Evaluation of Carbohydrates in Pukekohe Longkeeper and Grano Cultivars of *Allium cepa*

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The storage, soluble, and structural carbohydrates of two onion cultivars, the hard, pungent Pukekohe Longkeeper (PLK) and the softer, milder Houston Grano, were analyzed to determine differences that might be related to their response to sulfur nutrition received during growth as well as their postharvest attributes and end-use suitability. PLK tissue had 7 times more dry matter, composed of more fructan, sucrose, and glucose and less fructose, than Grano and a greater proportion of fructan with a degree of polymerization of 3–5. There were also differences in neutral sugar content, especially galactose, and the amount, size, and content of pectin fractions soluble in chelator and weak alkali. These two onion cultivars differed in their capacity to take up sulfur, but there was no statistical association between sulfur supply and any measured dry matter component.

KEYWORDS: Onion; texture; polysaccharide; cell wall; fructan; pectin

INTRODUCTION

Onions are valued for their flavor in the foods of many ethnicities and are increasingly recognized for their health-giving properties such as providing nondigestible dietary fiber from fructan (a nonstructural storage carbohydrate) and antiplatelet activity from organosulfur compounds (*1*). Dry matter content is the primary characteristic of onion bulb quality, determining appropriate end use (e.g., as salad, fresh, or dehydrated onions), storability, flavor, pungency, and texture. Dry matter >15% (and often 20%) of onion weight is required for dehydrator use, whereas <15% dry matter is regarded as better for fresh consumption (*2*). Sweet or "salad" onions, which are eaten without cooking, tend toward dry matters of <10%, whereas onions at the higher end of acceptable dry matter for fresh consumption are generally much firmer and have longer storage life before shoot growth and rots become limiting (*3–5*).

Nonstructural, soluble carbohydrates form a substantial part of onion dry matter, mainly as the fructose-containing polysaccharide fructan as well as glucose, fructose, and sucrose. Onion fructans are primarily of the trisaccharide to pentasaccharide size class, but variations to size profiles occur in relation to high, medium, or low dry matter contents (*3, 5–8*). Fructans of the inulin and inulin neoseries are present in onion (*9, 10*). High bulb dry matter and high soluble solids are generally linked to a greater accumulation of fructan rather than mono- and disaccharides.

Structural (cell wall-located) polysaccharides form another significant fraction of dry matter in onions. These polysaccharides of the cell wall include cellulose, hemicelluloses, and pectins. Several studies have shown that onion cell walls are rich in pectin and have high levels of the neutral sugar galactose, with significant differences in cell wall composition from the outer (older) to inner (younger) scales (leaves) as well as across the cell types of individual scales (*11–13*). Few linkages have been made between firmness of onion bulbs and cell wall composition, although changes to cell wall integrity are routinely associated with loss of firmness in other plant tissues (e.g., ripening fruit). Recent work (*14*) suggested that sulfur nutrition during growth could affect the accumulation of cell wall material in onion and hence could have an impact on bulb firmness. Although sulfur nutrition during growth has been linked to onion pungency (*15, 16*), the mechanisms linking cultivar-specific responses to sulfur fertility leading to increased dry matter have not been resolved. Pungency appears to be genetically linked to dry matter content as onion cultivars tend to be associated in high sulfur/highly pungent/high dry matter and low sulfur/sweet or mild pungency/low dry matter clusters. Comparative field trials (*17–19*) suggest the relationships between dry matter accumulation, soluble solids, sulfur levels, and subsequent pungency in onion bulbs are complex and are certainly influenced by the germplasm trialled and levels of sulfur fertility supplied during growth. Although the physiological links between sulfur fertility and dry matter may be unresolved, there is even less known about how sulfur fertility could affect cell wall content. It is likely that the effect is indirect (e.g., sulfur-

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dependent cell wall synthases) rather than a direct incorporation of sulfur into cell wall polysaccharides, because sulfur-containing polysaccharides appear to be restricted to marine algae.

The aims of this project were twofold. We wished to compare and contrast elements of the dry matter composition of two onion cultivars with markedly different dry matter and sensory qualities (a high dry matter/long-storage cultivar with a low dry matter/short-storage type) focusing on the nonstructural (storage and soluble) and structural carbohydrates in order to isolate components that might relate to differences in their texture and postharvest performance. Alongside this we aimed to investigate whether dry matter components, particularly cell wall polysaccharides, might be influenced by sulfur nutrition during growth.

MATERIALS AND METHODS

Two onion cultivars were used for this work: an open-pollinated selection from Pukekohe Longkeeper (PLK) and an inbred line from Houston Grano (Grano), as described (20). Onions were grown in a replicated hydroponic trial in a glasshouse, using three rates of sulfur fertility (0.5, 2, and 4 mequiv L^{-1}) supplied by varying the concentrations of MgSO$_4$, Mg(NO$_3$)$_2$, and Ca(NO$_3$)$_2$ in Hoagland media (15, 21), according to the procedure outlined by Lancaster et al. (14). There were four replicate trays per sulfur treatment for each cultivar (24 trays in total), and each replicate tray contained nine plants. Mature onions were harvested when stems were drying, and then bulbs were cured for a month. There were between six and nine usable (>2 g) bulbs from each treatment replicate, with >75% of the plots containing eight or nine usable onions. For each treatment replicate, tissue was combined from quadrants of each bulb and was then either homogenized and freeze-dried or used to prepare ethanol-insoluble residues (EIR) (22). The EIR were treated with Tris-buffered phenol during preparation (23) to inactivate endogenous cell wall-degrading enzymes. Material solubilized by the phenol was partially retained and reprecipitated in the EIR preparations.

Sulfur content of freeze-dried onion powders was analyzed using a Leco CNS-2000 analyzer (Leco Corp., St. Joseph, MI). Samples were dried at 60 °C for 48 h before analysis.

Nonstructural Carbohydrates. Glucose, fructose, and sucrose were extracted from freeze-dried material (10 mg) in 1 mL of 62.5% methanol. Aliquots were dried under vacuum and then redissolved in water prior to high-performance liquid chromatography (HPLC) separation on a Rezex RCM monosaccharide column (Phenomenex, Torrance, CA), operating at 85 °C with a flow rate of 0.6 mL min^{-1} water. Evaporative light scattering (ELSD 1000, Polymer Laboratories, Church Stretten, Shrop., U.K.) was used for detection. Sugars were quantified by comparison of peak area with peak area standard curves for sucrose, glucose, and fructose (Sigma, St. Louis, MO).

Minimal starch was detected in freeze-dried tissue (starch kit, Boehringer Mannheim, Darmstadt, Germany). Fructans in freeze-dried onion material and in EIR preparations were extracted in water at 80 °C for 15 min (in a ratio of 10 mg/1 mL of water). Fructans were quantified using a fructanase assay kit (Megazyme, Bray, County Wicklow, Ireland) following the procedure outlined in the accompanying Megazyme literature. Both the starch and fructan assays were adapted for microplate-scale determinations and absorbances read on a Molecular Devices SpectroMax 250 microplate reader (Sunnyvale, CA) using Softmax Pro software (version 1.2). For analysis of size classes of fructan, extracts of known fructan content were fractionated by HPLC on a Rezex RNO column (Phenomenex) at 85 °C with water as eluant at a flow rate of 0.3 mL min^{-1}. The elution positions of oligosaccharides with a degree of polymerization (DP) of 3−8 were determined by calibration with fructo-oligosaccharides from *Helianthus tuberosus* (24). Eluted material was detected by differential refractometry, and the distribution of fructans of DP 3−8 and DP >8 was determined from relative peak areas.

Structural Carbohydrates. Noncellulosic neutral sugars in 2 N trifluoroacetic acid hydrolysates of EIR were derivatized to their corresponding alditol acetates (25) and quantified by gas chromatography using a BPX-70 column (0.53 mm × 25 m, SGE, Victoria,

Australia), with temperature program and flame ionization detection as previously described (22). Cellulose in EIR preparations was hydrolyzed (26) and quantified using the anthrone assay (27) with D-glucose (Sigma) as a standard. The total pectin content of EIR was determined following controlled hydrolysis (28). The uronic acids released were quantified using 3-hydroxydiphenyl (29) adapted for microplate-scale assay (22), using D-galacturonic acid (Sigma) as the standard.

To obtain soluble pectin fractions, EIR was extracted with 50 mM NaOAc, pH 6.0, containing 50 mM *trans*-1,2-diaminocyclohexane-*N*,*N*,*N*′,*N*′-tetraacetic acid (CDTA) as chelator overnight at room temperature (22). Residual material was re-extracted in 50 mM Na$_2$CO$_3$ containing 20 mM NaBH$_4$. Uronic acid and neutral sugar contents in these extracts were assayed as described above. Size classes of carbohydrates solublized by CDTA and Na$_2$CO$_3$ were separated on a Superose 6HR column (1.0 cm × 30 cm; Amersham Pharmacia Biotech AB, Uppsala, Sweden) with 30 mM NaOAc, pH 6.5, containing 20 mM EDTA and 10 mM NaCl as the elution buffer, at a flow rate of 0.5 mL min^{-1}. Fractions (0.5 mL) were assayed for uronic acids (as described above) and total carbohydrates (30).

Data Analysis. Statistical analysis was performed using the REML procedure in Genstat (31), fitting a random replicate effect and fixed cultivar and sulfur treatment effects, plus cultivar/treatment interaction. Unless otherwise stated, observations were weighted by the number of plants yielding usable onions per plot. Analysis of correlations between the carbohydrate constituents was also by Genstat, and a principal component analysis was based on the correlation matrix information. Unless otherwise described, quantitative results are presented as means for each cultivar and sulfur fertility rate with a test statistic (least significant difference, LSD) for these interactions, as well as main effect data for overall cultivar and sulfur fertility rate comparison, with LSDs for each.

RESULTS

Data for bulb weight and dry matter, water, and sulfur contents appear in **Table 1**. As expected, Grano bulbs were heavier than those of PLK but had lower dry matter content, confirming that both Grano and PLK had dry matters indicative of their suitability for fresh consumption and that Grano had dry matter typical of sweet onion cultivars. The amount of dry matter per average bulb of each cultivar was similar, with water accumulation during growth a significant point of difference. This suggests that the quality characteristics of the two cultivars rest as much with the composition of the dry matter as with dry matter quantity. Variations in sulfur fertility during growth had no impact on bulb weight, dry matter or bulb water content. As expected, PLK had higher total sulfur levels than Grano (fresh weight basis). PLK almost doubled in sulfur content as sulfur fertility rose from 0.5 to 2 mequiv L^{-1}, but the response to 4 mequiv L^{-1} was not nearly so marked. Grano sulfur content followed a similar pattern, although starting levels (at 0.5 mequiv L^{-1}) were significantly lower than for PLK and increases were not as great as for PLK.

Nonstructural Carbohydrates. Nonstructural carbohydrate compositions of the cultivars were significantly different (**Table 2**). Approximately half of the mono- and disaccharides content was glucose for PLK and Grano. In Grano the remainder was ∼40% fructose/∼10% sucrose, but this situation reversed in PLK (∼10% fructose/∼40% sucrose). The higher fructose and lower sucrose in Grano coupled with the relative sweetness index of these sugars (1.4:1:0.6 for fructose/sucrose/glucose) (32) underpin the greater sweetness of Grano bulbs compared to PLK. Main effects analysis indicated the highest sulfur rate was associated with lower glucose across both cultivars.

There were both quantitative and qualitative differences in the fructan of PLK and Grano tissue (**Tables 2** and **3**). PLK contained 7 times the level of fructan in Grano (fresh weight

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Table 1. Bulb Weight and Dry Matter, Water, and Sulfur Contents of PLK and Grano Onions, Including Interactions (LSD[a]) and Main Effects (LSD[b] for Cultivar Comparisons and LSD[c] for Sulfur Rate Comparisons)

cultivar	S (mequiv L⁻¹)	bulb wt (g)	dry matter (mg g⁻¹ of fresh wt)	dry matter (g onion⁻¹)	water content (g onion⁻¹)	S (mg g⁻¹ of fresh wt)
PLK	0.5	145.3	139.9	20.4	125.0	476
	2.0	193.1	140.8	27.2	166.0	933
	4.0	161.2	137.6	22.3	139.0	973
Grano	0.5	219.3	100.2	22.0	197.3	329
	2.0	207.0	102.0	21.2	185.8	562
	4.0	226.9	102.9	23.4	203.5	606
LSD[a]		54.1	3.5	7.3	46.9	91
cultivar means						
PLK		166.5	139.4	23.3	143.3	796
Grano		217.8	101.7	22.2	195.6	503
LSD[b]		30.8	1.9	4.2	26.9	57
S means	0.5	182.5	120.1	21.2	161.2	507
	2.0	200.0	121.4	24.2	175.9	750
	4.0	193.9	120.3	22.8	171.2	790
LSD[c]		38.4	2.3	5.2	33.3	70

[a] Degrees of freedom = 16 and P = 0.05.

Table 2. Nonstructural Carbohydrate Content of PLK and Grano Onions

cultivar	S (mequiv L⁻¹)	glucose	fructose	sucrose	fructan
		mg g⁻¹ of fresh wt			
PLK	0.5	19.2	3.2	12.0	50.8
	2.0	18.5	3.0	11.0	49.5
	4.0	18.1	3.5	10.7	46.9
Grano	0.5	20.1	14.7	5.3	6.8
	2.0	20.3	14.9	4.7	7.0
	4.0	18.5	13.7	4.8	6.8
LSD[a]		1.5	0.8	1.5	3.8
cultivar means					
PLK		18.6	3.2	11.2	49.1
Grano		19.6	14.4	4.9	6.9
LSD[b]		0.8	0.5	0.9	2.2
S means	0.5	19.7	8.9	8.6	28.8
	2.0	19.4	8.9	7.8	28.2
	4.0	18.3	8.6	7.7	26.6
LSD[c]		1.0	0.6	1.1	2.7

[a] Data includes interactions (LSD[a]) and main effects (LSD[b] for cultivar comparisons and LSD[c] for sulfur rate comparisons). Degrees of freedom = 16 and P = 0.05.

Table 3. Percentage Distribution of Fructan Size Classes in PLK and Grano Onions, Separated by HPLC

cultivar	S (mequiv L⁻¹)	DP3[a]	DP4	DP5	DP6	DP7	DP8	>DP8
		fructan size classes (% distribution)						
PLK	0.5	19.4	18.0	14.7	9.8	5.8	3.2	29.1
	2.0	18.4	17.3	14.4	9.5	5.6	3.0	31.8
	4.0	18.9	16.5	13.5	9.0	5.3	3.1	33.7
Grano	0.5	15.2	6.7	5.0	3.4	2.2	1.4	66.0
	2.0	13.8	6.3	4.8	3.4	2.1	1.3	68.3
	4.0	13.7	6.0	4.5	3.0	2.0	1.2	69.6

[a] DP, degree of polymerization.

basis, **Table 2**). Fructan quantity was not influenced by the sulfur fertility received by plants of either cultivar during growth. Size exclusion chromatography was used to describe the size distribution profile of fructans present (**Table 3**). The system used did not discriminate between the linkage types (inulin or inulin neoseries fructans). Results are presented as a percentage of the total weight of fructan present (fresh weight basis). The majority of fructan in PLK (52%) had a DP of 3−5, whereas

~30% of the fructan had a DP >8. Grano, on the other hand, had only 27% of fructan in the size range DP 3−5 and more than two-thirds as polymers DP >8. Size distributions did not appear to change according to levels of sulfur applied.

Structural Carbohydrates. Ethanol extraction followed by buffered-phenol treatment was used to inactivate endogenous enzymes during preparation of EIR for subsequent cell wall polysaccharide identification and quantitation. EIR recoveries ranged from 319 to 359 mg g⁻¹ of dry matter for PLK and from 203 to 213 mg g⁻¹ of dry matter for Grano, but there were no sulfur treatment-related recovery trends. EIR was prepared in this way to retain all cell wall polysaccharides larger than DP 3, even if soluble *in planta*. However, EIR material of both cultivars also contained coextracted fructan, although quantities were not influenced by sulfur treatments (data not shown). Noncellulosic neutral sugars in EIR preparations were identified by alditol acetate derivatization. Glucose was present in the samples, but because the EIR of both cultivars contained a small amount of coextracted fructan, it was impossible to quantify the proportion of noncellulosic glucose associated with cell wall polysaccharides. Data for arabinose, xylose, and mannose were log-transformed to stabilize variability for statistical analysis (**Table 4**). The untransformed means are in parentheses alongside the transformed data. The main noncellulosic neutral sugar in cell walls of both cultivars was galactose, followed by xylose. All sugars (with the exception of mannose) were present in greater quantities in PLK than in Grano. There were no differences in the amounts of noncellulosic neutral sugars in relation to sulfur supply.

Cellulose and pectin contents were similar for PLK and Grano (fresh weight basis, **Table 5**), although they formed a smaller proportion of the PLK dry matter (data not shown). There were, however, quantitative and qualitative differences in polyuronide solubilized from EIR by CDTA (a calcium chelator) followed by Na_2CO_3 (weak alkali). For Grano the quantities of polyuronide solubilized by CDTA and Na_2CO_3 (fresh weight basis) were fairly similar, whereas for PLK Na_2CO_3-soluble polyuronide levels were more than twice that of CDTA-soluble polyuronide (**Table 5**). Grano had more CDTA-soluble polyuronide and less Na_2CO_3-soluble polyuronide than PLK. Neutral sugars in each soluble polyuronide fraction are shown in **Table 6**, presented as a ratio to the uronic acid content. Galactose was by far the most prevalent neutral sugar in both extracts for both

Table 4. Noncellulosic Neutral Sugars in PLK and Grano Onions[a]

| cultivar | S (mequiv L^{-1}) | mg g^{-1} of fresh wt | | | | |
		rhamnose	arabinose[d]	xylose[d]	mannose[d]	galactose
PLK	0.5	0.995	*−0.003 (1.087)*	*0.440 (1.649)*	*−0.564 (0.658)*	3.782
	2.0	1.157	*0.194 (1.278)*	*0.566 (1.856)*	*−0.333 (0.778)*	4.071
	4.0	0.868	*0.033 (1.150)*	*0.437 (1.610)*	*−0.458 (0.714)*	3.807
Grano	0.5	0.716	*−0.403 (0.680)*	*0.010 (1.037)*	*−0.671 (0.521)*	2.200
	2.0	0.737	*−0.406 (0.667)*	*0.122 (1.151)*	*−0.625 (0.541)*	2.592
	4.0	0.667	*−0.571 (0.584)*	*−0.045 (0.984)*	*−0.933 (0.435)*	2.172
LSD[a]		0.276	*0.420*	*0.359*	*0.531*	0.750
cultivar means						
PLK		1.007	*0.075*	*0.481*	*−0.452*	3.887
Grano		0.706	*−0.460*	*0.029*	*−0.743*	2.321
LSD[b]		0.155	*0.236*	*0.202*	*0.298*	0.422
S means	0.5	0.855	*−0.203*	*0.225*	*−0.618*	2.991
	2.0	0.947	*−0.106*	*0.244*	*−0.479*	3.331
	4.0	0.767	*−0.269*	*0.196*	*−0.696*	2.989
LSD[c]		0.192	*0.291*	*0.250*	*0.370*	0.524

[a] Glucose was detected, but values are not reported due to the presence of fructan in EIR preparations. Data are presented as interactions (LSD[a]) and main effects (LSD[b] for cultivar comparisons and LSD[c] for sulfur rate comparisons). Degrees of freedom = 16, and P = 0.05. [d] Data for arabinose, xylose, and mannose were log-transformed to stabilize variability; transformed means and LSDs are in italics, nontransformed means are alongside in parentheses.

Table 5. Structural Carbohydrates in PLK and Grano Onions[a]

| cultivar | S (mequiv L^{-1}) | mg g^{-1} of fresh wt | | | |
		cellulose	pectin	CDTA	Na$_2$CO$_3$
PLK	0.5	4.42	5.03	0.91	2.15
	2.0	4.52	5.13	0.89	2.44
	4.0	4.03	4.84	0.89	2.18
Grano	0.5	3.91	5.01	1.24	1.16
	2.0	3.81	4.71	1.18	1.33
	4.0	3.81	5.53	1.18	1.20
LSD[a]		0.78	0.77	0.18	0.35
cultivar means					
PLK		4.32	5.00	0.90	2.26
Grano		3.84	5.08	1.20	1.23
LSD[b]		0.45	0.43	0.10	0.20
S means	0.5	4.16	5.02	1.08	1.66
	2.0	4.16	4.92	1.04	1.88
	4.0	3.92	5.19	1.04	1.69
LSD[c]		0.56	0.54	0.13	0.25

[a] Analyses include cellulose, total pectin, and uronic acid contents of CDTA- and Na$_2$CO$_3$-soluble pectins. Data are presented as interactions (LSD[a]) and main effects (LSD[b] for cultivar comparisons and LSD[c] for sulfur rate comparisons). Degrees of freedom = 16 and P = 0.05.

cultivars. Again, glucose was detected but is not reported because it may have originated from fructan coprecipitating during the EIR preparation. Ratios between the neutral sugars and uronic acids present suggest the possibility that CDTA-soluble extracts contain some branched pectins (**Table 6**). Those from PLK may contain pectin species with more frequent branches (the ratio of rhamnose to uronic acid is twice that for Grano), whereas branches in CDTA-soluble pectins from Grano may be longer (higher galactose or arabinose/uronic acid). These branched species may be water soluble, as approximately half the amount of the polyuronide solubilized from EIR in the first CDTA extraction was freely soluble in water alone (data not shown), and CDTA is generally believed to release tracts of primarily unbranched polyuronide associated through calcium linkages. Alternatively, the galactose and arabinose may together form separate water-soluble arabinogalactans in this extract. Linkage analysis would be needed to clarify this.

Na$_2$CO$_3$-soluble extracts from both cultivars had nearly 4 times the galactose/uronic acid ratio of CDTA extracts (**Table 6**), suggesting a greater level of branched pectin present in these Na$_2$CO$_3$-soluble extracts. Assuming that the galactose and arabinose are both associated with pectins, via attachment to rhamnose as side chains to the polyuronide backbone, the data suggest that Na$_2$CO$_3$-soluble pectins in Grano onions may contain a larger number of side branches and that the less frequent side branches for PLK onions may have a higher number of galactose units.

Size exclusion chromatography was used to determine the molecular weight profile of carbohydrates solubilized from EIR first by CDTA, followed by Na$_2$CO$_3$. Size exclusion profiles for the sulfur fertility treatments were not substantially different for PLK or Grano, and so only those related to the rate of 2 mequiv L^{-1} sulfur are shown in **Figure 1**. CDTA-soluble polyuronide size profiles were similar for PLK and Grano for the major size grouping of high molecular weight (corresponding to an average size of >500 kDa). The total carbohydrate profile associated with these polymers was slightly offset from the uronic acid profile. The phenol/sulfuric acid assay used to obtain the total carbohydrate profile is also positive for uronic acids, and this slight offset indicates that neutral sugars were present and in slightly higher quantities in Grano extracts, particularly in polymers of sizes corresponding to the dextran markers of 73−500 kDa. In addition, PLK contained significantly more uronic acid associated with oligomers eluting close to the inclusion limit of the Superose 6HR column. Because low molecular weight fructan could have been coextracted with CDTA, total carbohydrates were not sampled in fractions below 9.3 kDa and are not presented for Na$_2$CO$_3$-soluble extracts for comparative purposes. The size profile of uronic acids in Na$_2$CO$_3$-soluble extracts of PLK indicates fractions of high molecular weight eluting close to the void volume of the column, whereas the Grano Na$_2$CO$_3$-soluble polyuronide distribution is much broader and does not contain polymers of such length as PLK. The size profile of total carbohydrates suggests there are significant neutral sugars (possibly as pectin side branches) associated with these polymers of high molecular weight.

Correlations. Analysis of correlations between the carbohydrate constituents assayed (standardized quantitative data, **Table 7**) indicated that very strong correlations ($|r| \geq 0.8$) existed between nonstructural components (e.g., fructan, fructose, and sucrose), among structural components (e.g., Na$_2$CO$_3$-soluble pectin and a number of the neutral sugars), and between

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Table 6. Ratios of Noncellulosic Neutral Sugars to Uronic Acid in CDTA-Soluble and Na$_2$CO$_3$-Soluble Extracts of EIR from PLK and Grano Onions[a]

cultivar	S (mequiv L^{-1})	ratio to uronic acid content					
		rhamnose	arabinose	xylose	mannose	galactose	uronic acid
		CDTA-Soluble					
PLK	0.5	0.055	0.065	0.029	0.044	0.207	1.000
	2.0	0.064	0.061	0.019	0.043	0.186	1.000
	4.0	0.059	0.059	0.018	0.043	0.192	1.000
Grano	0.5	0.032	0.070	0.020	0.019	0.156	1.000
	2.0	0.029	0.061	0.017	0.021	0.152	1.000
	4.0	0.035	0.065	0.016	0.019	0.181	1.000
		Na$_2$CO$_3$-Soluble					
PLK	0.5	0.100	0.068	0.013	0.009	0.826	1.000
	2.0	0.097	0.071	0.010	0.018	0.839	1.000
	4.0	0.103	0.071	0.015	0.018	0.821	1.000
Grano	0.5	0.113	0.051	0.013	0.009	0.728	1.000
	2.0	0.144	0.076	0.006	0.010	0.558	1.000
	4.0	0.126	0.044	0.014	0.011	0.553	1.000

[a] Glucose was detected, but values are not reported due to the presence of fructan in EIR preparations.
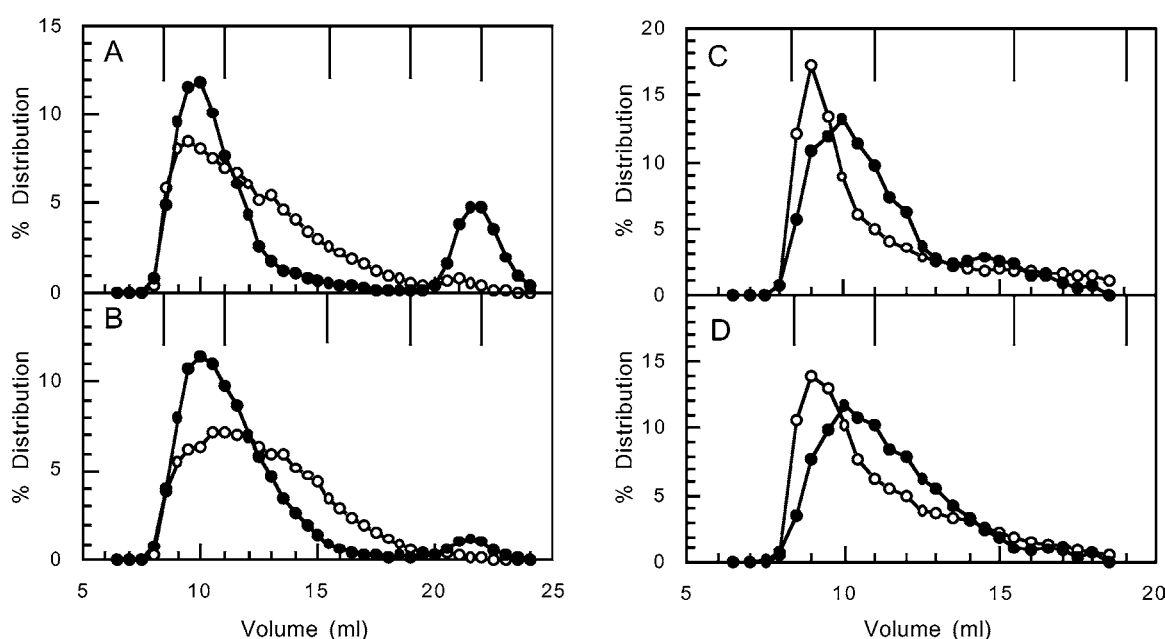

Figure 1. Molecular size distribution of polysaccharides extracted from EIR of onions supplied with 2 mequiv L^{-1} S fertility, first with 50 mM NaOAc, pH 6.5, containing 50 mM CDTA (●), followed by 50 mM Na$_2$CO$_3$ containing 20 mM NaBH$_4$ (○). Extracts were assayed for uronic acids (**A**, PLK; **B**, Grano) and total carbohydrates (**C**, PLK; **D**, Grano). Bars at the top of each graph indicate the elution positions of dextran size markers in the order 5 × 10^3, 500, 73, and 9.3 kDa, with glucose the last marker in **A** and **B** only.

Table 7. Correlation Matrix for Nonstructural and Structural Carbohydrates in Onions (Standardized Quantitative Data)

	fructan	cellulose	pectin	CDTA	carbonate	glucose	fructose	sucrose	rhamnose	arabinose	xylose	mannose	galactose
fructan	1.000												
cellulose	0.443	1.000											
pectin	−0.116	0.503	1.000										
CDTA	**−0.785**	−0.120	0.307	1.000									
carbonate	**0.894**	0.603	0.134	−0.656	1.000								
glucose	−0.339	−0.413	−0.617	0.266	−0.503	1.000							
fructose	**−0.987**	−0.489	−0.117	**0.768**	**−0.919**	0.466	1.000						
sucrose	**0.957**	0.531	−0.008	**−0.739**	**0.897**	−0.427	**−0.961**	1.000					
rhamnose	0.637	0.518	0.169	−0.314	**0.722**	−0.269	−0.664	0.641	1.000				
arabinose	0.588	0.384	0.147	−0.163	0.674	−0.358	−0.618	0.614	**0.815**	1.000			
xylose	0.601	0.496	0.245	−0.182	**0.722**	−0.416	−0.647	0.639	**0.873**	**0.959**	1.000		
mannose	0.361	0.434	0.296	0.115	0.530	−0.281	−0.400	0.403	0.680	**0.878**	**0.905**	1.000	
galactose	**0.833**	0.554	0.117	−0.476	**0.889**	−0.406	**−0.856**	0.845	0.766	0.775	**0.814**	0.650	1.000

nonstructural and structural components (particularly fructan, fructose, sucrose, Na$_2$CO$_3$-soluble pectin, and galactose). Several correlations with lower but strong coefficients (0.7 ≤ |r| ≤ 0.8) also existed. Principal component analysis confirms these

relationships (data not shown). One principal component, accounting for 56% of the variation, contrasts fructose levels with fructan, sucrose, Na$_2$CO$_3$-soluble polyuronide, rhamnose, arabinose, xylose, and galactose levels. This principal component

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grouping indicates that these components tend to be aligned, and shift in the same way in onion cultivars.

DISCUSSION

PLK is the mainstay of the New Zealand domestic and export market and is a brown globe-type onion, derived from long-term grower selection of Spanish lines in New Zealand volanic soil conditions. It is used as a "fresh" onion, as oppposed to a dehydrator type. PLK has a hard texture, is highly pungent, and can be stored for exceptionally long periods—a valuable attribute for export from New Zealand to Japan and Europe. In contrast, the cultivar Houston Grano is of North American origin and is softer textured, with a milder, sweeter flavor and lower dry matter content than PLK, typical of a salad-type fresh onion, although not grown widely for this purpose in New Zealand. Its long-term storage characteristics are poor, being more susceptible to postharvest rots and sprouting than PLK.

Response to Sulfur Supply. Field, hydroponic, and tissue culture trials worldwide (e.g., refs *14*, *17*, *18*, and *33−35*) have shown that manipulating the sulfur content of onions by varying sulfur supply during growth has mixed results, highly dependent on the germplasm type and the extent of variation of sulfur supply, and perhaps other seasonal and environmental influences as well. Our work supports the view that there is a cultivar-dependent "window of response" to sulfur fertility as hydroponically grown PLK and Grano significantly increased sulfur content in response to changes in sulfur supply between 0.5 and 2 mequiv L^{-1} only. An upper limit in sulfur uptake of field-grown spring onions was also noted in response to increasing sulfur fertility (*35*). Grano bulbs required higher sulfur supply to achieve a sulfur content similar to that of PLK, suggesting fundamental differences in their sulfur uptake mechanisms.

Although varying sulfur supply during bulb growth clearly affected the amount of sulfur taken up by bulbs in our trial, it had no effect on bulb weight or dry matter accumulation and no consistent influence on the cell wall-related components of dry matter. For many cultivars and new germplasm tested (e.g., refs *17* and *33−35*), there appears to be a sulfur fertility range within which dry matter accumulation may be influenced, although typically high and low dry matter-type onions respond differently. Our data further underscore a fundamental genetic difference between cultivars that are designated high/low pungency and high/low dry matter, because even if sulfur contents are equivalent or higher for the "mild" Grano bulbs (e.g., at 2 and 4 mequiv L^{-1} treatments) compared to pungent PLK (at 0.5 mequiv L^{-1}), dry matter contents are still distinctively different.

One of our aims was to examine the nature of cell wall accumulation influenced by sulfur fertility, as indicated by Lancaster et al. (*14*) where a variation of 100−300 mg of cell wall material g^{-1} of dry weight was found when sulfur supply to PLK or Grano bulbs increased from 0.5 to 4 mequiv L^{-1}. Surprisingly, although we confirmed tissue sulfur content was altered, indicating that the sulfur fertility treatments had been successfully applied, there was no indication that quantities of whole cell wall material or any of the cell wall polysaccharide fractions we isolated had been affected in either cultivar to the extent found in the earlier work. Factors such as slight genetic differences in the germplasm used (PLK is open-pollinated and prone to some seasonal variation) or differences in tissue preparation (and hence solubilization) for carbohydrate analysis could have contributed to this, although our EIR isolation procedure was chosen to retain as much cell wall material as possible.

Cultivar Differences for Nonstructural Carbohydrates. PLK and Grano were clearly different in the quantity and composition of nonstructural carbohydrates, with PLK containing much higher dry matter including higher proportions of fructan and lower levels of fructose than Grano, in agreement with similar trends found for onions of similar dry matter composition (e.g., refs *2*, *5*, *8*, and *36*). Whereas the total dry matter contents per bulb were similar for PLK and Grano, the higher amount of water per Grano bulb is likely to be linked to the elevated levels of mono- and disaccharides and lower quantities of fructan present compared to PLK, creating higher osmotic potential during the bulbing growth phase (*2*, *36*). Fructans accumulate over the entire bulbing period in high dry matter onions, whereas accumulation is restricted to a shorter period in low dry matter types (*37*). Jaime et al. (*8*) raise the possibility that high dry matter onions become so because they can (or are "genetically or enviromentally forced" to) generate fructans of high DP, thereby reducing osmotic potential and limiting water uptake. Our results suggest the ability to make high DP fructans may not necessarily be the key factor in dry matter accumulation, because the majority of Grano's fructans are DP >8, but rather that the efficiency of the first committed step to incorporate osmotically active sugars into less osmotically active oligomers could determine the high dry matter phenotype, because PLK is richer in fructans of the range DP 3−5. Fructans are used by the plant as an energy store, and it is thought that fructan hydrolysis supports subsequent sprout growth in mature onion bulbs. Distinct fructan size distributions have previously been found in onions with a wide range of dry matters (*3*, *5*, *6*, *8*). These differences suggest there may be genetically programmed advantages for specific patterns of fructan synthesis affecting later fructan hydrolysis to control the ability and timing of the onion to generate and grow new sprouts. This could subsequently influence storage quality of onion cultivars.

Cultivar Differences for Structural Carbohydrates. There are cultivar-related differences in structural polysaccharides of PLK and Grano, although these polysaccharides represent a smaller fraction of the dry matter than the nonstructural carbohydrates, especially in PLK. The main differences center on the CDTA- and Na_2CO_3-soluble fractions of pectin, in relative quantity, size, and neutral sugar composition (especially galactose) indicating potential differences in branching patterns. Pectins are found in the primary cell wall and middle lamella regions as matrix polysaccharides. They have a cementing role, enabling cells to adhere to each other and contribute to the mechanical strength and porosity of the cell wall. Previous comparisons of cell wall characteristics of onions of different dry matter contents and usage (*12*, *13*) did not uncover such distinct differences, although pectin fractions were not reported. The bulbing phase of onion growth is a period of intense water uptake and organ expansion. Cell walls of growing plants respond to the increasing turgor pressure and subsequent stress this places on the wall by specific and controlled relaxation of load-bearing polymers (*38*) while maintaining mechanical strength. Cell wall synthesis may also be occurring. The contribution of pectins to cell wall loosening as cells expand has not been fully explored, although it is assumed they play some role (*39*, *40*); the cellulose network and the tethering xyloglucans are usually more associated with active wall extension (*38*). The differences in bulb size and water content for PLK and Grano suggest that the cell walls in these cultivars have to adapt differently to accommodate this growth period, in relation to the osmotic potential of the tissue (likely to be

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higher for Grano than for PLK). Indeed, principal component analysis and correlation analysis show that for PLK and Grano onion cultivars, the quantities of fructose and fructan present were associated with Na_2CO_3-soluble pectin and several neutral sugars, including galactose, suggesting that cell wall composition is tailored to the requirements generated by fructan or fructose presence. However, study of an extended range of cultivars would be needed to determine if these relationships broadly exist for the wider onion population. It is not fully understood how pectins that are linked through calcium, and those that are branched, physically contribute to aspects of wall strength and wall flexibility, although immunolocalization studies have found that galactan branches of pectin and unbranched pectins of differing esterification states are located in specific parts of the wall, implying very precise roles (*40–42*). The differences in the amounts and neutral sugar composition of CDTA-soluble and Na_2CO_3-soluble pectins in PLK and Grano may arise at synthesis or from postsynthetic modifications in the wall in response to expansion needs. Other genetic factors apart from response to dry matter accumulation (especially mono- and disaccharides) may also drive differences in cell wall characteristics.

ABBREVIATIONS USED

CDTA, *trans*-1,2-diaminocyclohexane-*N,N,N′,N′*-tetraacetic acid; DP, degree of polymerization; EIR, ethanol-insoluble residue; HPLC, high-performance liquid chromatography; PLK, Pukekohe Longkeeper.

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LITERATURE CITED

(1) Galmarini, C. R.; Goldman, I. L.; Havey, M. J. Genetic analyses of correlated solids, flavor, and health-enhancing traits in onion (*Allium cepa* L.). *Mol. Genet. Genomics* **2001**, *265*, 543–551.

(2) Sinclair, P. J.; Blakeney, A. B.; Barlow, E. W. R. Relationships between bulb dry matter content, soluble solids concentration and non-structural carbohydrate composition in the onion (*Allium cepa*). *J. Sci. Food Agric.* **1995**, *69*, 203–209.

(3) Darbyshire, B.; Henry, R. J. Differences in fructan content and synthesis in some *Allium* species. *New Phytol.* **1981**, *87*, 249–256.

(4) Rutherford, P. P.; Whittle, R. The carbohydrate composition of onions during long-term cold storage. *J. Hortic. Sci.* **1982**, *57*, 349–356.

(5) Suzuki, M.; Cutcliffe, J. A. Fructans in onion bulbs in relation to storage life. *Can. J. Plant Sci.* **1989**, *69*, 1327–1333.

(6) Darbyshire, B.; Henry, R. J. The association of frutans with high percentage dry weight in onion cultivars suitable for dehydrating. *J. Sci. Food Agric.* **1979**, *30*, 1035–1038.

(7) Jaime, L.; Martínez, F.; Martín-Cabrejas, M. A.; Mollá, E.; López-Andréu, F. J.; Waldron, K. W.; Esteban, R. M. Study of total fructan and fructooligosaccharide content in different onion tissues. *J. Sci. Food Agric.* **2000**, *81*, 177–182.

(8) Jaime, L.; Martín-Cabrejas, M. A.; Mollá, E.; López-Andréu, F. J.; Esteban, R. M. Effect of storage on fructan and fructo-oligosaccharide of onion (*Allium cepa* L.). *J. Agric. Food Chem.* **2001**, *49*, 982–988.

(9) Shiomi, N. Properties of fructosyltransferases involved in the synthesis of fructan in liliaceous plants. *J. Plant Physiol.* **1989**, *134*, 151–155.

(10) Ernst, M. K.; Chatterton, N. J.; Harrison, P. A.; Matitschka, G. Characterization of fructan oligomers from species of the genus *Allium* L. *J. Plant Physiol.* **1998**, *153*, 53–60.

(11) Redgwell, R. J.; Selvendran, R, R. Structural features of cell-wall polysaccharides of onion *Allium cepa. Carbohydr. Res.* **1986**, *157*, 183–199.

(12) Ng, A.; Smith, A. C.; Waldron, K. W. Effect of tissue type and variety on cell wall chemistry of onion (*Allium cepa* L.). *Food Chem.* **1998**, *63*, 17–24.

(13) Jaime, L.; Mollá, E.; Fernández, A.; Martín-Cabrejas, M. A.; López-Andréu, F. J.; Esteban, R. M. Structural carbohydrate differences and potential source of dietary fiber of onion (*Allium cepa* L.) tissues. *J. Agric. Food Chem.* **2002**, *50*, 122–128.

(14) Lancaster, J. E.; Farrant, J.; Shaw, M. L. Sulfur nutrition affects cellular sulfur, dry weight distribution, and bulb quality in onion. *J. Am. Soc. Hortic. Sci.* **2001**, *126*, 164–168.

(15) Randle, W. M.; Lancaster, J. E.; Shaw, M. L.; Sutton, K. H.; Bussard, M. L. S fertility affects flavor precursors and precursor intermediates in onion. *J. Am. Soc. Hortic. Sci.* **1995**, *120*, 1075–1081.

(16) Lancaster, J. E.; Farrant, J.; Shaw, M. L. Effect of sulfur supply on alliinase, the flavor generating enzyme in onions. *J. Food Biochem.* **2000**, *24*, 353–361.

(17) Randle, W. M. Sulfur nutrition affects nonstructural water-soluble carbohydrates in onion germplasm. *HortScience* **1992**, *27*, 52–55.

(18) Randle, W. M.; Bussard, M. L. Pungency and sugars of short-day onions as affected by sulfur nutrition. *J. Am. Soc. Hortic. Sci.* **1993**, *118*, 766–770.

(19) McCallum, J. A.; Grant, D. G.; McCartney, E. P.; Scheffer, J.; Shaw, M. L.; Butler, R. C. Genotypic and environmental variation in bulb composition of New Zealand adapted onion (*Allium cepa*) germplasm. *N. Z. J. Crop Hortic. Sci.* **2001**, *29*, 149–158.

(20) McCallum, J. A.; Pither-Joyce, M.; Shaw, M. Sulfur deprivation and genotype affect gene expression and metabolism of onion roots. *J. Am. Soc. Hortic. Sci.* **2002**, *127*, 583–589.

(21) Hoagland, D. R.; Arnon, D. I. The water culture method for growing plants without soil. *Calif. Agric. Exp. Stn. Circ.* **1950**, *No. 347*.

(22) O'Donoghue, E. M.; Somerfield, S. D.; Heyes, J. A. Organization of cell walls in *Sandersonia aurantiaca* floral tissue. *J. Exp. Bot.* **2002**, *53*, 513–523.

(23) Huber, D. J. The inactivation of pectin depolymerase associated with isolated tomato fruit cell wall: implications for the analysis of pectin solubility and molecular weight. *Physiol. Plant.* **1992**, *86*, 25–32.

(24) Sims, I. M.; Cairns, A. J.; Furneaux, R. H. Structure of fructans from excised leaves of New Zealand flax. *Phytochemistry* **2001**, *57*, 661–668.

(25) Blakeney, A. B.; Harris, P. J.; Henry, R. J.; Stone, B. A. A simple and rapid preparation of alditol acetates for monosaccharide analysis. *Carbohydr. Res.* **1983**, *113*, 291–299.

(26) Updegraff, D. M. Semimicro determination of cellulose in biological material. *Anal. Biochem.* **1969**, *32*, 420–424.

(27) Dische, Z. Qualitative and quantitative colorimetric determination of heptoses. *J. Biol. Chem.* **1953**, *204*, 983–997.

(28) Ahmed, A. E.; Labavitch, J. M. A simplified method for accurate determination of cell wall uronide content. *J. Food Biochem.* **1977**, *1*, 361–365.

(29) Blumenkrantz, N.; Asboe-Hansen, G. New method of quantitative determination of uronic acids. *Anal. Biochem.* **1973**, *54*, 484–489.

(30) Dubois, M. K. A.; Hamilton, J. K.; Rebers, P. A.; Smith, F. Colorimetric method for determination of sugars and related substances. *Anal. Chem.* **1956**, *28*, 350–356.

(31) *Genstat 6.1 Committee, Reference Manual*; VSN International: Oxford, U.K., 2002.

(32) Corrigan, V. K.; Irving, D. E.; Potter, J. F. Sugars and sweetness in buttercup squash. *Food Qual. Prefer.* **2000**, *11*, 313–322.

(33) Bakr, A. A.; Gawish, R. A. Current studies on onion and its pungency II. Interactive effects of sulfur fertilization and drying under the aspect of solar energy utilization. *Nahrung* **1998**, *42*, 94–101.

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O'Donoghue et al.

(34) Hamilton, E. K.; Yoo, K.-S.; Pike, L. M. Changes in pungency of onions by soil type, sulphur nutrition and bulb maturity. *Sci. Hortic.* **1998**, *74*, 249−256.

(35) Abbey, L.; Joyce, D. C.; Aked, J.; Smith, B. Genotype, sulphur nutrition and soil type effects on growth and dry-matter production of spring onion. *J. Hortic. Sci. Biotechnol.* **2002**, *77*, 340−345.

(36) Sinclair, P. J.; Neeson, R. J.; Barlow, E. W. R. Osmotic potential and soluble solids concentration in onion (*Allium cepa*) bulbs. *J. Sci. Food Agric.* **1995**, *69*, 211−214.

(37) Kahane, R.; Vialle-Guerin, E.; Boukema, I.; Tzanoudakis, B.; Bellamy, C.; Chamauz, C.; Kik, C. Changes in non-structural carbohydrate composition during bulbing in sweet and high-solid onions in field experiments. *Environ. Exp. Bot.* **2001**, *45*, 73−83.

(38) Cosgrove, D. J. Enzymes and other agents that enhance cell wall extensibility. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **1999**, *50*, 391−417.

(39) Darley, C. P.; Forrester, A. M.; McQueen-Mason, S. J. The molecular basis of plant cell wall extension. *Plant Mol. Biol.* **2001**, *47*, 179−195.

(40) Willats, W. G. T.; McCartney, L.; Mackie, W.; Knox, J. P. Pectin: cell biology and prospects for functional analysis. *Plant Mol. Biol.* **2001**, *47*, 9−27.

(41) Jones, L.; Seymour, G. B.; Knox, J. P. Localization of pectic galactan in tomato cell walls using a monoclonal antibody specific to (1→4)-β-D-galactan. *Plant Physiol.* **1997**, *113*, 1405−1412.

(42) Willats, W. G. T.; Gilmartin, P. M.; Mikkelsen, J. D.; Knox, J. P. Cell wall antibodies without immunization: generation and use of de-esterified homogalaacturonan block-specific antibodies from a naïve phage display library. *Plant J.* **1999**, *18*, 57−65.