Sulfur Nutrition Affects Cellular Sulfur, Dry Weight Distribution, and Bulb Quality in Onion

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ABSTRACT. Three onion (*Allium cepa* L.) cultivars, 'Southport White Globe', 'Grano', and 'Pukekohe Longkeeper' were grown at low to high S (at 0.5, 1.8, 3.0 or 4.0 meq·L⁻¹) in hydroponic culture. Differential solvent extractions of bulbs were used to isolate quantitatively cell contents, cell wall proteins, and cell wall residue. The weight of the cell fractions, their S content, and the S content of intact bulbs were determined. Bulb characteristics of fresh weight (FW), firmness, soluble solids concentration (SSC), and soluble sugars were also determined. For all three cultivars, bulb FW increased with S from 0.5 to 4.0 meq·L⁻¹. Sulfur had a significant effect on bulb firmness. Onion bulbs grown with S at 0.5 meq·L⁻¹, the lowest S concentration, were significantly softer than onion bulbs grown at the highest concentration of 4.0 meq·L⁻¹. Varying the S supply had a major effect on dry weight (DW) allocation to the cell wall residue. Bulbs of all three cultivars grown at the lowest S had significantly less DW in the cell walls compared to S at 3.0 or 4.0 meq·L⁻¹. In contrast to the effect of S supply on DW allocation, varying S supply had no effect on total bulb S, free SO₄²⁻, and on the S content of the cell contents and the cell wall residue and only a minor effect on cell wall proteins. There was no significant effect of S supply on either SSC or soluble sugars. At low S nutrition, which is limiting to the growth of onion bulbs, cell wall deposition is reduced, with a consequent decrease in bulb firmness. The S composition of the cellular components is maintained at the expense of bulb growth.

A distinguishing feature of the 500 or so *Allium* L. sp. is their unique metabolic network of organic S compounds. The edible *Allium* sp., onion and garlic, also have a higher S content than many other plant species (Nielson et al., 1991). Roots actively take up S, mainly as SO₄²⁻ (Mengel and Kirkby, 1982). The SO₄²⁻ is translocated to the leaves and reduced in chloroplasts to organic forms (Andersen, 1980). In *Allium* sp., the S-alk(en)yl cysteine sulfoxides (ACSOs), give rise to numerous volatile S compounds which have caused *Allium* sp. to be valued as herbal medicines and as a food. Intact *Allium* cells have no odor, but when cells are disrupted, the enzyme alliinase (EC 4.4.1.4) hydrolyzes the ACSOs to produce pyruvate, ammonia, and the many volatile S compounds associated with flavor (Lancaster and Boland, 1990).

The partitioning of S to the ACSOs and its consequent effect on flavor has been shown to be affected by S supply (Randle et al., 1995). They found that at low S supply there was a reduction in some ACSOs and a consequent reduction in flavor. Despite this reduction in flavor at low S supply, nearly 95% of the total bulb S could be attributed to ACSOs and their biosynthetic intermediates. Therefore, onions preferentially store S in the form of ACSOs even when S levels are limiting. Consequently, at low S supply S-containing compounds other than ACSOs and S-dependent cellular processes will have a greatly reduced S supply.

Sulfur is also incorporated into glutathione, into the biosynthetic intermediates to ACSOs, into proteins via amino acids cysteine and methionine, and into cell walls as sulfated polysaccharides (Chiovitti et al., 1998; Lancaster and Boland, 1990). It is commonly stored as SO₄²⁻ in the vacuole in most plant cells (Mengel and Kirkby, 1982). This SO₄²⁻ is mobile within the plant

and is redistributed extensively from mature leaves to new, developing leaves (Sunarpi and Andersen, 1996).

The following research was undertaken to determine the effect of S supply on S partitioning within onion bulb cells, and its subsequent effect on bulb quality attributes other than flavor. Onion bulbs were separated into soluble cell contents, including free SO₄²⁻, cell wall proteins, and insoluble cell wall material using differential solvent extractions. The effect of S supply and cultivar on dry weight (DW) and S content of each of these cell fractions was determined and also their effect on the quality attributes of bulb weight, bulb firmness, and soluble sugars.

Materials and Methods

PLANT MATERIAL. Three onion cultivars were used that represent a range of germplasm: 'Southport White Globe', a pungent, firm, high dry matter onion; 'Grano', a mild, soft, low dry matter onion; and 'Pukekohe Longkeeper', a pungent, firm onion, of dry matter intermediate between the other two cultivars.

PLANT CULTURE. Bulbs were grown in hydroponic culture (October 1997 to January 1998) in a glasshouse in Christchurch, New Zealand, latitude 43 °S (Randle et al., 1995). No auxiliary lighting was used. Days/nights were 20–25 °C/4–10 °C. Daylength was 12 h in October, increasing to 14 h in January. There were six replicate containers for each S treatment, and these were planted with three bulbs of each of three cultivars, thus giving a split-plot design.

Sulfur treatments were arranged in six randomized blocks. Seeds were germinated in early October 1997 in 25 mm cubes of artificial medium (Oasis Horticultural Foam, 9100 LC Thin Cut; Smithers-Oasis, Kent, Ohio.). At the flag leaf stage the cubes were transferred to 2-cm holes in the lids of 20 L containers, nine plants per container. The containers held 18 L of nutrient solution (Hoagland and Arnon, 1950; Randle et al., 1995), varied for S levels, by adjusting the MgSO₄ to MgCl₂ ratio to obtain solutions with S at 0.5, 1.8, 3.0, or 4.0 meq·L⁻¹. Only the SO₄²⁻ and Cl⁻ levels varied, and all other nutrients were kept constant between treat-

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ments. Containers were aerated continuously with aquarium pumps and sandstone bubblers. Solutions were replaced completely every 2 weeks, and water levels were maintained daily with deionized water during the 2 weeks. Plants were grown to maturity as indicated by pseudostem softening and foliar lodging. Bulbs were harvested in early Jan. 1998, cured for 1 month, and weighed.

BULB QUALITY MEASUREMENTS. Bulb firmness of each onion was determined by compression using a Materials Testing System (Instron, Canton, Mass.). A 6 cm wide flat head probe was used with a cross head speed of 200 mm·min⁻¹ and a 1.5 mm compression distance. Results are reported in N (force × compression distance).

Soluble solids concentration (SSC) were determined on juice, expressed with a garlic press, with a hand-held refractometer.

CELLULAR DISTRIBUTION OF DW AND S. Quadrants from each of the three bulbs per cultivar per container were combined, freeze dried, and weighed. A second set of quadrants were used for solvent extraction. Solvent extraction procedures were used to separate soluble cell contents from cell wall proteins and insoluble cell wall residue quantitatively. Quadrants from each of the three bulbs per cultivar per container were combined, weighed, and extracted in 12 methanol : 5 chloroform : 3 water (by volume) (Lancaster and Kelly, 1983). Phases were separated by addition of 4.5 mL water and 5.5 mL chloroform for every 10 mL extract. The lower, chloroform phase containing lipids was discarded. The methanol/water phase, containing free SO₄²⁻, sugars, amino acids, ACSOs, and their biosynthetic intermediates was freeze dried and weighed.

The tissue residue was air dried and then ground, in a blender (Virtis, Gardiner, N.Y.) in 150 mL 2 phenol : 1 acetic acid : 1 water (PAW) (by volume) to extract intracellular proteins and glycoproteins (Grant et al., 1992; Redgwell et al., 1990). Samples were stirred overnight in the PAW and filtered through Whatman 1 filter paper. The residue was reextracted in 150 mL PAW for 4 h, followed by washing in deionized water to remove any residual PAW. The combined filtrates were dialyzed exhaustively over 4 d against deionized water using dialysis tubing in a 10 L bucket with 10 changes of water. The protein precipitate that formed in the tubing was collected by filtration. The filtrate was reduced in volume under vacuum to recover further protein and combined precipitates were freeze dried and weighed. The tissue residue from the PAW/H₂O extractions was washed with deionized water, freeze dried, and weighed. Freeze dried intact bulb tissue, and the freeze-dried cell components were ground to a powder and analyzed for S content utilizing a Leco S analyzer (Leco Corp., St Joseph, Mich.).

ANALYSIS OF SUGARS. One gram DW of the freeze-dried methanol/water extract of bulb (see above) was used for analysis of sugars by high-performance liquid chromatography (HPLC). The dried material was dissolved in 1 mL water, centrifuged at 14,000 g_n for 15 min and analyzed for free glucose, fructose, and sucrose by HPLC. Fructan levels were not determined. A liquid chromatograph (Waters, Milford, Mass.) consisting of a model 626 pump and controller, model 717 plus autosampler and a model 410 refractive index detector were used. The detector signal (output at attenuation setting 64) was stored, integrated, and manipulated using Waters 'Millennium32' software. Sugars were separated with a 220 × 4.6 mm Applied Biosystems (Applied Biosystems Inc., San Jose, Calif.) Brownlee AMINO column fitted with a 15 × 3.2 mm Applied Biosystems Brownlee NewGuard AMINO guard column at 30 °C. The mobile phase was HPLC-grade 82.5 acetonitrile : 17.5 water (v/v). Solvent flow rate was 1.5 mL·min⁻¹. Injection volume for both sugar standards and onion extracts was 10 mL.

Identification of each sugar was based on HPLC retention times. Detector response to all sugars was linear over the concentration

range 0 to 2 mg·mL⁻¹. Standard sugars exhibited <2% variability in individual sugar concentrations between triplicate injections of the same sample.

Determination of free SO₄²⁻. Freeze-dried methanol/water extract of bulbs was redissolved in deionized water to about 2 mg·mL⁻¹. The samples were centrifuged at 14,000 g_n for 15 min before electrophoresis. Capillary ion analysis was carried out on a capillary electrophoresis system (Biofocus 3000; Bio-Rad, Hercules, Calif.) fitted with a standard cartridge containing an uncoated 36 cm × 50 mm i.d. fused silica capillary. A separation voltage of 20 kV negative to positive and a background electrolyte containing 2.25 mM pyromellitic acid, 6.5 mM NaOH, 0.75 mM hexamethonium hydroxide, and 1.6 mM triethanolamine, pH 7.5 were used. Indirect ultra-violet detection was carried out at 250 nm. A sample was introduced onto the column by pressure injection at 351.5 g·cm⁻²·s⁻¹. Column and carousel temperature were set at 20 °C. Sulfate standards showed a linear response over the concentration range of 1 to 25 mg·L⁻¹. Sulfate is reported as percentage (g/100 g) initial freeze dried material.

STATISTICAL ANALYSIS. Data were subjected to analysis of variance procedures and LSDs were calculated for mean separation. LSDs to compare between S levels are based on a weighted sum of the between and within plot variances. Satterthwaites method was used to obtain associated df in a similar manner. All analyses was carried out with Genstat 5 release 4.1 (Genstat 5 committee, 1998, Genstat 5 Release 4.1 Reference Manual Supplement).

Results

BULB FRESH WEIGHT (FW). There were significant differences ($P = 0.05$) in the FW of bulbs of the three cultivars (Fig. 1). 'Grano' bulbs were the heaviest with a mean weight of 187 g followed by 'Pukekohe Longkeeper' at 103 g and 'Southport White Globe' at 64 g. There was a trend for bulb FW to increase with S supply from 0.5 to 4.0 meq·L⁻¹. 'Southport White Globe' increased by 27%, 'Grano' by 22%, and 'Pukekohe Longkeeper' by 114% from the lowest to the highest S concentration for all three cultivars. However, this increase was significant ($P = 0.05$) only for 'Pukekohe Longkeeper'. There was no significant S level × cultivar interaction.

BULB FIRMNESS. Bulb firmness differed significantly with culti-

Fig. 1. Effect of hydroponic solution concentrations of S at 0.5, 1.0, 3.0, or 4.0 meq·L⁻¹ on bulb FW (g) for 'Southport White Globe' (SPWG), 'Grano', and 'Pukekohe Longkeeper' (PLK) onions. Vertical bars represent LSDs ($P = 0.05$): (a) is the LSD (25.1, df = 52) for comparison of means at varying levels of S and (b) is the LSD (24.3, df = 40) for comparison of means at the same level of S.

Fig. 2. Effect of hydroponic solution concentrations of S at 0.5, 1.0, 3.0, or 4.0 meq·L⁻¹ on bulb firmness for 'Southport White Globe' (SPWG), 'Grano' and 'Pukekohe Longkeeper' (PLK) onions. Vertical bars represent LSDs ($P = 0.05$): (a) is the LSD (11.4, df = 47) for comparison of means at varying levels of S and (b) is the LSD (10.3, df = 40) for comparison of means at the same level of S.

var (Fig. 2). For overall cultivar means, 'Grano' bulbs were softest at 124 N, whereas 'Pukekohe Longkeeper' and 'Southport White Globe' onions were significantly firmer at 196 and 192 N, respectively.

The S supply had a significant effect ($P = 0.05$) on bulb firmness. 'Pukekohe Longkeeper' and 'Grano' onions increased in firmness between the S levels of 0.5 and 3.0 meq·L⁻¹. This difference was

significant ($P = 0.05$) for 'Pukekohe Longkeeper' onions. Firmness at a S level of 4.0 meq·L⁻¹ was similar to that at 3.0. 'Southport White Globe' onions increased in firmness between the lowest S concentration of 0.5 and 1.8 meq·L⁻¹. However, subsequent S concentrations did not produce a significant increase in bulb firmness. There was no significant S level $\times$ cultivar interaction.

BULB SUGARS. All cultivars showed significant differences in SSC. 'Southport White Globe' onions had the highest SSC of 17.4%, 'Pukekohe Longkeeper' onions had 12.1% SSC, and 'Grano' bulbs had a SSC of 8.9% (Table 1). There were also significant cultivar differences in fructose, glucose, and sucrose levels (Table 1). 'Grano' bulbs had the highest fructose and glucose levels. 'Southport White Globe' onions had the lowest fructose and glucose levels. Sucrose levels in 'Grano' were similar to those of 'Southport White Globe' (64.4 mg·g⁻¹). 'Pukekohe Longkeeper' onions had fructose and glucose levels intermediate between those of the other two cultivars and a higher sucrose level of 116.5 mg·g⁻¹. 'Southport White Globe' and 'Pukekohe Longkeeper' onions contain fructan polymers in addition to the free sugars. These fructans contribute to the higher dry matter and SSC of these cultivars.

However, there was no significant effect of S supply on either SSC or sugars, and no significant cultivar $\times$ S interactions (data not presented).

BULB TOTAL S CONTENT. There were significant cultivar differences in total bulb S content (Fig. 3). Bulb S content for overall cultivar means was highest for 'Southport White Globe' (0.66%), and for 'Pukekohe Longkeeper' (0.65%) and lower for 'Grano' (0.57%). There was no significant effect of S supply on total bulb S

Table 2. Percentage distribution of DW and percentage S in cell contents (C), cell wall proteins (P), residual cell walls (W), and percentage free SO₄²⁻ for three onion cultivars ['Southport White Globe' (SPWG), 'Grano', and 'Pukekohe Longkeeper' (PLK)] grown hydroponically at four S levels (0.5, 1.8, 3.0 or 4.0 meq·L⁻¹). LSD at $P = 0.05$.

Cultivar	S concn (meq·L ⁻¹)	Dry wt (%)			% S of component			Free SO ₄ ²⁻ (%)
		C	P	W	C	P	W	
SPWG	0.5	88.1	2.5	9.3	1.03	1.34	0.044	4.8
	1.8	75.3	1.4	23.3	0.99	1.32	0.091	4.3
	3.0	76.4	2.0	21.7	1.01	1.14	0.092	9.1
	4.0	66.0	8.0	26.1	1.03	0.33	0.113	5.5
Grano	0.5	78.9	1.1	20.1	0.72	1.12	0.075	9.7
	1.8	75.7	1.0	23.2	0.71	1.07	0.058	9.7
	3.0	70.2	0.5	29.4	0.73	1.05	0.047	8.5
	4.0	63.9	3.9	32.2	0.71	0.61	0.091	10.1
PLK	0.5	78.9	1.9	19.3	0.87	1.31	0.074	8.3
	1.8	87.9	1.8	10.3	0.82	1.25	0.074	8.7
	3.0	77.3	0.7	22.1	0.89	1.37	0.079	11.2
	4.0	55.7	1.5	42.8	0.80	0.76	0.032	11.8
LSD ^z (df) ^y		7.7(41)	1.7(39)	7.2(45)	0.19(39)	0.23(53)	0.033(43)	5.0(38)
LSD ^x (df = 38)		6.6	1.4	6.5	0.15	0.24	0.029	4.1
Mean S over cultivar	0.5	81.9	1.8	16.2	0.87	1.26	0.064	7.6
	1.8	79.6	1.4	19.0	0.84	1.21	0.075	7.6
	3.0	74.6	1.0	24.4	0.88	1.19	0.073	9.6
	4.0	61.9	4.5	33.7	0.85	0.57	0.079	9.5
LSD (df = 15)		5.9	1.3	5.0	0.15	0.12	0.025	3.9
Mean	SPWG	76.4	3.5	20.1	1.01	1.03	0.085	5.9
	Grano	72.2	1.6	26.2	0.72	0.96	0.068	9.5
	PLK	74.9	1.5	23.6	0.85	1.17	0.065	10.3
LSD (df = 38)		3.3	0.7	3.3	0.08	0.13	0.014	2.0

^zFor comparison of different levels of S.

^yMissing values in some columns.

^xFor comparison within the same S level.

Fig. 3. Effect of hydroponic solution concentrations of S at 0.5, 1.0, 3.0, or 4.0 meq·L⁻¹ on bulb S concentration for bulbs of 'Southport White Globe' (SPWG), 'Grano', and 'Pukekohe Longkeeper' (PLK) onions. Vertical bars represent LSDs ($P = 0.05$): (a) is the LSD (0.11, $df = 54$) for comparison of means at varying levels of S and (b) is the LSD (0.12, $df = 40$) for comparison of means at the same level of S.

content and no significant S level $\times$ cultivar interaction.

CELLULAR DISTRIBUTION OF DW. For all cultivars the highest percentage DW was in the cellular components of sugars, polysaccharides, proteins, ACSOs, and their intermediates and free SO_4^{2-} (Table 2). (The lipid component was discarded.) 'Southport White Globe' contained 76.4% of the cell DW as cell contents, followed by 'Pukekohe Longkeeper' at 74.9% and 'Grano' at 72.2%. The insoluble cell wall residue represented a substantial, but lesser, component of cell DW. For this cellular component 'Southport White Globe' contained 20.1%, compared to 'Pukekohe Longkeeper' at 23.6% and 'Grano' at 26.2%. The cell wall proteins represented a minor component of the DW. 'Grano' and 'Pukekohe Longkeeper' had similar cell wall DW distributions at 1.5% and 1.6%, respectively, and 'Southport White Globe' was higher at 3.5%.

Varying the S supply had a major effect on the allocation of DW to the cell fractions, and in particular the DW of the cell walls. Bulbs, averaged over three cultivars, grown at the lowest S level had significantly less DW in the cell walls (16.2%) compared to the S at 3.0 and 4.0 meq·L⁻¹ treatments (24.4% and 33.7% respectively). For each cultivar the percentage distribution of DW in the cell walls increased at S levels between 0.5 and 4.0 meq·L⁻¹. However, values for cell wall percentage DW for 'Southport White Globe' at S level of 3.0 meq·L⁻¹ and 'Pukekohe Longkeeper' at a S level of 1.8 meq·L⁻¹ were lower than would be expected from a parallel pattern of increasing percentage distribution of DW in the cell wall with S level. These lower values account for the significant S level $\times$ cultivar interaction.

The percentage distribution increases reflected actual weight changes in the cell wall residues from about 0.1 g·g⁻¹ bulb DW at the lowest S level to 0.3 g·g⁻¹ bulb DW at the highest S level. The cell wall residue at low S supply was also different qualitatively. During sample preparation for Leco analysis it was observed that the cell wall residue from the higher S supply bulbs was able to be ground to a powder in a pestle and mortar. In contrast the cell wall residues of the bulbs grown at low S supply did not fracture to a powder with grinding but remained as a fibrous sheet. This observation applied to all three cultivars.

Sulfur nutrition also had a significant effect on the DW of the cell contents. There was less DW in the cell contents at higher S levels.

There was also a significant S level $\times$ cultivar interaction, probably because of the higher value for DW percentage distribution in the cell contents for 'Pukekohe Longkeeper' at a S level of 1.8 meq·L⁻¹. The effect of S supply on cell wall proteins is less clear although the percentage DW in them was highest at the S level of 4.0 meq·L⁻¹ for all cultivars.

CELLULAR DISTRIBUTION OF S. Percentage distribution of S in the tissue fractions differed from the percentage DW distribution. The cell wall proteins had the highest S percentage. 'Pukekohe Longkeeper' had 1.17% S in cell wall proteins with 'Southport White Globe' at 1.03% S and 'Grano' at 0.96% S. Cell contents had lower S at 1.01%, 0.85%, and 0.72% for 'Southport White Globe', 'Pukekohe Longkeeper', and 'Grano', respectively. The cell wall residue had a very low S content, one tenth that of the other two cell components. 'Southport White Globe' cell walls had 0.085% S, 'Grano' 0.068%, and 'Pukekohe Longkeeper' 0.065%.

Varying the S supply had a lesser effect on the S content of the cell fractions than on DW allocation. There was no significant effect of S supply on the S percentage of cell contents, or the cell wall residue. At a S level of 3.0 or 4.0 meq·L⁻¹, the percentage S of the cell wall proteins was less.

Distribution of S as a portion of the DW was predominantly in the cell contents (percentage of total S was 52% for 'Grano', 63.6% for 'Pukekohe Longkeeper', and 77.2% for 'Southport White Globe'). 'Southport White Globe' had a total S content of 3.6%, 'Grano' 1.5%, and 'Pukekohe Longkeeper' 1.7% in the cell wall proteins. For all cultivars, total S in the cell wall residue was between 1.5% and 1.8%.

BULB FREE SO_4^{2-} . Direct measurement by capillary electrophoresis of the free SO_4^{2-} component of the soluble cell contents showed significant cultivar differences in free SO_4^{2-} content (Table 2). 'Southport White Globe' had the lowest free SO_4^{2-} content at 5.9%, compared to 'Grano' and 'Pukekohe Longkeeper' at 9.5% and 10.3%, respectively.

There was no significant effect of S supply on free SO_4^{2-} content although at the lower S levels of 0.5 and 1.8 meq·L⁻¹ levels of free SO_4^{2-} were lower than at the two higher S levels (7.6% and 7.6% vs 9.5% and 9.6%). There was no significant S level $\times$ cultivar interaction.

Discussion

Results indicate that at growth limiting S supply, cell wall deposition is reduced, and onion bulb firmness is decreased. The effects were measured for 'Southport White Globe', 'Grano' and 'Pukekohe Longkeeper' onions, which represent cultivars with a range of bulb firmness from soft to hard. This is the first report of an effect of S supply on bulb firmness. Previous papers on S supply have shown that lower S supply reduces ACSOs and pungency (Freeman and Mossadeghi, 1970; Randle et al., 1995,

Table 1. Fructose, glucose, and sucrose concentration and soluble solids concentration (SSC) for onion bulbs of 'Southport White Globe' (SPWG), 'Grano' and 'Pukekohe Longkeeper' (PLK) onions ($n = 38$).

Cultivar	Fructose	Glucose	Sucrose	SSC
	(mg·g ⁻¹ soluble cell contents)			(%)
SPWG	16.2	27.8	64.4	17.4
Grano	212.8	257.2	74.1	8.9
PLK	52.2	180.3	116.5	12.1
LSD _{0.05}	9.3	8.4	6.0	0.5

1999), and alters nonstructural water-soluble carbohydrates for some onion cultivars (Randle, 1992). Effects on other quality characteristics have not been reported.

Most of the S taken up by the bulb was found in the cell contents. Less S was found in the cell wall proteins and the cell wall residue than in the cell wall contents. However, the amounts of S in the cell wall proteins and cell wall residue were similar. The cellular component most affected by a reduction in S supply was the cell wall residue. With reduced S supply there was a concomitant reduction in the DW of the cell wall residue. There was a 2.8, 1.6, and 2.2 fold reduction in cell wall residue for 'Southport White Globe', 'Grano' and 'Pukekohe Longkeeper', respectively, from the S treatments of 4.0 to 0.5 meq·L⁻¹. The cell wall residue for all three cultivars at low S supply was more fibrous and less able to be ground, compared to the higher S supply.

In contrast to the effect of S supply on DW allocation, varying S supply had no effect on total bulb percentage S, the percentage S content of the cell contents, and the cell wall residue and only a minor effect on cell wall proteins. These results show that at limiting levels of S, the onion plant responds by reducing growth and thus maintaining the S composition of the cellular components. Although the three cultivars had quantitatively different partitioning of S and DW allocation to the cell components, their response to reductions in S supply was similar qualitatively.

This apparent tendency of onions towards nutrient homeostasis has been observed in other species (Cabrera and Devereaux, 1999). Plants have the ability to compensate for resource limitations and imbalances by adjusting growth and allocation responses that increase the efficiency with which they use limiting resources (Bloom, 1986; Bloom et al., 1985; Chapin, 1991; Chapin et al., 1987).

The role of S in the development of cell walls is not well characterized. Sulfated polysaccharides have been characterized in algae (Chiovitti et al., 1998; Witvrouw et al. 1997) but not in other plant species. Studies on the effects of SO₂ from polluted air, and S dust on *Pinus contorta* Loudon x *Pinus banksiana* Lambert trees (Mayo et al., 1992) showed that S increased the levels of phenolic acid and lignin and altered cell wall elastic properties. Ferulic acid increased in grasses grown in SO₂ polluted air (Akin and Hogan, 1983; Koziol, 1991). Addition of S alone to creeping bentgrass (*Agrostis palustris* L.) grown in sand increased the DW of this grass (Hodges and Campbell, 1997). These effects of S may be via the enzymes of phenolic acid synthesis in cell walls. Further experimentation is required on S and cell walls.

Our results corroborate those of Randle et al. (1995) in showing that the cell contents, which contain the ACSOs, are the major sinks for S in onion cells. In a later study Randle et al. (1999) also showed that free SO₄⁻² and total bulb percentage S were reduced in very low S treatments. This contrasts with our results. The differences may result from the manner by which the plants are grown. Although the levels of S supplied in the nutrient solutions were comparable, in the sand culture system used by Randle et al. (1999) the S supply becomes limiting more rapidly and to a greater extent than in the hydroponic system used in the present investigation.

In our experiment, S supply did not affect total soluble solids or the sugar content of the three cultivars. Thus, changes in bulb firmness with reduced S supply were not the result of osmotic differences in the bulb. The type of sugar accumulated, i.e., glucose, fructose, sucrose, or fructans, varies depending on the dry-matter accumulation potential among cultivars (Darbyshire

and Steer, 1990). Cultivars relatively low in dry matter accumulate mostly glucose, fructose, and sucrose, while high-dry-matter cultivars accumulate mostly fructans. Randle (1992) found that some cultivars require high S supply for maximum soluble solids accumulation, while others accumulate maximum soluble solids at low S supply. Because of its high S content and the economic importance of S to pungency and firmness in bulbs, onion represents a useful plant system in which to study the effect of S on various plant physiological processes.

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