

Selenium Affects the S-alk(en)yl Cysteine Sulfoxides among Short-day Onion Cultivars

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ABSTRACT. Four cultivars of onion (*Allium cepa* L. 'Primavera', 'Granex 33', 'Pegasus', and 'Sweet Success') were grown to maturity in modified nutrient solutions with or without $2.0 \text{ mg} \cdot \text{L}^{-1} \text{Na}_2\text{SeO}_4$ ($1.51 \text{ mg} \cdot \text{L}^{-1} \text{SeO}_4^{2-}$). Selenium did not affect total flavor precursor content (ACSO) in 'Granex 33', 'Pegasus', and 'Sweet Success'. However, Se affected several individual ACSOs and precursor intermediates. Selenium decreased γ -L-glutamyl-S-(1-propenyl)-L-cysteine sulfoxide and trans(+)-S-(1-propenyl)-L-cysteine sulfoxide content in all four cultivars. (+)-S-Methyl-L-cysteine sulfoxide content was higher while (+)-S-propyl-L-cysteine sulfoxide content was lower with the added Se for two cultivars. Selenium lowered total bulb S content in all cultivars, and increased the percentage of total S accumulated as SO_4^{2-} in three cultivars. The effect of Se on the flavor pathway was similar to that found when onions were grown under low S-concentrations. Flavor changes can be expected when onions are grown in a high Se environment, however, specific changes may be cultivar dependent.

Sulfur, an important plant macroelement, and Se, a nonessential plant element, have similar oxidation states and can substitute for one another in plants. To be utilized by plants, SO_4^{2-} is absorbed by the roots, transported to the leaves, and assimilated into cysteine by a light-dependent reaction (Anderson, 1980). Cysteine can then be metabolized through a number of different plant pathways. The uptake of SO_4^{2-} is aided by a high affinity permease (Clarkson and Lüttge, 1991). When selenate (SeO_4^{2-}) is present in high concentrations, it can competitively inhibit SO_4^{2-} uptake because they compete for similar binding sites to the permease (Leggett and Epstein, 1956). Sulfate and SeO_4^{2-} also share a common uptake mechanism and are competitive substrates in the anaerobic microorganism *Clostridium pasteurianum* Winogradsky, (Bryant and Laishley, 1988). Once absorbed, SeO_4^{2-} can substitute for SO_4^{2-} in the pathways leading to amino acid and protein synthesis (Anderson and Scarf, 1983). In doing so, Se analogues of cysteine and methionine are formed. Substitution of Se-amino acids into S-proteins, however, can alter the tertiary structure of Se-substituted proteins and cause drastic changes in catalytic activity (Brown and Shrift, 1982). These catalytic changes are considered a cause of Se toxicity in plants.

Onion (*Allium cepa*) flavors are dominated by a unique group of S-compounds derived from the precursors collectively known as S-alk(en)yl-L-cysteine sulfoxides (ACSO). The flavor precursors are nonprotein S-amino acids. The starting point of the proposed onion biosynthetic flavor pathway is the cysteine containing tripeptide glutathione (Lancaster and Boland, 1990). Glutathione, may become a temporary storage compound for reduced S (Rennenberg, 1984). Depending on conditions and variety, S is then selectively metabolized through a series of peptides in the biosynthetic pathways leading to ACSO synthesis. Onions possess three of the four ACSOs identified in *Allium*, and include (+)-S-methyl-L-cysteine sulfoxide (MCSO); (+)-S-propyl-L-cysteine sulfoxide (PCSO); and trans(+)-S-(1-propenyl)-L-cysteine sulfoxide (1-PrenCSO). Two measurable intermediates leading to

ACSO synthesis are S-2-carboxypropyl glutathione (2-carb) and γ -L-glutamyl-S-(1-propenyl)-L-cysteine sulfoxide (GPECSO). Onion flavor is experienced only following cellular disruption, where the enzyme alliinase is released from the vacuole and decomposes the ACSOs to produce pyruvate, ammonium, and the S-flavor compounds (Block, 1992).

Selenium is an essential micronutrient for maintaining mammalian health. Benefits attributed to proper Se nutrition range from immune system enhancement to cancer suppression (Schrauzer and Sacher, 1994; Vandenbrandt et al., 1993). This micronutrient is required for proper glutathione peroxidase function associated with antioxidant activity in cells. However, when consumed in high quantities, Se can accumulate in tissues and become toxic (Combs and Combs, 1986). The vegetables belonging to *Allium* were determined to be an excellent delivery system for Se that suppressed mammary cancer in mice (Ip et al., 1992; Ip and Lisk, 1994). A synergistic effect of the *Allium* S-compounds with the Se in cancer suppression was also reported. Selenium-flavor analogues exist in onion and garlic (*Allium sativum* L.) (Cai et al., 1994). Moreover, the Se-enriched onion and garlic had minimal Se accumulation in body tissues which were normally found with the standard Se supplement selenomethionine. Also, these Se-enriched onions and garlic did not alter the production and function of mammalian selenoenzyme (Ip and Lisk, 1994).

Because onions are a potentially beneficial source of Se in our diet, the effects of Se on the ACSO biosynthetic pathway and flavor development needs to be understood. Therefore, this study was undertaken to determine whether or not SeO_4^{2-} would influence flavor precursor and precursor intermediate accumulation in bulb onions.

Materials and Methods

PLANT CULTURE. On 12 Dec. 1996, four short-day onion cultivars ['Primavera' (Peto Seeds, Saticoy, Calif.), 'Granex 33' (Asgrow Seeds, Kalamazoo, Mich.), 'Pegasus' (Asgrow Seeds, Kalamazoo, Mich.), 'Sweet Success' (Sun Seeds, Hollister, Calif.)] were seeded into growing cubes (Grodan A/S, Dk-2640, Hedehusene, Denmark). Fertilization was applied with $200 \text{ mg} \cdot \text{L}^{-1}$ Peter's 20N–

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8.8P–16.6K water-soluble fertilizer (Grace-Sierra Co., Malpitas, Calif.) as needed once cotyledons emerged. The seedlings were greenhouse grown (25 °C day and 20 °C night set points at 12 h for each temperature) for 5 weeks under natural photoperiods ($\approx 34^\circ$ N latitude). A broad-spectrum fungicide (Bravo 720 (chlorothalonil), Fermenta Plant Protection Co., Mentor, Ohio) was applied as needed.

Tanks ($2.74 \times 1.22 \times 0.18$ m) with prefabricated black liners were each filled with 500 L of a half-strength Hoagland's nutrient solution (Hoagland and Arnon, 1950). The S concentration was $246.5 \text{ mg}\cdot\text{L}^{-1} \text{ MgSO}_4\cdot 7\text{H}_2\text{O}$ ($67.3 \text{ mg}\cdot\text{L}^{-1} \text{ SO}_4^{2-}$). Solutions were circulated and aerated using a Teel pedestal pump (model 3P611E; Dayton Electric Mfg. Co., Chicago, Ill.) attached to polyvinyl chloride (PVC) piping. Solution levels were maintained in the tanks with a mechanical float attached to a deionized water source. Styrofoam insulation boards were placed in the tanks to support the plants. The boards were suspended over the solutions with a PVC pipe framework. Wire fencing was placed 20.3 cm over the boards to support the foliage.

On 17 Jan. 1997, the onions were transferred to the Styrofoam boards and allowed to grow in the nutrient solution until 14 Feb. 1997, when $2.0 \text{ mg}\cdot\text{L}^{-1} \text{ Na}_2\text{SeO}_4$ ($1.51 \text{ mg}\cdot\text{L}^{-1} \text{ SeO}_4^{2-}$) (ICN Biochemicals, Cleveland, Ohio) was added to one tank. No Se was added to the other. The selenium level used in this experiment was based on results of Kopsell and Randle (1997a) who reported that SO_4^{2-} use by onions was unaffected at $2.0 \text{ mg}\cdot\text{L}^{-1} \text{ Na}_2\text{SeO}_4$ concentrations, yet Se accumulation was highest among their treatments. The experimental design was a split plot with four replications and 14 plants per replication. The Se treatments were the main plots and onion cultivars were the subplots. A complete solution change was made every 2 weeks in each tank to restore the initial nutrient conditions of each treatment. Onions were grown and allowed to mature under natural photoperiods. Each cultivar was harvested when 50% of the plants had a soft pseudostem, indicating plant maturity. 'Primavera' and 'Sweet Success' were harvested 18 Apr. 1997, and 'Granex 33' and 'Pegasus' were harvested on 2 May 1997. Upon harvest, the roots were removed and the plants were allowed to dry at ambient greenhouse temperatures for 7 d. Leaves were removed from the bulbs prior to analysis. Eight of the most uniform bulbs were selected for analysis. Wedges (8–10 mm) were cut longitudinally and grouped from each eight-bulb replication. One wedge group was used for total Se, total S, and SO_4^{2-} determination, a second wedge group was used to measure flavor precursors and precursor intermediates, and a third wedge group was used to determine gross bulb pungency, as measured by total enzymatic pyruvic acid (EPY) content, and soluble solids content (SSC).

TOTAL SE AND S DETERMINATION. The combined wedge groups were placed into paper bags and allowed to dry at 60 °C in a forced-air drying oven (model 630; National Appliance Co., Portland, Ore.) for no less than 48 h. The dried tissue was then ground to pass a 0.5-mm screen in a Cyclotec mill grinder (model 1093; Tector, Höganäs, Sweden). For total Se analysis, a wet acid digest was used. One gram of onion powder was placed into a 125-mL Erlenmeyer flask with 10 mL of concentrated nitric acid (70% HNO_3) and set on a hot plate (type 2200; Thermolyne, Dubuque, Iowa) for 4 h at 165 °C. A funnel test tube was inserted into each flask, and when filled with water, acted as a condenser to reduce sample loss during digestion. The flasks were then allowed to cool to room temperature ($\approx 26^\circ\text{C}$) and adjusted to a final volume of 50 mL with deionized water. The solutions were filtered through Whatman no. 1 filter paper (Maidstone, United Kingdom). Total Se was measured by graphite furnace atomic absorption spectro-

photometry (GFAA) (model 4100ZL; Perkin Elmer Corp., Norwalk, Conn.), with a background detection limit of $4.0 \mu\text{g}\cdot\text{L}^{-1} \text{ Se}$. Total S in the plant tissue samples was determined using an S determinator (model SC-232; Leco Corp., St. Joseph, Mo.). One gram of onion powder was combined with vanadium pentoxide accelerator (Leco Corp.) and combusted at 1371 °C with O_2 . Total S was measured as SO_2 with an infrared cell detector and quantified against S standards. Total SO_4^{2-} was measured by modifying a turbidimetric method for total S determination (Gaines and Mitchell, 1979). Magnesium nitrate was not added to the onion powder before ashing so only tissue SO_4^{2-} was detected.

FLAVOR PRECURSORS AND INTERMEDIATE ANALYSIS. Precursor intermediates and ACSOs were extracted using a 12 methanol : 3 water solution, and an 80% ethanol solution according to Lancaster and Kelly (1983). Percent recovery throughout ion exchange fractionation, derivitization, and analysis was quantified by adding S-methyl glutathione (MeGTH; at $0.5 \text{ mg}\cdot\text{g}^{-1}$ fresh weight), g-L-glutamyl-L-glutamic acid (γG ; at $0.2 \text{ mg}\cdot\text{g}^{-1}$ fresh weight), and ($\pm$)-S-1-butyl-L-cysteine sulfoxide (BCSO; at $1.0 \text{ mg}\cdot\text{g}^{-1}$ fresh weight), as internal standards. An extracted sample representing 1 g fresh weight of bulb tissue (15 mL of extract) was placed into a test tube, along with each internal standard, and blown to dryness using ambient air, and redissolved in 1 mL of deionized-distilled water. A 0.5-mL aliquot was then loaded onto an ion exchange column (10×40 -mm; Bio-Rad, Hercules, Calif.) with 3 mL Dowex 1×8 (200 to 400 mesh; Bio-Rad). Fractionation was carried out using acetic acid (HOAc) at four concentrations; 0.1, 1, 2, and 5 M. The 0.1 and 2-M fractions, containing the ACSOs and precursor intermediates, respectively, were collected and blown to dryness using ambient air. High-performance liquid chromatography (HPLC) sample preparation and analysis were carried out according to Randle et al. (1995). Each sample fraction was resuspended in 1 mL deionized-distilled water, and a 100- μL sample was transferred to a 1.5-mL microcentrifuge tube and reduced in vacuo using a concentrator (Centrivap; Labconco, Kansas City, Mo.). When dry, 250 μL of 1 ethanol : 1 triethylamine (TEA) : 1 water was added to each sample and reduced again. Derivitization was performed by adding 100 μL of freshly prepared 7 ethanol : 1 TEA : 1 phenylisothiocyanate (PITC) : 1 water to each sample. The samples were immediately flushed with nitrogen, capped, and held at room temperature for 20 min. The derivitization solution was then removed in vacuo using the centrivap. Samples were again redissolved using 1.0 mL of 2 acetonitrile (HPLC grade) : 7 water and transferred to 1.5-mL glass vials before injection on HPLC.

An HPLC separator module (2690; Waters, Milford, Mass.) with a 996 photodiode array detector was used for analysis. A Spheri-5 RP-18 5- μm , 250×4.6 -mm column (Applied Biosystems, Foster City, Calif.) fitted with a 15×3.2 -mm 7 μm guard column (RP-18 Newgard; Applied Biosystems) was used for separation. The column temperature was maintained at 30 °C. Eluted compounds were detected at 254 nm and data were collected and recorded using a personal computer with Millennium chromatography manager software (version 2.15.01; Waters). Peak assignment was carried out by comparing retention times with authentic standards (supplied by J.E. Lancaster, Crop and Food Research, Christchurch, New Zealand) as described by Randle et al. (1995). Solvents were A) aqueous acetonitrile (60%) and B) 0.14 M sodium acetate with 0.05% TEA buffered to pH 6.5 using glacial acetic acid. Solvents were filtered through a 0.45-mm Magna nylon filter (MSI, Westboro, Mass.). Forty-microliter sample volumes were injected onto the column. A flow rate of $1.0 \text{ mL}\cdot\text{min}^{-1}$ was used. The solvent gradient used was 15% A for 1.10

Table 1. Bulb enzymatically formed pyruvic acid (EPY) ($\mu\text{mol}\cdot\text{g}^{-1}$ fresh weight), percent soluble solids content (SSC), total bulb Se ($\mu\text{g}\cdot\text{g}^{-1}$ Se dry weight), total bulb S ($\mu\text{g}\cdot\text{g}^{-1}$ S dry weight), total bulb SO_4 ($\mu\text{g}\cdot\text{g}^{-1}$ SO_4 dry weight), and bulb S as SO_4 (percent S as SO_4) of four onion cultivars grown with or without $2.0\text{ mg}\cdot\text{L}^{-1}$ Na_2SO_4 ($1.51\text{ mg}\cdot\text{L}^{-1}$ SeO_4^{2-}) in the nutrient solutions.

Cultivar	Treatment	EPY	SSC	Bulb Se	Bulb S	Bulb SO_4	S (%) as SO_4
Granex 33	No Se	6.3	8.4	ND ²	8130	3310	41.16
	Se	6.5	8.7	156.2	6730	2680	39.96
	Significance	NS	NS	***	***	NS	NS
Pegasus	No Se	5.0	9.2	ND	8610	2180	25.50
	Se	3.8	9.0	143.3	7720	2920	37.87
	Significance	NS	NS	***	NS	***	**
Primavera	No Se	6.8	7.6	ND	8190	3340	40.84
	Se	5.6	7.8	146.2	7500	4260	56.98
	Significance	NS	NS	***	***	**	***
Sweet Success	No Se	10.7	9.0	ND	7420	2400	32.38
	Se	9.0	9.7	143.2	6810	3530	52.06
	Significance	***	***	***	**	**	**

²ND = nondetected (below detection limits of GFAA).

NS, *, **, *** Nonsignificant or significant at $P = 0.10$, 0.05 , or 0.01 , respectively by a paired t test.

min, 15% to 45% A over 21.1 min, 45% to 100% A over 1 min, and a hold at 100% A for 14 min. The flow was returned to the initial solvent gradient over 1 min, and the column was re-equilibrated for 12.9 min before the next sample was injected.

BULB PUNGENCY AND SOLUBLE SOLIDS ANALYSIS. Onion pungency for each treatment combination was determined using the pyruvic acid method of Randle and Bussard (1993). Enzymatically formed pyruvic acid (EPY) is routinely used to measure an onion's gross flavor intensity (Lancaster and Boland, 1990), and has been used to measure onion pungency in response to increasing Se concentrations (Kopsell and Randle, 1997b).

Wedges from each eight bulb group were juiced in a pneumatic press and subjected to the analysis. Juice was also applied to hand-held refractometer (Kernco, Tokyo, Japan) to measure soluble solids content (SSC). Soluble solids content is highly correlated with sugar content in onions (Jones and Mann, 1963).

STATISTICAL ANALYSIS. Data were analyzed by the GLM procedure of SAS (Cary, N.C.). Differences among Se treatments were detected by paired t test ($P = 0.05$ or $P = 0.01$) with StatMost software (DataMost, Salt Lake City, Utah).

Results and Discussion

Onions grown in the Se supplemented nutrient solutions accumulated significant levels of Se. Bulb Se accumulation, however, differed only between Na_2SeO_4 treatments ($F = 5073.42$; $P = 0.0001$). Bulb Se was not significantly different among cultivars, and Se averages ranged from 143.2 to $156.2\text{ mg}\cdot\text{g}^{-1}$ dry weight with $2.0\text{ mg}\cdot\text{L}^{-1}$ Na_2SeO_4 ($1.51\text{ mg}\cdot\text{L}^{-1}$ SeO_4^{2-}) in the nutrient solution (Table 1). When no Se was added to the nutrient solutions, bulb Se was below the limits of GFAA detection. The highest level of bulb Se previously reported with $2.0\text{ mg}\cdot\text{L}^{-1}$ Na_2SeO_4 added to the nutrient solutions was $112.9\text{ }\mu\text{g}\cdot\text{g}^{-1}$ dry weight (Kopsell and Randle, 1997b). In that experiment, however, the S concentration was $493\text{ mg}\cdot\text{L}^{-1}$ $\text{MgSO}_4\cdot 7\text{H}_2\text{O}$ ($134.6\text{ mg}\cdot\text{L}^{-1}$ SO_4^{2-}) of nutrient so-

lution, while in the current experiment, the S concentration was $246.5\text{ mg}\cdot\text{L}^{-1}$ $\text{MgSO}_4\cdot 7\text{H}_2\text{O}$ ($67.3\text{ mg}\cdot\text{L}^{-1}$ SO_4^{2-}). Selenium content in plant materials has been shown to decrease with increasing SO_4^{2-} in the growing environment (Barak and Goldman, 1997;

Fig. 1. Typical chromatograms for S-alk(en)yl-L-cysteine sulfoxides (ACSO) of 'Granex 33' onions grown with and without $2.0\text{ mg}\cdot\text{L}^{-1}$ Na_2SeO_4 . Identified peaks are 1) (+)-S-methyl-L-cysteine sulfoxide (MCSO), 2) trans-(+)-S-(1-propenyl)-L-cysteine sulfoxide (1-PrenCSO), 3) (+)-S-propyl-L-cysteine sulfoxide (PCSO), and the internal standards 4) butyl-L-cysteine sulfoxide (BCSO(S)), 5) butyl-L-cysteine sulfoxide (BCSO(R)).

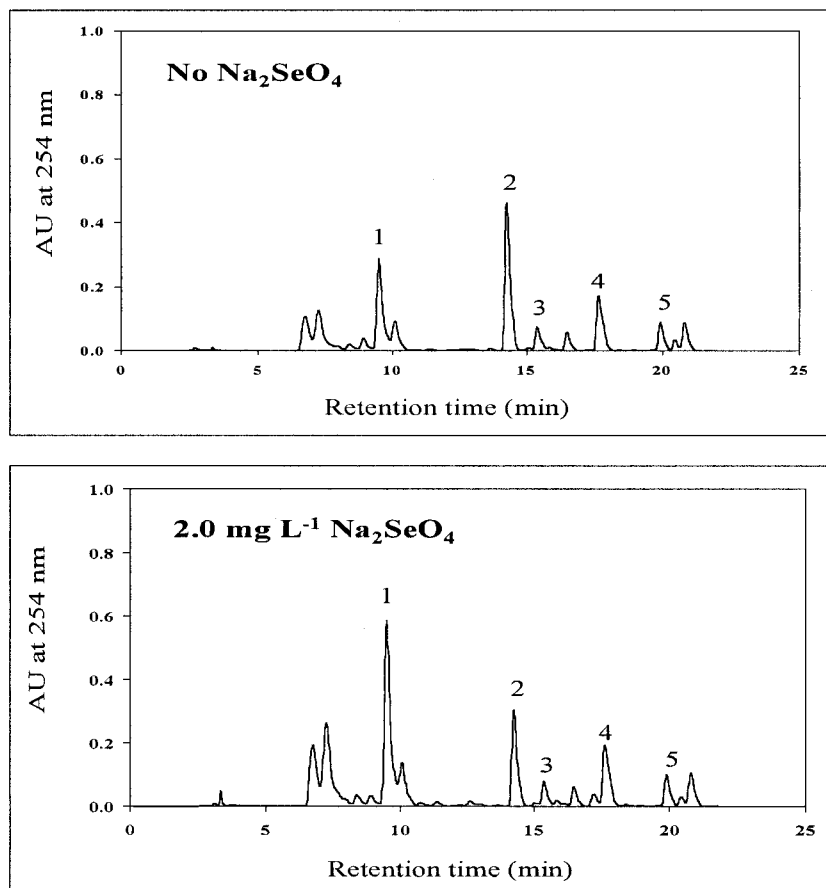


Table 2. Flavor precursors and precursor intermediates^z (mg·g⁻¹ fresh weight) of four onion cultivars grown with and without 2.0 mg·L⁻¹ Na₂SeO₄ (1.51 mg·L⁻¹ SeO₄²⁻) in the nutrient solutions.

Cultivar	Treatment	MCSO	1-PrenCSO	PCSO	ACSO	γGPECSEO	2-carb
Granex 33	No Se	1.159	1.206	0.264	2.628	0.274	0.450
	Se	1.535	0.745	0.205	2.485	0.191	0.463
	Significance	**	***	**	NS	***	NS
Pegasus	No Se	1.226	1.032	0.288	2.546	0.274	0.500
	Se	1.312	0.713	0.320	2.673	0.225	0.480
	Significance	NS	***	NS	NS	*	***
Primavera	No Se	1.382	1.233	0.270	2.884	0.259	0.489
	Se	1.171	0.773	0.182	2.126	0.183	0.512
	Significance	NS	***	*	*	**	NS
Sweet Success	No Se	1.031	1.285	0.264	2.580	0.212	0.488
	Se	1.987	0.767	0.312	3.065	0.173	0.461
	Significance	**	***	NS	NS	***	NS

^zMCSO = (+)-S-methyl-L-cysteine sulfoxide, 1-PrenCSO = trans(+)-S-(1-propenyl)-L-cysteine sulfoxide, PCSO = (+)-S-propyl-L-cysteine sulfoxide, ACSO = total S-alk(en)yl cysteine sulfoxides, γGPECSEO = γ-L-glutamyl-S-(1-propenyl)-L-cysteine sulfoxide, 2-carb = S-2-carboxypropyl glutathione.

NS, **, *** Nonsignificant or significant at $P = 0.10$, 0.05 , or 0.01 , respectively by a paired t test.

Mikkelsen et al., 1989; Shrift, 1954). Onion Se content has been reported as high as 1000 μg·g⁻¹ dry weight with 8 mg·L⁻¹ Se (Barak and Goldman, 1997).

Selenium concentrations caused changes in the flavor precursor profile in onions (Fig. 1). However, we could not completely eliminate the possibility that the Se-analogs to the individual flavor precursors may have coeluted with the ACSOs during HPLC analysis. To date, we do not have direct quantitative measures for the Se-ACSO analogs. However, indirect evidence suggested that coelution was either trivial or separate elution occurred. First, total Se accumulation was between 43 and 54 times less than S accumulation among the cultivars tested (Table 1). Assuming the formation of Se analogs was proportional to total Se accumulation, Se analogs would be insignificant in the chromatograms if they coeluted. Second, the photodiode array detection of our method allows for a spectral analysis of important peaks. When we compared the ACSO peak spectral data from the Se-enriched plants with nonenriched plants, the probability that coeluted peak occurred was very low (data not shown). Spectral changes could be expected with the Se analogs. And third, several unidentified small peaks appeared on the Se-enriched chromatogram that did not appear on the S-only chromatograms (Fig 1.). In the future, identification and quantification of the Se-ACSO analogs will be important if Se-enriched *Alliums* are to be used as Se sources in our diets. Se-Cysteine-substituted proteins have the ability to alter the catalytic activity of enzymes in normal S metabolism (Brown and Shrift, 1982), and can reduce the rate of protein synthesis (Eustice et al., 1981).

Total ACSO, PCSO, and 2-carb content did not differ significantly in the statistical model (Table 2). Methyl cysteine sulfoxide content in bulb tissues was significantly different between Na₂SeO₄ treatments ($F = 4.62$; $P = 0.042$) and for the Na₂SeO₄ treatments and cultivar interaction ($F = 3.14$; $P = 0.044$). 'Granex 33' and 'Sweet Success' MCSO content significantly increased with 2.0 mg·L⁻¹ Na₂SeO₄ ($P = 0.05$; Table 2). With no Na₂SeO₄ in the solution, MCSOs were 44%, 48%, 47%, and 40% of the total ACSO content for 'Granex 33', 'Pegasus', 'Primavera', and 'Sweet Success', respectively. At 2.0 mg·L⁻¹ Na₂SeO₄, MCSO content increased to 62%, 49%, 55%, and 65% of total ACSO for 'Granex 33', 'Pegasus', 'Primavera', and 'Sweet Success', respectively. Even though S was maintained at a level which should have been sufficient for proper growth and flavor development in

both nutrient solutions, the elevated MCSO content with 2.0 mg·L⁻¹ Na₂SeO₄ followed the response of MCSO measured under low S concentrations (Randle et al., 1995). In that study, MCSO content increased relative to the other individual ACSO as SO₄²⁻ decreased in concentration to S-stress levels. Sufficiently high Se uptake and metabolism in onions thus gives the semblance of S stress in the flavor pathway.

The concentration of 1-PrenCSO differed significantly among cultivars ($F = 5.83$; $P = 0.004$) and Na₂SeO₄ treatments ($F = 245.3$; $P = 0.0001$). Significant decreases in 1-PrenCSO content with 2.0 mg·L⁻¹ Na₂SeO₄ were found for all of the cultivars tested (Table 2). With no Se, 1-PrenCSO was 46%, 41%, 43%, and 50% of the total ACSO content for 'Granex 33', 'Pegasus', 'Primavera', and 'Sweet Success', respectively. With 2.0 mg·L⁻¹ Na₂SeO₄, 1-PrenCSO decreased to 30%, 27%, 36%, and 25% of the total ACSO content for 'Granex 33', 'Pegasus', 'Primavera', and 'Sweet Success', respectively. Further evidence of the affect of Se on the 1-PrenCSO pathway is evident with GPECSEO, which is the penultimate compound leading to 1-PrenCSO synthesis. Concentrations of GPECSEO differed among cultivars ($F = 6.50$; $P = 0.002$) and between Na₂SeO₄ treatments ($F = 42.56$; $P = 0.0001$). Selenium significantly decreased GPECSEO content for 'Granex 33', 'Primavera', and 'Sweet Success' (Table 2). Decreases in GPECSEO content should cause decreased 1-PrenCSO content. These results are similar to the decreases in GPECSEO and 1-PrenCSO previously reported with decreased S concentration (Randle et al., 1995), giving further support to the effects of Se causing S stress like symptoms in the flavor pathway.

The effect of Se on S metabolism in onions was also evident when examining total bulb S and SO₄²⁻ (Table 1). Bulb S accumulation differed among cultivars ($F = 8.22$; $P = 0.0006$) and Na₂SeO₄ treatments ($F = 30.85$; $P = 0.0001$). Selenate and SO₄²⁻ can be antagonistic in plants (Arthur et al., 1992; Mikkelsen et al., 1989; Shrift, 1954), but the concentration of each ion in the nutrient solution is also important in determining how much of each ion is absorbed. Low Se concentrations in the nutrient solution actually enhanced S uptake by onions (Kopsell and Randle, 1997a). In this study, Se in the nutrient solution decreased total bulb S significantly in 'Granex 33', 'Primavera', and 'Sweet Success', displaying the antagonism of the competing ions (Table 1). Concurrently, SO₄²⁻ content increased significantly in 'Pegasus', 'Primavera', and 'Sweet Success' when Se was added to

the nutrient solution (Table 1). Therefore, not only did Se decrease total S uptake and accumulation, it also decreased the amount of S that entered the flavor pathway by storing a greater percentage of the absorbed S as SO_4^{2-} . Excess SO_4^{2-} is normally stored in the vacuole (Mengel and Kirkby, 1982) and increasing S concentration in the nutrient solutions will increase the percentage of total S that is stored as SO_4^{2-} (Randle et al., 1999).

Pungency, as indicated by EPY, differed among cultivars ($F = 31.48$; $P = 0.0001$) and Na_2SeO_4 treatments ($F = 5.39$; $P = 0.029$). Bulb sugars, as indicated by SSC, differed among cultivars ($F = 28.06$; $P = 0.0001$) and Na_2SeO_4 treatments ($F = 2.95$; $P = 0.098$). Values for EPY and SSC were within the range previously reported for onions grown under similar Na_2SeO_4 treatments (Kopsell and Randle, 1997b). 'Sweet Success', however, was the only cultivar in which EPY and SSC differed significantly between Na_2SeO_4 treatments (Table 1).

Onions have the ability to accumulate large amounts of Se which, in turn, affects the S-based flavor precursor and precursor intermediates. The effect of Se on the flavor pathway was cultivar dependent, but Se generally decreased 1-PrenCSO and increased MCSO content. Selenium has also been shown to form analogs of the S-flavor compounds in the edible tissues of different *Allium* species (Cai et al., 1994). The ACSO changes resulting from Se supplementation would result in milder tasting onions because the heat and tear producing compounds arise from the decomposition of 1-PrenCSO (Block, 1992). These responses were comparable to the ACSO changes associated with low S concentrations in the nutrient solution. Changes in the flavor profile provides further evidence of the competitive nature of S and Se in plants, and needs to be considered if onions are to be used as a Se delivery source to our diet.

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