The search for means to improve the C7 sugar pool in 'Hass' avocado

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ABSTRACT

Avocado contains a number of "unusual" sugars, consisting of a C7 versus the usual C6 carbon backbone. Although the role of these sugars remains unknown, there are several indications that these compounds contribute to fruit growth and development, and are, hence, related to fruit quality. Therefore, possibilities to increase the size of the C7 sugar pool in avocado were explored. Firstly, the foliar application of micronutrients (B and Zn) was tested as a means to improve the leaf C7 sugar concentration; while, secondly, the effect of girdling fruit-bearing branches on the concentration of fruit sugars was examined. Leaf analysis carried out after the application of three levels of B and two levels of Zn showed that B application positively influenced leaf mannoheptulose levels, while Zn seemed to reduce levels of this C7 sugar. Combining the three levels of B with the lower Zn applications did not improve leaf mannoheptulose concentration, while the higher Zn application even reduced leaf mannoheptulose levels. Similar trends were observed in leaf perseitol and glucose concentrations. Girdling of individual branches during the later fruit growth period affected fruit sugar concentration negatively. Although the expected result of increased C7 sugar levels were not observed, it is anticipated that particularly mannoheptulose levels can be influenced through timed fertilizer application as well as other tree manipulation practices. The quest for the role of the other dominant C7 sugar, perseitol, is, however, ongoing.

INTRODUCTION

The isolation of the special sugar 'perseitol' from avocado was published by Jones and Wall in one of the most prestigious scientific journals, "Nature", in 1961. At a similar time Bean (1958) reported a decreasing trend in sugar levels of late-harvested fruit in 'Zutano' and a 'Mexican Seedling'. Storing fruit also reduced total sugar levels. Similar trends as in total sugars were reported for 'keto-sugars' – a group that comprises fructose, mannoheptulose and the fructose part of sucrose. As mannoheptulose and perseitol are the main sugars in avocado flesh (Li *et al*., 1999) and mannoheptulose is a "keto-sugar", it is likely that not the C7 sugar pool as such (comprising mainly of mannoheptulose and perseitol), but the concentration of the C7 sugar mannoheptulose on its own, is an indicator of fruit quality. It has been furthermore suggested that one of the possible functions of mannoheptulose is to act as a mesocarp antioxidant (Bertling *et al*., 2007).

A further function of these C7 sugars could be to act as carbon storage compounds. Such a function is highly likely since avocado contains only little fruit starch (Li *et al*., 1999), although starch is present in larger amounts in avocado tree trunks (Whiley *et al*., 1992; Kaiser and Wolstenholme, 1993).

Furthermore, certain sugar alcohols partake in the osmotic adjustment process during water deficit (e.g. sorbitol in apple) (Mills *et al*., 1996) or act as a sugar transport molecule (e.g. sorbitol in peach) (Makinen and Soderling, 1980). The latter function of the C7 sugars in avocado has been suggested by Li *et al*. (1997). Additionally sugar alcohols can act as secondary messengers – like myo-inositol in seeds (Loewus and Loewus, 1983).

It is, hence, of great importance to find means of increasing the C7 sugars in avocado tissue, in order to demonstrate whether an increase in C7 sugars, and particularly mannoheptulose, will result in increased fruit quality, a parameter that is closely aligned to storability and shelf life in general. In temperate fruit the management practice of girdling has been used extensively to increase the sugar concentration in fruit on the girdled branch (Berueter and Feusi, 1997). Bellaloui *et al*. (1999) reported that the transport of sugar alcohols is correlated with the transport of boron (B). Subsequently, it has also been reported that the remobilisation of B is, in certain species, correlated to the levels of sorbitol in the plant (Bellaloui *et al*., 2003). A similar effect, increasing the transport and mobility of sugar alcohols by B application, might also be possible, particularly in a situation where B is slightly deficient and, hence, hindering the formation of the sugar alcohol-B transport complex. The application of B as well as of zinc (Zn), a nutrient often co-transported with B that might, hence, also play a role in the sugar alcohol allocation, was, therefore, examined with respect to their ability to increase C7 sugar accumulation in leaves to improve the supply of sugars to the developing fruit.

MATERIALS AND METHODS

Thirty-six trees selected from four rows of ten-year-old 'Hass' avocado trees on 'Duke7' planted in the KZN Midlands were used for the experiments. Three concentrations of B (as Solubor® at 0.5, 1.5 and 2.5 gxL^{-1}), two concentrations of Zn (as Zn-chelate at 0.5 and 1 gxL^{-1}), as well as the combination of these onto selected branches were sprayed in September 2007 to drip-off. Leaf samples of the youngest mature leaf were taken prior to foliar application to determine their nutrient concentrations. Five weeks after application, the youngest fully expanded leaves were harvested and prepared for sugar analysis as previously described (Bertling *et al*., 2007). Furthermore, girdling treatments were applied in 2007 on March 20 or April 21 to six branches of a further three trees, using a girdling knife. Fruit samples from this experiment were taken off these branches of approximately 4 and 6 cm diameter in May 20 and June 21.

Leaf and fruit samples from these treatments were freeze-dried, milled and sugars extracted as previously described (Bertling *et al*., 2007). Separation of sugars was achieved using a Shimadzu HPLC system (and using a Rezex® RCM-Monosaccharide column, 300 x 7.80 mm 8 micron) and detection of sugars carried out by comparison with standards using a refractive index detector (RID-10A, SHIMADZU) as described previously (Bertling *et al*., 2007).

Statistical evaluations were carried out using GenStat® Version 9.0.

RESULTS

Zn and B applications

Prior to foliar application of Zn and B, both nutrients were deficient in leaves (**Table 1**), hence a beneficial effect of the fertilization was anticipated. The predominant sugar in leaves was mannoheptulose, followed by sucrose and perseitol.

Although there was a tendency towards an increase in leaf mannoheptulose concentration with an increase in the amount of Solubor® applied (**Table 2**), differences between B treatments and control were not significant. Application of the highest B concentration shifted the ratio between leaf sugars towards mannoheptulose as the dominant sugar present (**Table 2**), with 2.5 g*L^{-1} Solubor® resulting in the highest leaf mannoheptulose concentration. Zinc had a tendency to affect leaf mannoheptulose concentration negatively, particularly, when the lower Zn application rate was combined with the B applications; however, when the higher Zn application was combined with boron treatments, leaf mannoheptulose levels increased. The higher Zn plus B combinations had a tendency to increase leaf

Table 1. Percentage nutrient concentration (on a leaf dry matter basis) prior to B and Zn treatment (2006)

Row (R) / Tree (T) number	C (%)	N (%)	Ca (%)	Mg (%)	K (%)	Na mg/kg	Zn mg/kg	Mn mg/kg	Fe mg/kg	P (%)	B mg/kg
R1T1	48.34	2.26	1.56	0.76	0.81	347.5	51	535	335	0.16	22
R1T4	49.24	2.34	1.38	0.64	0.71	102.2	20	429	139	0.14	20
R2T2	49.43	2.62	1.35	0.58	0.85	61.1	22	366	85	0.14	16
R2T5	49.22	2.45	1.31	0.63	0.78	81.4	18	594	75	0.13	20
R3T3	49.26	2.46	1.47	0.67	0.71	40.7	18	324	88	0.13	18
R3T6	49.34	2.28	1.27	0.62	0.75	61.1	18	362	67	0.13	18
R4T1	49.71	2.72	1.27	0.55	0.95	60.9	18	293	81	0.14	22
R4T4	49.10	2.54	1.09	0.60	0.87	101.6	24	435	71	0.14	20
NORMS*	-	2.2-2.3	1.2-2.0	0.7-1.0	0.75-1.15	<15	40-100	50-250	50-150	0.15-0.20	50-80

* Leaf norms as per recommendation (Donkin, 2001 and SAAGA, 2005)

Table 2. Leaf sugar concentrations of youngest mature leaf 35 days after application (total mg/g DW and percentage of mannoheptulose, perseitol, sucrose and glucose pool)

	Manno-heptulose	Perseitol	Sucrose	Glucose	Sum of main 4 sugars	Manno-heptulose	Perseitol	Sucrose	Glucose
	(mg/g DW)					%			
C	6.67^{ab*}	5.24^{ab}	6.62^{ab}	3.91^{abc}	22.44	29.98	23.4	29.04	17.58
B_1	8.02^{ab}	5.85^{a}	6.55^{ab}	3.20^{abcd}	23.62	32.85	23.51	30.12	13.52
B_2	8.10^{ab}	4.55^{ab}	5.14^{ab}	4.31^{ab}	22.1	35.20	20.62	25.13	19.05
B_3	9.18^{a}	4.87^{ab}	6.61^{ab}	1.75^{bcde}	22.41	41.97	21.49	27.08	9.46
Z_1	4.78^{ab}	3.56^{ab}	5.81^{ab}	3.06^{abcde}	17.21	26.40	19.61	35.40	18.58
Z_2	4.09^{ab}	3.01^{ab}	5.12^{ab}	3.28^{abc}	15.5	27.91	20.24	35.47	16.39
Z_1+B_1	2.53^{b}	1.78^{b}	2.89^{b}	0.42^{cde}	7.62	32.91	23.44	38.37	5.28
Z_1+B_2	4.66^{ab}	3.96^{ab}	5.69^{ab}	1.37^{cde}	15.68	30.26	25.33	36.99	7.42
Z_1+B_3	2.59^{ab}	1.88^{b}	2.88^{b}	0.58^{de}	7.93	33.08	23.61	36.55	6.76
Z_2+B_1	7.64^{ab}	5.17^{ab}	7.90^{a}	3.81^{abc}	24.52	30.78	21.14	33.73	14.34
Z_2+B_2	6.69^{ab}	4.26^{ab}	8.56^{a}	3.93^{abc}	23.44	28.95	17.56	36.55	16.93
Z_2+B_3	7.64^{ab}	5.29^{ab}	6.02^{ab}	4.67^{a}	23.62	31.26	23.00	25.30	20.43

* = numbers within a column followed by different letters are significantly different at the 0.05% level

B_1= 0.5 g Solubor x L^{-1}, B_2= 1.5 g Solubor x L^{-1}, B_3 = 2.5 g Solubor x L^{-1};

Zn_1= 0.5 g Zn-Chelate x L^{-1}, Zn_2= 1 g Zn-Chelate x L^{-1}

$LSD_{(5\%)}$: Sucrose = 4.381; Mannoheptulose = 5.631; Glucose = 2.675; Perseitol = 3.65

Table 3. Fruit sugar concentrations of young fruit on girdled branches at indicated time intervals after the girdling

Soluble sugar (mg/g DW)	Date of girdle	Picked 24-05-2007	Picked 24-06-2007	Post-storage (28 days)
Mannoheptulose	None (control)	15.6	17.4	5.5
	20-03-2007	4.6	12.6	n.d.
	21-04-2007	6.4	12.0	n.d.
Perseitol	None (control)	10.9	11.3	8.4
	20-03-2007	7.3	8.5	n.d.
	21-04-2007	5.3	8.8	n.d.
Sucrose	None (control)	5.8	4.2	5.6
	20-03-2007	12.5	4.1	n.d.
	21-04-2007	5.6	4.1	n.d.

n.d.= not determined due to insufficient numbers of fruit maintained on girdled branches

sucrose levels, seemingly at the expense of the mannoheptulose level.

Girdling

Girdling reduced the concentration of both C7 sugars in fruit below the girdle (**Table 3**), while sucrose levels seemed to increase or remain unchanged in fruit of such branches. Two months after the treatment, however, these effects had faded or were no longer visible. The number of fruit retained on girdled branches was not sufficient to warrant a post-harvest determination of fruit sugars; in fruit from non-treated branches the perseitol levels, and more pronounced the mannoheptulose levels, declined post-harvest.

DISCUSSION

Application of Zn and B as well as girdling was expected to increase the sugar concentration in leaves / fruit below the girdle. As sugar alcohol transport positively affects B uptake (Bellaloui *et al.*, 1999), it was tested if the reciprocal action (B positively affects sugar transport) would also hold true. However, our data do not support such an assumption, as no accumulation of mannoheptulose occurred in leaves after B application. Application later in the season might result in an increase in fruit sugars. It should, furthermore, be tested if soil boron application impacts positively on leaf sugar – and ultimately fruit sugar – concentration.

Zink applications did not increase the level of any of the tested C6 or C7 sugars, but rather tended to decrease sugar levels. Although the concentration of perseitol in avocado honey has been reported to be highly correlated with its Zn concentration (Dag *et al.*, 2006), no such effect of a Zn leaf application on leaf C7 sugar concentration was found. Again, the timing of this application should be closer investigated, as possibly a later application could affect fruit C7 sugar levels positively. Girdling of main limbs of young avocado trees has been successfully applied by Köhne (1992) to increase fruit yield. Trochoulias and O'Neill (1976) have also reported that girdling increases yield of girdled branches and that multiple girdling increases avocado fruit size. However, both groups of authors applied the first girdle at earlier stages of fruit development than carried out in our experiment. This later girdling time was chosen as it was not intended to manipulate early flower/fruit development but rather sugar accumulation in the fruit or leaves on girdled branches. Analysis of immature and mature fruit from these branches would clarify if the increased yield, reported by Trochoulias and O'Neill (1976) as well as Köhne (1992), following girdling treatments are due to C7 sugar accumulation. It is, however, clear from our girdling experiment that C6 sugars are affected differently to C7 sugars by this practise. The March as well as the April girdle decreased the C7 sugar concentration in fruit on the first sampling after treatment. However, in the following month when fruit were almost ready for harvest, C7 levels had risen again. Therefore, it might be possible that by applying the girdle one month earlier, more C7 sugars and particularly mannoheptulose are transported into the fruit, possibly improving fruit quality and shelf life. The main C6 sugar present in major amount in fruit in May and June was sucrose and levels of this sugar remained constant post-treatment, indicating that C6 and C7 sugars react differently to treatments and, therefore, have a different function in avocado tree physiology. It is furthermore evident that not, as initially anticipated, mannoheptulose and perseitol are present in similar amounts, but that mannoheptulose decreases towards harvest and that, hence, the two major C7 sugars fulfil different roles in fruit development and ripening.

CONCLUSIONS

C7 sugars in avocado, and particularly mannoheptulose, seem to fulfil a variety of roles, ranging from acting as transport sugar to antioxidants protecting against stress to a role in fruit quality. Although current efforts to increase C7 sugars in avocado leaves and fruit have not been successful, changing the timing of fertilization or girdling might be successful in improving C7 and particularly mannoheptulose levels prior to harvest.

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