl coenzyme a reductase, active oxygen species and the role of C7 sugars. *South African Journal of Botany* **70**, 75–82.

Cowan AK, Moore-Gordon CS, Bertling I, Wolstenholme BN (1997) Metabolic control of avocado fruit growth. *Plant Physiology* **114**, 511–518.

Cutting JGM, Bower JP (1987) Effect of harvest date and applied abscisic acid on browning potential of avocado fruit. *South African Avocado Growers Association Yearbook* **10**, 130–132.

Häfliger B, Kindhauser E, Keller F (1999) Metabolism of D-glycero-D-heptitol, volemitol, in polyanthus. Discovery of a novel ketose reductase. *Plant Physiology* **119**, 191–198. doi:10.1104/pp.119.1.191

Hershkovitz V, Friedman H, Goldschmidt EE, Pesis E (2010) Ethylene regulation of avocado ripening differs between seeded and seedless fruit. *Postharvest Biology and Technology* **56**, 138–146. doi:10.1016/j.postharvbio.2009.12.012

Ish-Am G, Eisikowitch D (1991) Possible routes of avocado tree pollination by honeybees. *Acta Horticulturae* **288**, 225–233.

Landahl S, Meyer MD, Terry LA (2009) Spatial and temporal analysis of textural and biochemical changes of imported avocado cv. Hass during fruit ripening. *Journal of Agricultural and Food Chemistry* **57**, 7039–7047. doi:10.1021/jf803669x

Liu X, Robinson PW, Madore MA, Witney GW, Arpaia ML (1999) 'Hass' avocado carbohydrate fluctuations. II. Fruit growth and ripening. *Journal of the American Society for Horticultural Science* **124**, 676–681.

Liu X, Sievert J, Arpaia ML, Madore MA (2002) Postulated physiological roles of the seven-carbon sugars, mannoheptulose, and perseitol in avocado. *Journal of the American Society for Horticultural Science* **127**, 108–114.

McOnie AJ, Wolstenholme BN (1982) Avocado fruit growth and maturity in two Natal localities. *South African Avocado Growers' Association Yearbook* **5**, 76–77.

Meyer MD, Terry LA (2010) Fatty acid and sugar composition of avocado, cv. Hass, in response to treatment with an ethylene scavenger or 1-methylcyclopropene to extend storage life. *Food Chemistry* **121**, 1203–1210. doi:10.1016/j.foodchem.2010.02.005

Moore-Gordon CS, Cowan AK, Bertling I, Botha CEJ, Cross RHM (1998) Symplastic solute transport and avocado fruit development: a decline in cytokinin/ABA ratio is related to appearance of the 'Hass' small fruit variant. *Plant & Cell Physiology* **39**, 1027–1038.

Morgan JM (1984) Osmoregulation and water stress in higher plants. *Annual Review of Plant Physiology* **35**, 299–319. doi:10.1146/annurev.pp.35.060184.001503

Nadwodnik J, Lohaus G (2008) Subcellular concentrations of sugar alcohols and sugars in relation to phloem translocation in *Plantago major, Plantago maritima, Prunus persica*, and *Apium graveolens. Planta* **227**, 1079–1089. doi:10.1007/s00425-007-0682-0

Noiraud N, Maurousset L, Lemoine R (2001) Transport of polyols in higher plants. *Plant Physiology and Biochemistry* **39**, 717–728. doi:10.1016/S0981-9428(01)01292-X

Oliveira CM, Priestley CA (1988) Carbohydrate reserves in deciduous fruit trees. *Horticultural Reviews* **10**, 403–430.

Richtmyer NK (1970) The isolation of volemitol and other polyhydric alcohols from avocado seeds. *Carbohydrate Research* **12**, 135–138. doi:10.1016/S0008-6215(00)80234-7

Schroeder CA (1953) Growth and development of the Fuerte avocado fruit. *Proceedings of the American Society for Horticultural Science* **61**, 103–109.

Sluiter A, Sluiter J (2005) Determination of starch in solid biomass samples by HPLC: laboratory analytical procedure (LAP), NREL/MRI, National Renewable Energy Laboratory. Technical Report NREL/TP-510-42624, Issue Date 07/17/2005.

Steyn EMA, Robertse PJ, Smith D (1993) An anatomical study of ovary-to-cuke development in consistently low-producing trees of 'Fuerte' avocado (*Persea americana* Mill.) with special reference to seed abortion. *Sexual Plant Reproduction* **6**, 87–97. doi:10.1007/BF00227653

Tesfay SZ (2009) Special carbohydrates of avocado – their function as 'sources of energy' and 'anti-oxidants'. PhD Thesis, University of KwaZulu–Natal, Republic of South Africa.

Tesfay SZ, Bertling I, Bower JP (2010) Anti-oxidant levels in various tissues during the maturation of 'Hass' avocado (*Persea americana* Mill.). *The Journal of Horticultural Science & Biotechnology* **85**, 106–112.

Tesfay SZ, Bertling I, Bower JP (2011) *D*-Mannoheptulose and perseitol in 'Hass' avocado: metabolism in seed and mesocarp tissue. *South African Journal of Botany.* doi:10.1016/j.sajb.2011.10.006

Zimmermann MH, Ziegler H (1975) List of sugars and sugar alcohols in sieve-tube exudates. In 'Transport in plants. Encyclopedia of plant physiology, 1'. (Eds MH Zimmermann MH, JA Milburn) pp. 479–503. (Springer: Berlin)