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Selenium Influences Glucosinolate and Isothiocyanates and Increases Sulfur Uptake in *Arabidopsis thaliana* and Rapid-Cycling *Brassica oleracea*

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ABSTRACT: This study investigated the impact of Se on glucosinolates (GSs) and isothiocyanates (ITCs). Plants of *Arabidopsis thaliana* cv. Columbia and a rapid-cycling base population of *Brassica oleracea* were grown hydroponically under different Se and S concentrations. The objective was to determine the effects of increasing Se and S concentrations on the GSs and ITCs. The results indicate that S and Se concentrations increased in *A. thaliana* and *B. oleracea* leaf tissue in response to increasing Se treatments. Aliphatic and total GSs decreased significantly ($P \leq 0.001$) from 0.0 to 3.2 mg Se L^{-1} in *B. oleracea* and *A. thaliana* leaf tissues. Consequently, aliphatic and total ITCs decreased significantly ($P \leq 0.001$) from 0.0 to 3.2 mg Se L^{-1} in *B. oleracea* and *A. thaliana* leaf tissues. Data demonstrate that high levels of anticarcinogenic GSs can be maintained as the Se concentration is increased to 0.8 mg L^{-1}. Thus, it is feasible to increase Se to beneficial dietary levels without compromising GS concentrations.

KEYWORDS: *isothiocyanates, selenium, anticarcinogens, HPLC, phytonutrients*

INTRODUCTION

Glucosinolates (GS) are hydrophilic, nonvolatile plant secondary metabolites containing a β-D-thioglucose moiety, a sulfate attached through a sulfonated oxime (C═N bond), and a variable side chain.[1] Glucosinolates are derived from amino acids and can be divided into three groups: aliphatic GSs, derived from Ala, Leu, Ile, Val, and Met; benzenic GSs, derived from Phe or Tyr; and indolic GSs, derived from Trp. There are three independent stages of GS biosynthesis: chain elongation, formation of the core GS structure, and secondary modification of the amino acid side chain.[2] Side-chain elongation and secondary modification are responsible for approximately 120 known GSs. Glucosinolates are hydrolyzed by myrosinase [β-thioglucosidase (EC 3.2.3.1)], which is physically separated within intact plant cells. When chopping or chewing disrupts cells, myrosinase comes in contact with GSs and catalyzes their hydrolysis, producing isothiocyanates (ITCs), thiocyanates, and nitriles. Epithionitriles, oxazolidinethiones, and amines may also result depending on substrate, pH, and availability of ferrous ions.[1,3,4]

Approximately 20 GSs are found in commercially important brassica crops, which include *B. oleracea*, *B. napus*, *B. rapa*, and *B. juncea*.[5] These metabolites are a major part of *Brassica* species defense mechanisms against pest and disease infections. Research has shown that GSs provide a defense against pests by making the plant inedible, in part because of ITCs, which become toxic to a wide range of organisms when in contact with S-containing groups in proteins.[6] Glucosinolates and ITCs increase disease resistance of plants by inhibiting growth of fungal pathogens such as *Fusarium* and *Rhizoctonia*.[7] Additionally, it has been demonstrated that ITCs are toxic to black vine weevil and wireworms.[8,9]

Previous research indicates that a diet high in brassica vegetables reduces the risk of developing certain cancers, such as colorectal,[10,11] lung,[12−14] stomach,[15] breast,[16] bladder,[6] and prostate cancers.[17−19] The chemopreventative properties of brassica vegetables are often linked to GS degradation products such as ITCs. ITCs are powerful electrophiles that readily react with sulfur (S), nitrogen (N), and oxygen(O)-based nucleophiles. Previous studies show that ITCs are extremely potent and have shown remarkable ability to affect all three phases of the carcinogenesis process: tumor initiation, promotion, and progression.[20] In addition, they are capable of suppressing the final steps of carcinogenesis, that is, angiogenesis and metastasis. Some ITCs, such as benzyl, allyl, 2-phenylethyl, and 4-(methylsulfinyl)butyl, can induce apoptosis in colorectal adenocarcinoma cells (HT29) by rapidly blocking proliferating cancer cells.[20] Furthermore, sulforaphane, a well-researched ITC, is a hydrolysis product of 4-(methylsulfinyl)butyl GS and a powerful natural inducer of phase II detoxifying enzymes,[21] such as glutathione-*S*-transferase,[22] UDP-glucuronosyl transferase (UGT), NAP(P)H:quinone oxidoreductase (NQO1), thioredoxinreductase 1 (TR1), and heme oxygenase 1 (HO-1).[23]

Sulfur and selenium (Se) nutrition are particularly interesting because of their regulation of GS metabolism[24] and function in nutritional metabolites. Selenium is an essential micronutrient in mammalian nutrition. This nutrient is a component of the enzymes glutathione peroxidase, selenoprotein P, and tetraiodothyronine 5′-deiodinase.[25,26] Research has shown that this powerful antioxidant can inhibit experimentally induced carcinogenesis in animal models and reduce cancer incidence in human clinical trials.[27,28] Selenium is similar to S in chemical characteristics, and it often serves as a substitute for S in physiological and metabolic processes in plants. Sulfur is an

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essential plant nutrient that is incorporated into proteins through amino acids Cys and Met. Sulfur is involved in many oxidative/reduction reactions in plant metabolism and is incorporated into secondary metabolites such as GSs.[29] For example, Rosen et al.[30] have demonstrated that increasing S and N concentrations had positive effects on cabbage (*Brassica oleracea* var. *capitata*) by increasing GS concentrations, thus increasing its nutritional quality. Toler et al.[24] found that Se increases S uptake by preventing its down-regulation at the plant's roots. In other words, increasing Se helps S uptake into the plants more than increasing S fertilizer concentrations alone.

Various Se fertilizer regimens can affect GS production in rapid-cycling *B. oleracea*. Charron et al.[31] studied *B. oleracea* and have found that increasing Se levels decreased total GSs. Because Se readily accumulates in *Brassica* species through the S assimilation pathway, the analysis of these metabolites is necessary to evaluate the mechanisms by which GSs and ITCs are affected metabolically. Furthermore, it is important to evaluate any subsequent effects Se might have on their anticancer properties as well. Thus, this research is important because additions of both Se and GS-containing brassica vegetables in an individual's diet may offer sizable health benefits.

The objective of this study was to look at how nutrients affect antioxidants in *B. oleracea* and *Arabidopsis thaliana*. These species were selected because they accumulate Se in large quantities and synthesize similar GSs. Furthermore, they are important scientific models in plant physiology, biochemistry, and molecular biology. In addition, it is important to add to the growing knowledge of *B. oleracea* because of its economic importance in production agriculture. In detail, this study evaluates how Se influences S uptake into the leaf tissue and determines how increasing Se and S levels affect major defense compounds in *Brassica* species, specifically GS and ITC concentrations. Previous studies have mainly focused on how Se regulates GS metabolism. Isothiocyanates, the actual bioactive components, have not been studied in relation to Se in detail. Thus, hypotheses are (a) Se will increase S uptake into the leaf tissue in *B. oleracea* and *A. thanliana*, (b) solution containing both Se and S treatments will decrease GS and ITC concentrations in *B. oleracea* and *A. thanliana*, and (c) solution containing only S treatment will increase GS and ITC concentrations in *B. oleracea* and *A. thanliana*.

MATERIALS AND METHODS

***B. oleracea* and *A. thaliana* Growing Conditions.** Experiments were set in a randomized complete block design and conducted in a factorial arrangement. Seeds of a rapid-cycling base population of *B. oleracea* (Crucifer Genetics Cooperative, Department of Plant Pathology, University of Wisconsin, Madison, WI, USA) and *A. thaliana* ecotype Columbia (*Arabidopsis* Biological Resource Center, The Ohio State University, Columbus, OH, USA) were sown into Oasis cubes (Smithers-Oasis North America, Kent, OH, USA) and germinated in a growth chamber (Conviron Controlled Environments Inc., Pimbenia, ND, USA) at 20 °C and with a 16 h photoperiod. Treatments were initiated at the appearance of the first true leaves 1 week after germination. *B. oleracea* and *A. thaliana* plants were grown hydroponically with half-strength Hoagland's nutrient solution[32] in 11 L reservoirs. Half-strength Hoagland's nutrient solution consisted of 2.5 mM $Ca(NO_3)_2$, 2.5 mM KNO_3, 0.5 mM $MgSO_4$, 0.5 mM KH_2PO_4, 40 μM Fe-EDTA, 25 μM H_3BO_3, 2.25 μM $MnCl_2$, 1.9 μM $ZnSO_4$, 0.15 μM $CuSO_4$, and 0.05 μM $(NH_4)_6Mo_7O_{24}$, pH 5.8−6.0). Four blocks contained four replications of each treatment for both species, with individual reservoirs representing an experimental unit. Each reservoir contained six plants. The treatments consisted of the control (containing no Se) and four Se treatments (0.4, 0.8, 1.6, and 3.2 mg Se L^{-1}), one Se and S combination treatment (0.8 mg Se L^{-1} and elevated 37 mg S L^{-1}), and one S treatment (elevated 37 mg S L^{-1}). The elevated sulfate treatments were added to the 96 mg SO_4 L^{-1} already in a half-strength nutrient solution to give a total of 133 mg SO_4 L^{-1}. Selenium treatments were given in the form of sodium selenate, and S treatments were given as sulfate. These treatments are based on previous research looking at Se effects on GSs.[24,31] Plants of *A. thaliana* were grown in a controlled environment growth chamber at 18 °C under an 8 h photoperiod. Light intensity was measured at 354 μmol m^{-2} s^{-1} ± 4% photosynthetically active radiation (PAR). *A. thaliana* was grown in the growth chamber due to lower light requirements, therefore preventing photooxidation. Plants of *B. oleracea* were grown in a greenhouse at 23 °C under a 16 h photoperiod and at 18 °C under an 8 h dark period. Average light intensity measured 854 μmol m^{-2} s^{-1} ± 4% PAR.

All plants were harvested just before anthesis 28−31 days after seeds were sown; leaves and stems were immediately separated into equal halves and frozen in an ultralow −80 °C freezer (Isotemp, Fisher Scientific, Waltham, MA, USA). When frozen, half of the tissue was lyophilized (Labconco, Kansas City, MO, USA) to remove water content and prevent GS degradation. Lyophilized leaves were ground to a powder before analysis to ensure homogenization.

Leaf Tissue Glucosinolate Analysis. For GS analysis, 200 ± 0.1 mg of lyophilized leaf tissue samples was combined with 1 mL of benzylglucosinolate (Chromadex, Irvine, CA, USA) solution (1 mM) as an internal standard, 2.0 mL of methanol, and 0.3 mL of barium lead acetate (0.6 mM) in a 16 mm × 100 mm culture tube and vortexed at 60 rpm for 1 h. Barium lead acetate was added to remove protein content in the samples to isolate GSs in the sample. Each tube was centrifuged at 2000g_n for 10 min. An aliquot of 0.5 mL of supernatant was added to a 1 mL column (Visiprep solid phase extraction vacuum manifold, Supelco, St. Louis, MO, USA) containing 0.3 mL of DEAE Sephadex A-25 (Sigma-Aldrich, St. Louis, MO, USA) and desulfated according to the procedure of Raney and McGregor.[33]

Extracted desulfoglucosinolates were separated in a high-performance liquid chromatography (HPLC) unit with a photodiode array detector (1100 series, Agilent Technologies, Santa Clara, CA, USA) using a reverse-phase 250 mm × 4.6 mm i.d., 5 μm Luna C_{18} column (Phenomenex, Inc., Torrance, CA, USA) at a wavelength of 230 nm. The column temperature was set at 40 °C, with a flow rate of 1 mL min^{-1}. The gradient elution parameters were 100% water for 1 min, followed by a 15 min linear gradient to 75% water/25% acetonitrile. Solvent levels were then held constant for 5 min and returned to 100% water for the final 5 min. Desulfoglucosinolates were identified by comparison with retention times of authentic standards (Chromadex).

Leaf Tissue Isothiocyanate Analysis. Isothiocyanates were extracted, with modifications, according to the procedure of Brown et al.[34] In brief, 200 ± 0.5 mg of lyophilized plant material was added to 16 mm test tubes, defatted with 5 mL of hexane, and centrifuged at 20 °C and 2000g_n for 10 min. The hexane was discarded, and 10 mL of methylene chloride (MeCl), 5 mL of RO water (<18.0 MΩ), and 2 mL of 100 μmol methyl isothiocyanates (MITC) as an internal standard were added, and the mixture was vortexed. The mixture was first added to an orbital shaker for 2 h at 150 rpm and then centrifuged. The organic fraction was placed into a new test tube, and the pellet was re-extracted with 2 mL of MeCl and 1 mL of RO water according to the steps above. In the next step, 2 g of anhydrous sodium sulfate was added to the combined organic fractions and allowed to set for 1 h. The organic fraction was filtered through a 0.2 μm nylon syringe filter and dried under a stream of nitrogen gas. The dried residue was reconstituted in 100 μL of MeCl for analysis. For volatile headspace analysis, 200 ± 0.5 mg of lyophilized plant material was placed in a 20 mL headspace vial and hydrated with 4 mL of RO water. The 20 mm cap (rubber/Teflon septa) was immediately crimped to minimize volatile compounds loss. The sample was vortexed for 30 s then placed on the carousel for analysis with a G1888 headspace analyzer (Agilent Technologies Inc., Wilmington, DE,

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USA). Hydrated plant material was incubated for 30 min at 45 °C, and vials were pressurized at 0.965 bar.

Sample analysis was conducted using a Hewlett-Packard 5890 series II gas chromatograph (GC) using He as a carrier gas, flame ionization detector (FID), and a 7673A autosampler. A HP-5MS (5% phenyl) column was used for separation with dimensions of 30 m × 0.25 mm × 0.25 μm. The following conditions were used with the FID: injection, 1 μL; inlet, 250 °C; He flow rate, 37 cm/s; detector, 260 °C; purge time after injection, 0.5 min.; initial oven temperature, 35 °C for 2 min, increasing at 10 °C/min to 250 °C for 8 min. Total run time with cool down and equilibration was approximately 40 min. A 6890 GC (Agilent Technologies Inc., Wilmington, DE, USA) with the same column, coupled to a 5973N quadrapole mass selective (MS) detector, was used for identification of the compounds. The MS parameters were as follows: injection, 1 μL; inlet, 250 °C; MS source, 230 °C; MS quad, 150 °C; aux-2 temperature, 280 °C. For headspace analysis the GC oven ranged from 35 °C for 2 min, increasing at 20 °C/min, to 220 °C. Total run time was approximately 18 min.

Chemicals. Reference chemicals used in the study for GS analysis were obtained from Chromadex. All ITC reference chemicals were obtained from LKT Laboratories, Inc. (St. Paul, MN, USA).

Leaf Tissue Selenium and Sulfur Nutrient Analysis. Nutrient analysis for Se and S was performed using a 100 mg subsample of lyophilized plant tissue, which was combined with 10 mL of 70% HNO_3 and digested in a microwave digestion unit (Ethos model, Milestone Inc., Shelton, CT, USA). The microwave temperature was ramped to 140 °C for 5 min at 1000 W and 2000 kPa, followed by an increase to 210 °C for 10 min at 1000 W and 3000 kPa. Furthermore, microwave temperature was held at 210 °C for 10 min at 1000 W and 4000 kPa and cooled for 10 min at 0 W and 2000 kPa. The digest was then allowed to cool to 20 °C. A 100 μL subsample of the digest was diluted with 9900 μL of ICP-MS matrix consisting of 2% HNO_3 and 0.5% HCl (v/v). The sample was measured by inductively coupled plasma mass spectroscopy (ICP-MS; Agilent Technologies, Inc., Wilmington, DE, uSA) equipped with an ASX-510 (CETAC, Omaha, NE, USA) autosampler.

Statistical analysis of data was performed using SAS (version 9.1.3 for Windows, SAS Institute, Cary, NC, USA). Data were analyzed using a mix model ANOVA.

RESULTS AND DISCUSSION

Impact of Selenium and Sulfur Treatments on Glucosinolates in *B. oleracea* and *A. thaliana*. Glucosinolates extracted from *B. oleracea* and *A. thaliana* leaf tissue and identified as desulfoglucosinolates were glucoiberin [3-(methylsufinyl)propyl], progoitrin [2(*R*)-2-hydroxy-3-butenyl], glucoraphanin [4-(methylsufinyl)butyl], sinigrin (2-propenyl), glucosinalbin (4-hydroxybenzyl), 4-hydroxyglucobrassicin (4-hydroxyindol-3-ylmethyl), glucoerucin [4-(methylthio)butyl], gluconapin (3-butenyl), glucobrassicin (indole-3-methyl), 4-methoxyglucobrassicin (4-methoxyindol-3-ylmethyl), and neoglucobrassicin (1-methoxyindol-3-ylmethyl) (Tables 1 and 3). Glucosinolates in this study have been identified in other *B. oleracea* vegetables, including cabbage, Brussels sprouts (*B. oleracea* var. *gemmifera*), kale (*B. oleracea* var. *acephala*), and cauliflower (*B. oleracea* var. *Botrytis*).[35,36] They were also identified in other studies with *A. thaliana*.[37] Although concentrations of gluconasturtiin (2-phenylethyl) have been identified in *B. oleracea* plants,[6,35,37] they were under the detection limit.

Isothiocyanates from *B. oleracea* and *A. thaliana* leaf tissues were identified by GC-FID and GC-MS as either volatile headspace samples or extracted compounds in methylene chloride (MeCl). Isothiocyanates identified by GC-FID included 2-propenyl (allyl), 3-butenyl, 3-methylsulfinylpropyl, 3-methylsulfinylbutyl (sulforphane), 2(*R*)-2-hydroxyl-3-butenyl, 4-methylthiobutyl, and 2-phenylethyl isothiocyanate (Tables

Table 1. Glucosinolate (GS) Concentrations (Mean ± SE) in Rapid-Cycling *Brassica oleracea* Leaf Tissue Grown at Different Selenium (Se) Concentrations

	GS[a] concn (μmol g^{-1} of DW)										
Se/S mg L^{-1}	GI	PGN	GR	SN	SB	GN	GBN	NGBN	aliphatic	indole	total
0.0	0.28 ± 0.08	3.29 ± 0.78	0.19 ± 0.07	3.07 ± 0.42	0.42 ± 0.13	2.91 ± 1.02	1.93 ± 0.43	1.03 ± 0.35	9.73 ± 1.47	3.41 ± 0.71	13.56 ± 1.73
0.4	0.18 ± 0.08	0.55 ± 0.78	0.20 ± 0.07	2.13 ± 0.42	0.33 ± 0.13	4.92 ± 1.02	1.18 ± 0.43	0.87 ± 0.35	7.97 ± 1.47	2.39 ± 0.13	10.68 ± 1.73
0.8	0.20 ± 0.08	2.17 ± 0.78	0.04 ± 0.07	1.79 ± 0.42	0.39 ± 0.13	1.65 ± 1.02	1.25 ± 0.43	0.25 ± 0.35	5.86 ± 1.47	1.81 ± 0.13	8.06 ± 1.73
1.6	0.18 ± 0.08	0.60 ± 0.78	0.07 ± 0.07	1.61 ± 0.42	0.24 ± 0.13	2.85 ± 1.02	0.80 ± 0.43	0.54 ± 0.35	5.31 ± 1.47	1.64 ± 0.13	7.18 ± 1.73
3.2	0.08 ± 0.08	0.18 ± 0.78	0.01 ± 0.07	1.10 ± 0.42	0.24 ± 0.13	1.55 ± 1.02	0.98 ± 0.43	0.51 ± 0.35	2.92 ± 1.47	1.79 ± 0.13	4.94 ± 1.73
0.8/37	0.22 ± 0.08	0.23 ± 0.78	0.07 ± 0.07	2.29 ± 0.42	0.27 ± 0.13	4.97 ± 1.02	1.59 ± 0.43	0.32 ± 0.35	7.78 ± 1.47	2.39 ± 0.13	10.43 ± 1.73
37	0.56 ± 0.08	4.28 ± 0.78	0.27 ± 0.07	3.79 ± 0.42	0.57 ± 0.13	3.50 ± 1.02	2.53 ± 0.43	0.27 ± 0.35	12.40 ± 1.47	3.26 ± 0.13	16.22 ± 1.73
F test[b]	**	**	NS	***	NS	*	NS	NS	***	NS	***

[a]GI, glucoiberin; PGN, progoitrin; GR, glucoraphanin; SN, sinigrin; SB, sinalbin; GN, gluconapin; GBN, glucobrassicin; NGBN, neoglucobrassicin. [b]NS, *, **, and *** indicate nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

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Table 2. Isothiocyanate (ITC) Concentrations (Mean ± SE) in Rapid-Cycling *Brassica oleracea* Leaf Tissue Grown at Different Selenium (Se) Concentrations

	ITC[a] concn (μmol g^{-1} of DW)								
Se/S mg L^{-1}	AITC	3-butenyl	goitrin	PEITC	iberin	SF	IC	aliphatic	total
0.0	1.12 ± 0.03	1.07 ± 0.05	0.86 ± 0.05	0.22 ± 0.00	0.08 ± 0.00	0.06 ± 0.01	0.68 ± 0.06	2.34 ± 0.06	4.11 ± 0.10
0.4	0.47 ± 0.03	0.39 ± 0.05	0.72 ± 0.05	0.15 ± 0.00	0.06 ± 0.00	0.03 ± 0.01	0.41 ± 0.06	1.01 ± 0.06	2.31 ± 0.06
0.8	0.08 ± 0.03	0.40 ± 0.05	0.53 ± 0.05	0.16 ± 0.00	0.07 ± 0.00	0.02 ± 0.01	0.41 ± 0.06	0.97 ± 0.06	2.08 ± 0.06
1.6	0.08 ± 0.03	0.39 ± 0.05	0.12 ± 0.05	0.12 ± 0.00	0.06 ± 0.00	0.02 ± 0.01	0.32 ± 0.06	0.57 ± 0.06	1.15 ± 0.06
3.2	0.07 ± 0.03	0.33 ± 0.05	0.12 ± 0.05	0.09 ± 0.00	0.05 ± 0.00	0.02 ± 0.01	0.32 ± 0.06	0.49 ± 0.06	1.03 ± 0.06
0.8/37	0.80 ± 0.03	0.70 ± 0.05	0.10 ± 0.05	0.14 ± 0.00	0.09 ± 0.00	0.03 ± 0.01	0.41 ± 0.06	1.68 ± 0.06	2.33 ± 0.06
37	1.12 ± 0.03	1.08 ± 0.05	1.05 ± 0.05	0.19 ± 0.00	0.09 ± 0.00	0.13 ± 0.01	0.69 ± 0.06	2.43 ± 0.06	4.37 ± 0.06
F test[b]	***	***	***	***	***	***	**	***	***

[a]AITC, allyl; goitrin, 2(*R*)-2-hydroxy-3-butenyl; iberin, 3-methylsulfinylpropyl; PEITC, 2-phenethyl; SF, sulforaphane; IC, indole-3-carbinol. [b]NS, *, **, and *** indicate nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

2 and 4), with low/trace amounts of 4-hydroxybenzyl isothiocyanate and indole-3-carbinol. Other ITCs from their respective GSs were not detected or identified due to their unstable nature as ITCs.

The results show that the total GS concentration in *B. oleracea* decreased 63.6% while the leaf Se concentration increased from 1.6 to 3.2 mg Se L^{-1}. Specifically, plants exposed to 3.2 mg Se L^{-1} treatment reduced the production of glucoiberin, singrin, and progoitrin ($P < 0.05$) by 74.1, 64.2, and 94.5%, respectively (Table 1). Diminishing GS concentrations when exposed to increasing concentrations of Se have been observed in *B. oleracea* in a number of studies. For example, Charron et al.[31] found a significant decline in GSs when plants were exposed to Se concentrations up to 9.0 mg $NaSeO_4$ L^{-1}. Toler et al.[24] found similar results while exposing plants to Se fertilization at 1.5 mg L^{-1}. In *A. thaliana*, total GSs decreased 46.2% in 1.6 and 3.2 mg Se L^{-1} Se treatments, whereas GSs in all five Se treatments (0.0−3.2 mg Se L^{-1}) decreased 72.2%. Individually, the productions of glucoiberin and glucoraphanin were negatively affected with increasing Se concentration in the nutrient solution ($P < 0.01$). Plants exposed to 3.2 mg Se L^{-1} treatment exhibited 86.8 and 72.8% reductions in the production of glucoiberin and glucoraphanin, respectively, when compared to plants in the control treatment (Table 3). These significant decreases in GS concentrations were comparable to the decline in GSs in *B. oleracea*. Recent studies in our laboratory have shown a significant decrease ($P < 0.01$) in GSs when plants were exposed to Se fertilizer concentrations ranging from 0.0 to 3.2 mg Se L^{-1} applied to a soilless medium (data not shown). Therefore, GS levels in both species decreased with increasing Se treatment.

Total ITCs decreased significantly in *B. oleracea* ($P < 0.05$) from 0.0 to 3.2 mg Se L^{-1} with a 63.6% drop. Sulforaphane was significantly affected ($P < 0.05$) by increasing concentrations of Se treatments, with a 94.7% decrease from 0.0 to 3.2 mg Se L^{-1} (Table 2). Total ITCs decreased significantly ($P < 0.001$) in *A. thaliana* from 0.0 to 3.2 mg Se L^{-1}, dropping 99.1%. Sulforaphane, 3-methylsulfinylpropyl, and 3-butenyl were the predominant ITCs in *A. thaliana* leaf tissue. Sulforaphane was highest with 71.6% of the total ITCs present in the control treatment, with 3-methylsulfinylpropyl and 3-butenyl making up 12.7 and 4.9%, respectively. Sulforaphane decreased significantly ($P < 0.001$) with increasing concentration of Se treatments with a 97.2% decrease in production from 0.0 to 3.2 mg Se L^{-1}. 3-Methylsulfinylpropyl exhibited an 87.3% decrease in production and 3-butenyl a 90.6% decrease when by comparisonn of 0.0 to 3.2 mg Se L^{-1} treatments (Table 4).

These results indicate that GS and ITC levels in both species decreased with increasing Se treatment. It has been demonstrated that this synthesis of Se−amino acids and their incorporation into proteins adversely affect GS synthesis and metabolism.[25,38,39] In other words, although the health benefits of Se are being added, the plant is losing GSs and ITCs, which are essential for plants' defense against pest and diseases and have nutritional qualities.

The highest concentration of total GSs in *B. oleracea* was found in the elevated sulfate treatment. Glucoiberin increased by 51.7% in the elevated sulfate treatment compared to the control and by 87.1% compared to the 3.2 mg Se L^{-1} treatment. Notably, both sinigrin and progoitrin showed significant increases in concentration of 19 and 23.1% in the elevated sulfate treatment compared to the control. However, despite a 16.4% difference, the total GSs in the elevated sulfate treatment did not change significantly when compared to the control (Table 1). Similar results were found in *A. thaliana* (Table 3). Therefore, whereas increasing levels of S increase some individual GSs, on a cumulative level S does not positively influence total GSs. These results are comparable to previous research.[24,40]

Elevated sulfate treatment in *B. oleracea* increased ITCs by 6.3% when compared to the control treatment. Interestingly, sulforaphane was not affected by the elevated sulfate treatment when compared to the control. Although there were no significant differences in 3-methylsulfinylpropyl and 2(*R*)-2-hydroxyl-3-butenyl, these ITCs showed 37.5 and 86% increases in concentration in the elevated sulfate treatment, respectively (Table 2). In addition, in *A. thaliana* the elevated sulfate treatment increased total ITCs by 22.2% when compared to the control treatment. The majority of individual ITCs increased significantly in the elevated sulfate treatment, whereas 4-methylthiobutyl and 2-phenylethyl were not affected. 3-Butenyl increased the most, by 55%, when compared to the control treatment (Table 4).

The results show that on a cumulative level S does not positively influence total GSs. Furthermore, increasing levels of S did not significantly increase individual GSs and ITCs in both species. These results are not consistent with previous studies,[41,42] which found that S fertilization leads to increases in GS content in most cases with up to a 10-fold increase.[40] For example, Omirou et al.[41] treated broccoli plant with a 15-fold difference in S fertilization and found a 60.1% increase in total

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Table 3. Glucosinolate (GS) Concentrations (Mean ± SE) in *Arabidopsis thaliana* Leaf Tissue Grown at Different Selenium (Se) Concentrations

	GS[a] concn (μmol g^{-1} of DW)												
Se/S mg L^{-1}	GI	GR	SN	SB	4-HGB	GE	GBN	4-MOGB	NGB	aliphatic	aromatic	indole	total
0.0	2.3 ± 0.2	18.2 ± 1.6	0.4 ± 0.3	1.0 ± 0.4	0.3 ± 0.0	1.3 ± 0.2	12.5 ± 1.2	3.3 ± 0.3	3.2 ± 0.6	24.2 ± 1.7	1.0 ± 0.4	19.4 ± 1.9	44.8 ± 3.2
0.4	1.5 ± 0.2	10.3 ± 1.6	0.8 ± 0.3	0.7 ± 0.4	0.2 ± 0.0	1.6 ± 0.2	9.9 ± 1.2	2.1 ± 0.3	1.8 ± 0.6	15.5 ± 1.7	0.7 ± 0.4	14.2 ± 1.9	30.5 ± 3.2
0.8	1.3 ± 0.2	8.1 ± 1.6	0.6 ± 0.3	0.4 ± 0.4	0.2 ± 0.0	1.7 ± 0.2	9.7 ± 1.2	2.0 ± 0.3	1.4 ± 0.6	13.4 ± 1.7	0.4 ± 0.4	13.5 ± 1.9	27.3 ± 3.2
1.6	0.9 ± 0.2	4.9 ± 1.6	0.3 ± 0.3	0.2 ± 0.4	0.1 ± 0.0	2.7 ± 0.2	9.5 ± 1.2	1.9 ± 0.3	1.3 ± 0.6	9.8 ± 1.7	0.2 ± 0.4	12.9 ± 1.9	23.1 ± 3.2
3.2	0.4 ± 0.2	2.0 ± 1.6	0.6 ± 0.3	0.1 ± 0.4	0.0 ± 0.0	1.9 ± 0.2	6.1 ± 1.2	3.2 ± 0.3	0.8 ± 0.6	6.4 ± 1.7	0.1 ± 0.4	10.2 ± 1.9	16.7 ± 3.2
0.8/37	2.3 ± 0.2	16.8 ± 1.6	0.5 ± 0.3	0.2 ± 0.4	0.3 ± 0.0	1.5 ± 0.2	11.1 ± 1.2	4.1 ± 0.3	2.7 ± 0.6	22.7 ± 1.7	0.2 ± 0.4	18.3 ± 1.9	41.3 ± 3.2
37	2.8 ± 0.2	17.4 ± 1.6	0.6 ± 0.3	1.0 ± 0.4	0.2 ± 0.0	2.1 ± 0.2	11.8 ± 1.2	4.3 ± 0.3	1.9 ± 0.6	24.6 ± 1.7	1.0 ± 0.4	18.3 ± 1.9	44.0 ± 3.2
F test[b]	***	***	NS	NS	***	**	*	***	**	***	NS	*	***

[a]GI, glucoiberin; GR, glucoraphanin; SN, sinigrin; SB, sinalbin; 4-HGB, 4-hydroxyglucobrassicin; GE, glucoerucin; GBN, glucobrassicin; 4-MOGB, 4-methoxyglucobrassicin; NGBN, neoglucobrassicin. [b]NS, *, **, and *** indicate nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

GSs under growth at a normal N supply. It can be argued that the difference in outcome of the present study resulted from an insufficient increase in S fertilization (Tables 1 and 3). The effects of S fertilization on ITCs have not been studied in detail beyond synthetic synthesis.[43,44] However, one previous study did demonstrate that the concentration of ITCs corresponds with the concentration of GSs. Specifically, Gerendas et al.[45] found that, at increasing levels of S supply, sinigrin and corresponding allyl ITC did not change. Future studies should look further at ITCs and how S affects their production from GSs in more detail.

Selenium and S combination treatment did not significantly influence total GS levels when compared to the control and elevated S treatments. In *B. oleracea*, total GSs decreased 23 and 35.7% when compared to the control and elevated S treatments, respectively. In *A. thaliana*, total GSs at the combination treatment decreased 7.8 and 6.1% when compared to the control and elevated treatments, respectively (Table 1). Furthermore, on the individual level most GSs did not change significantly when compared to the control in both species (Table 3). However, when compared to the elevated S treatment, there were inconsistencies in change of individual GS levels. For example, in *B. oleracea* there was no change in glucoraphanin concentrations when compared to the control, whereas in comparison to the elevated S treatment it increased significantly by 74.1% (Table 1). On the other hand, glucoraphanin concentrations in *A. thaliana* did not change significantly in either control or elevated S treatment. The only GS that changed significantly was glucoerucin, which increased 28.6% in the elevated S treatment (Table 3). Furthermore, the effect of the combination treatment on total ITCs in both species is somewhat controversial. Total ITCs decreased significantly ($P < 0.01$) by 43.3% in *B. oleracea* (Table 2) and increased significantly ($P < 0.05$) by 21.6% in *A. thaliana* (Table 4). On an individual level the majority of ITCs in both species follow the trend of total ITC levels (Tables 2 and 4).

The results indicate that Se and S combination treatment did not significantly change GS levels while exposing plants to Se concentrations that are at health benefit levels. These results are consistent with previous research.[24] In Se-treated plants the GS concentrations are affected by increasing Se supply. However, it could be argued that when Se is combined with S in the fertilizer supply, the influence of S on GS metabolism is stronger, therefore nullifying possible Se impact. Furthermore, the combination treatment decreased total ITCs in *B. oleracea* and increased them in *A. thaliana*. These data do not correspond to the results found on Se and S treatment impact on GSs. A possible explanation might be that environmental factors may affect the conversion of GSs to ITCs or other related metabolites. Future studies should look at how Se and S combination treatments influence GSs and ITCs, specifically myrosinase activity.

In addition, S content in the leaf tissue of *B. oleracea* increased with increasing concentrations in the nutrient solution. As Se concentrations increased in the nutrient solution, *B. oleracea* leaf tissue Se concentration increased to 2.05 mg Se g^{-1} dry weight (DW). Selenium treatment means ranged from 0.0 to 2.05 mg Se g^{-1} DW in the 0.0−3.2 mg Se L^{-1} treatments, respectively. Leaf Se increased significantly from 1.6 to 3.2 mg Se L^{-1} (Figure 1) Concurrently, there was a significant increase of S in the leaf tissue, leading to a 74.3% change from 1.6 to 3.2 mg Se L^{-1} (Figure 1). This trend was also demonstrated by Kopsell et al.[46] and Toler et al.,[24] who

Table 4. Isothiocyanate (ITC) Concentrations (Mean ± SE) in *Arabidopsis thaliana* Leaf Tissue Grown at Different Selenium and Sulfur Concentrations

	ITC[a] concn (μmol g^{-1} of DW)								
Se/S mg L^{-1}	AITC	3-butenyl	erucin	PEITC	iberin	SF	IC	aliphatic	total
0.0	0.01 ± 0.00	0.36 ± 0.09	0.93 ± 0.25	0.34 ± 0.12	0.26 ± 0.04	5.25 ± 0.76	0.15 ± 0.03	6.83 ± 0.83	7.33 ± 0.93
0.4	0.00 ± 0.00	0.14 ± 0.09	0.40 ± 0.25	0.05 ± 0.12	0.10 ± 0.04	1.40 ± 0.76	0.14 ± 0.03	2.14 ± 0.83	2.39 ± 0.93
0.8	0.01 ± 0.00	0.29 ± 0.09	0.85 ± 0.25	0.07 ± 0.12	0.17 ± 0.04	1.83 ± 0.76	0.12 ± 0.03	3.22 ± 0.83	3.47 ± 0.93
1.6	0.00 ± 0.00	0.10 ± 0.09	0.75 ± 0.25	0.02 ± 0.12	0.08 ± 0.04	0.47 ± 0.76	0.03 ± 0.03	1.36 ± 0.83	1.40 ± 0.93
3.2	0.00 ± 0.00	0.03 ± 0.09	0.01 ± 0.25	0.01 ± 0.12	0.04 ± 0.04	0.14 ± 0.76	0.11 ± 0.03	0.04 ± 0.83	0.07 ± 0.93
0.8/37	0.03 ± 0.00	0.77 ± 0.09	1.05 ± 0.25	0.08 ± 0.12	0.50 ± 0.04	6.74 ± 0.76	0.15 ± 0.03	9.12 ± 0.83	9.36 ± 0.93
37	0.03 ± 0.00	0.80 ± 0.09	1.15 ± 0.25	0.34 ± 0.12	0.49 ± 0.04	6.76 ± 0.76	0.13 ± 0.03	9.23 ± 0.83	9.43 ± 0.93
F test[b]	***	***	NS	NS	***	***	*	***	***

[a]AITC, allyl; erucin, 4-methylthiobutyl; PEITC, 2-phenethyl; iberin, 3-methylsulfinylpropyl; SF, sulforaphane; IC, indole-3-carbinol. [b]NS, *, **, and *** indicate nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

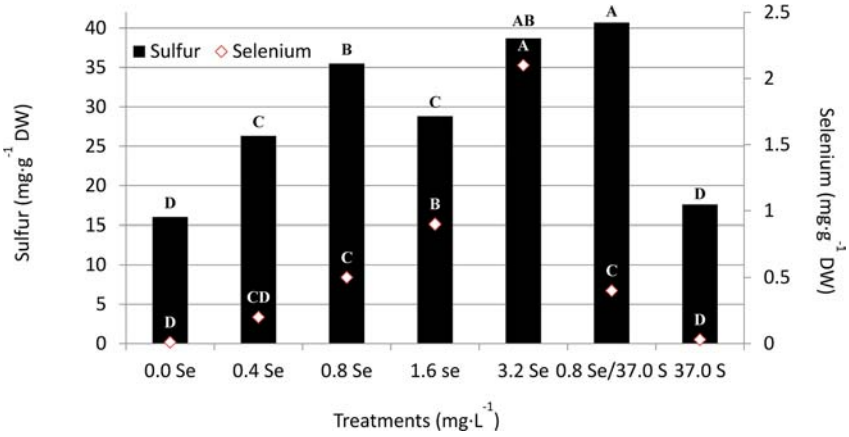

Figure 1. Sulfur and selenium concentrations (μg/g dry weight) in leaf tissue of hydroponically grown *Brassica oleracea* at 31 days. Plants were exposed to Se concentrations ranging from 0.0 to 3.2 mg L^{-1} or an elevated S of 37.0 2 mg L^{-1} more than the 96 2 mg L^{-1} sulfate concentrations preexisting in the nutrient solution ($P < 0.0001$). Letters represent significant mean separation by Fisher's LSD test.

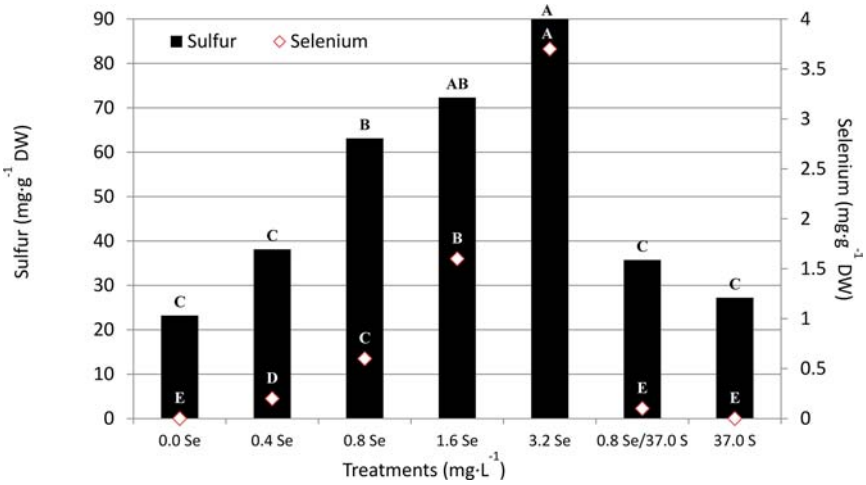

Figure 2. Sulfur and selenium concentrations (μg/g dry weight) in leaf tissue of hydroponically grown *Arabidopsis thaliana* at 28 days. Plants were exposed to Se concentrations ranging from 0.0 to 3.2 2 mg L^{-1} or an elevated S of 37.0 2 mg L^{-1} more than the 96 2 mg L^{-1} sulfate concentrations preexisting in the nutrient solution ($P < 0.0001$). Letters represent significant mean separation by Fisher's LSD test.

found that increasing concentrations of Se in the nutrient solution increased S concentrations in the leaf tissue. The data clearly show that Se has a positive impact on S uptake. In combination with S, Se can accumulate in the leaf tissue to acceptable levels for human health benefits when exposed to concentrations of 0.8 and 196 mg S L^{-1} in the nutrient solution.

In *A. thaliana*, Se concentration in the leaf tissue increased significantly ($P < 0.001$) as Se treatment concentrations increased in the nutrient solution. Selenium concentrations ranged from 0.00 to 3.72 mg Se g^{-1} DW and S concentration ranged from 2.31 to 9.01 mg S g^{-1} DW in *A. thaliana* leaf tissue (Figure 2). The dramatic increase of Se and S in the leaf tissue

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occurred in 1.6 and 3.2 mg Se L^{-1} treatments, leading to a decreased production of GSs and ITCs. Selenium concentration increased 95.4% from 0.4 to 3.2 mg Se L^{-1}, whereas there was an increase of 57.8% in S concentration (Figure 2). This is, to our knowledge, the first report on the effects of increasing Se treatment concentration in the nutrient solution compared to S accumulation in *A. thaliana* leaf tissue. The increase in S concentration with increasing Se has been observed previously in rapid-cycling *B. oleracea*.[24,31,47] At high ratios of Se/S fertilization, S and Se absorption and translocation are antagonistic, but at ratios of 1:125−1:500, Se fertilizations enhanced S uptake in onions (*Allium cepa* L.).[48]

Conclusions. Results indicate that Se treatment adversely affected GSs and ITCs, whereas S treatment did not affect them. Therefore, it can be argued that the most beneficial treatment for the plant and its health benefits is the Se and S combination treatment because the levels of GSs and ITCs remain unchanged while Se concentrations increased. Chemopreventive qualities of Se and degradation products of glucoraphanin, glucoiberin, glucoerucin, sinigrin, and indole-3-ylmethyl GSs have been extensively examined.[27,49−54] Because glucoraphanin, glucoiberin, glucoerucin, sinigrin, and indole-3-ylmethyl GSs were detected in both *B. oleracea* and *A. thaliana* under all Se and elevated sulfate treatments, it is possible to simultaneously deliver dietary Se and chemopreventative ITCs in brassica vegetable crops at concentrations potentially needed to prevent carcinogenesis. Consequently, the rapid decrease in glucoraphanin, glucoiberin, and glucoerucin with increasing Se treatments and subsequent Se accumulation in the leaf tissue is notable, because sulforaphane, 3-methylsulfinylpropyl, and 4-methylthiobutyl isothiocyanates are some of the most powerful natural inducers of phase II detoxification enzymes.[20,53,55,56] In addition to stimulating detoxification enzymes, these compounds protect against oxidative stresses by enhancing the synthesis of glutathione, an antioxidant, and by inducing enzymes with antioxidant functions.[55] Whether significant decreases in GSs and subsequent declines in ITCs resulting from Se treatment fertilization are acceptable needs to be tested at levels accumulated within the edible portions of the plant. This depends on the relative benefits of providing adequate concentrations of Se and GSs in the diet.

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Notes

The authors declare no competing financial interest.

ABBREVIATIONS USED

GS, glucosinolate; ITC, isothiocyanate; HPLC, high-performance liquid chromatography; GC, gas chromatography; FID, flame ionization detector; ICP-MS, inductively coupled plasma mass spectroscopy; DW, dry weight

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