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Effect of Selenium on Glucosinolate and Isothiocyanate Concentrations in *Arabidopsis thaliana* and Rapid-Cycling *Brassica oleracea*

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To the Graduate Council:

I am submitting herewith a thesis written by Thomas Casey Barickman entitled "Effect of Selenium on Glucosinolate and Isothiocyanate Concentrations in *Arabidopsis thaliana* and Rapid-Cycling *Brassica oleracea*." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Plant Sciences.

Carl E. Sams, Major Professor

We have read this thesis and recommend its acceptance:

Dennis Deyton, Dr. Dean A. Kopsell

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

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the Master of Science
Degree
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Thomas Casey Barickman
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Abstract

Brassica vegetables play a unique nutritional and sensory role in human diets around the world. Their characteristic flavors come from the break down products of glucosinolate (GS) compounds, a large group of nitrogen (N) and sulfur (S) containing glucosides. Glucosinolates are hydrolyzed by myrosinase to isothiocyanates (ITCs) which are biologically active. Mounting evidence of this process is of scientific interest due to the potential for high consumption of *Brassica* vegetables containing several GSs and their respective hydrolysis products that are associated with cancer chemoprevention. Glucosinolates are sulfur-rich hydrophilic, nonvolatile plant secondary metabolites; and. over the past few decades, their importance has increased following discoveries of their hydrolysis products, ITCs, as potential anticarcinogens. The importance of selenium (Se) to human health has increased in recent years due its antioxidant potential and cancer suppression properties. Recent studies have demonstrated that certain Se containing compounds like Se-methyl-Se-Cysteine and Se-methionine are effective chemoprotective agents, reducing the incidence of breast, liver, prostate, and colorectal cancers in model systems. *Brassicaa* species are able to hyperaccumulate selenium at concentrations of up to 10-15 mg $Se \cdot g^{-1}$ dry weight in their shoots while growing on naturally-occurring soils containing only 0.2-10 mg $Se \cdot kg^{-1}$. The non-specific integration of Se into the S assimilation pathway enables the plant to metabolize selenoamino acids, selenocysteine and selenomethionine, into proteins. The process is believed to be the major contributor of Se toxicity in plants. The ability of hyperaccumulators to accrue and tolerate high concentrations of Se is thought to be associated with a distinct metabolic capacity that enables the plants to convert these selenoamino acids into non-protein amino acids.

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Chapter 1: Introduction

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Introduction

The genus *Brassica* is one of fifty-one genera in the family *Brassicacea* and is the most economically important genus within the family, containing thirty-seven different species (Gomez-Campo, 1980). The *Brassicacea* plant family has essential functions in the world's economy as sources of vegetables for human nutrition and sources of edible and industrial oils, animal fodder, and soil amendments such as green manures and biofumigants (Williams and Hill, 1986). The wild species of the *Brassica oleracea* group are found in small isolated areas and make up distinct phenotypes (Snogerup, 1980). *Brassica oleracea* originated on the coasts of northern Spain, western France and southern and southwest England. It is a perennial species with a strong vegetative stock which develops over several years before it starts flowering. It has glabrous leaves which have a grayish surface (Rakow, 2004). It is generally believed that all six cultivated groups, *Brassica oleracea, Brassica junceae, Brassica napus, Brassica nigarea, Brassica rapa, and Brassica carninate* originated from western European wild *B. oleracea* (Snogerup, 1980).

The principle *Brassica* vegetable species is *B. oleracea* L., which provides a large range of unique types. The cultivated forms of *B. oleracea* include plant varieties which are staples of food consumption throughout the world. *Brassica rapa* varieties *pekinensis* and *chinensis* are Chinese cabbage types and are extensively grown as vegetables in China. *Brassica alboglabra,* or Chinese kale, is among the ten most important market vegetables in some southeastern Asian countries. Leaf mustards (*B. juncea*) are consumed in great quantities in China and other Asian counties, such as Thailand (Rakow, 2004).

Brassica vegetables play a unique nutritional and sensory role in human diets around the world. Their characteristic flavors come from the break down products of glucosinolate (GS) compounds, a large group of N and S containing glucosides. Glucosinolates are hydrolyzed by myrosinase [β-thioglucosidase, thioglucoside glucohydrolase (E.C. 3.2.3.1)], a class of functionally similar enzymes, and a glycoprotein with molecular mass usually ranging from 125-150 kDa (Björkman and Janson, 1972; Lönnerdal and Janson, 1973; Ohtsuru and Hata, 1973; Bones and Slupphaug, 1989). Myrosinase, which is located in specialty cells called idioblasts, also called myrosin cells (Thangstad et al., 1990; Hoglund et al., 1991), are physically separated from glucosinolates which are produced and mainly retained in the cell's choloroplast and vacuole. When the cells are disrupted by chopping or chewing, myrosinase comes in contact with GSs and catalyzes their hydrolysis. Mounting evidence of this process is of scientific interest due to the potential for high consumption of *Brassica* vegetables containing several glucosinolates and their respective hydrolysis products that are associated with cancer chemoprevention (Zhang et al., 1994; Fahey et al., 1997; Juge et al., 2007; Nestle, 1998; Pereira et al., 2002). In addition, GS degradation products are suppressive to a number of plant pathogens (Charron and Sams, 1999; Charron et al., 2002; Harvy et al., 2002) and have been tested in the feasibility of controlling soil-borne pathogens by incorporation of *Brassica* residues into the soil (Chan and Close, 1987; Muehlchen, 1990; Subbarao and Hubbard, 1996; Larkin and Griffin, 2007; Njoroge et al., 2008).

Rapid cycling *Brassica oleracea*:

A specific variety of *B. oleracea* designed for a wide range of physiological, biochemical, and molecular biological research is rapid cycling *B. oleracea*. Rapid cycling *B. oleracea* plants were selected by interpollenating diverse early flowering types of *B. oleracea* grown in

environments of high plant densities under continuous low intensity light (Willams and Hill, 1986). Through subsequent generations plants were selected for experimentally favorable plant attributes such as minimum time from sowing to flowering, rapid seed maturation and absence of seed dormancy, small plant size, and high female fertility. This selection resulted in a base population of genetically similar *B. oleracea* plants with a mean life cycle of 31 days (seed to flower) (Williams and Hill, 1986). The short life cycle and small plant size thus allows for multiple experiments that can be conducted quickly in a laboratory setting. The production of results within the rapid cycling *B. oleracea* system has a unique standing because of its facile relationship with vegetable crops and its close phylogenetic relationship with Arabidopsis thaliana (Williams and Hill, 1986).

***Arabidopsis thaliana*:**

Arabidopsis thaliana is a small dicotyledonous plant that is used as a model organism for plant biology. *Arabidopsis thaliana* has over seven hundred and fifty natural accessions from around the world with considerable morphological and physiological diversity. *Arabidopsis thaliana* is a weedy plant species in the *Brassicacea* family with significant advantages for basic and advanced research in physiology, biochemistry, and molecular biology. The completion of *A. thaliana* genome provided an integral opportunity to observe and analyze the organization of a plant genome in a finite scale. As a model plant for biochemistry and molecular biology, *A. thaliana* presents an opportunity to provide key insights into gene functions that can affect crop production (Boyes et al., 2001).

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Glucosinolates

Glucosinolates are sulfur-rich hydrophilic, nonvolatile plant secondary metabolites once know as mustard oil glycosides and have long been a part of the human diet because of the pungent flavor and tastes they elicit in *Brassica* vegetables. Over the past few decades the importance of GSs has increased further following discoveries of their hydrolysis products, isothiocynates (ITCs), as potential anticarcinogens, crop-protection compounds, and agricultural biofumigants (Halkier and Gerhenzon, 2006). The approximately 120 described GSs share a chemical structure consisting of a β-D-thioglucose moiety, a sulfate attached through a sulfonated oxime, and a variable side chain (R-group). About twenty GSs are found in commercial *Brassica* crops (Mithen et al., 1987). Glucosinolates can be classified by their precursor amino acid and the types of modifications to the R-group. Compounds derived from Al anine (Ale), Cystine (Cys), Leucein (Leu), Isoleucine (Ile), Methionine (Met), or Valine (Val) are called aliphatic GSs, those derived from Phenylalanine (Phe) or Tyrosine (Tyr) are called aromatic GSs, and those derived from Tryptophan (Trp) are called indole GSs (Halkier and Gerhenzon, 2006).

Glucosinolate Biosynthesis:

It is generally agreed that there are three core independent stages through which GSs are formed (Halkier and Du, 1997; Fahey et al., 2001). The first stage of biosynthesis comprises a common pathway in which protein amino acids, or their chain elongated derivatives, converge to produce homologs, such as the conversion of methionine derivatives dihomomethionine and trihomomethionine to their respective aldoximes (Halkier, 1999; Fahey et al., 2001; Rosa et al. 1997). Aldoximes are early intermediates in the pathway and undergo oxidation reactions by the

flavin containing NADPH-dependant cytochrome P450 (cytP450) monooxygenase via cytP450 reductase (Underhill, 1980; Bennett et al., 1995; Bennett et al., 1996). After the production of the aldoximes, they quickly are oxidized to nitro or nitrile compounds, which in turn are converted into S-alkyl-thiohydroximate by integrating S from cysteine (Wittstock and Halkier, 2002; Halkier, 1999; Halkier and Gerhenzon, 2006). Finally, the reactive intermediate, thiohydroximic acid is released for the S-alkyl-thiohydroximate by a C:S lyase and is glucosylated by UDPG: thiohydroximate glucosyltransferase [EC 2.4.1.-] to form a desulfoglucosinolate. The GS is produced by the addition of sulfate catalysed by desulfoglucosinolate sulfotransferase [EC 2.8.2.-] (Wittstock and Halkier, 2002).

Enzymatic Hydrolysis of Glucosinolates:

Enzymatic hydrolysis of GSs occurs in the presence of water and myrosinase in which the D-glucose is cleaved to produce an unstable thiohydroximate-O-sulfonate. Thiohydroximate-O-sulfonate releases a sulfate ion and spontaneously rearranges (by a Lossen rearrangement) to a wide range of N and S based products (Bones and Rossiter, 2006). These hydrolysis products, many with biological activity, include substituted isothiocyanates (ITCs), nitriles, thiocyanates, epithionitriles, and oxazolidinethiones, which vary depending on the plant species studied, side-chain substitution, cell pH, cell iron concentration, and other protein elements (Vaughn and Berhow, 2005; Bones and Rossiter, 2006). Glucosinolate degradation products, ITCs, are of interest because of their biocidal activity and chemoprotective properties.

Brassica Vegetables and Disease Prevention:

The widespread belief that oxidative damage and chronic inflammation play a major role in induction of cancer, aging, and a number of unremitting diseases has focused scientific and public attention to the health benefits of phytonutrients in fruit and vegetables and their function in impeding the effects of these processes. Like most other vegetables, *Brassica* vegetables are good sources of vitamins, minerals, and phytonutrients (Holts and Williamson, 2004). One characteristic that sets *Brassica* vegetables apart from others is their high GS content (van Poppel, 1999). Isothiocyanates may help prevent cancers by enhancing the elimination of carcinogens before they can damage DNA, or by altering cell signaling pathways in ways that help prevent normal cells from being transformed into cancerous cells (Zhang, 2004). Some ITCs may alter the metabolism or activity of hormones like estrogen in ways that inhibit the development of hormone-sensitive cancers (Auborn et al., 2003). The protective properties of *Brassica* vegetables are also known to inhibit Phase I and induce Phase II detoxifying enzymes, therefore increasing the ability for anticancer activities (Fahey and Talalay, 1997).

In numerous case-studies there are findings that people diagnosed with lung cancer had significantly lower intakes of *Brassica* vegetables than people in cancer-free groups (Verhoeven et al., 1996). Smaller clinical trials found that the consumption of 250 $g \cdot day^{-1}$ of broccoli and 250 $g \cdot day^{-1}$ of Brussels sprouts significantly increased the urinary excretion of a potential carcinogen found in well-done meat, suggesting that high *Brassica* vegetable intakes might decrease colorectal cancer risk by enhancing the elimination of some dietary carcinogens (Walters et al., 2004).

The results of recent epidemiological studies suggest that the protective effects of *Brassica* vegetable consumption may be influenced by inherited differences in the capacity of individuals to metabolize and eliminate isothiocyanates (Lin et al., 1998; Slattery et al., 2000; Seow et al., 2002; Turner et al., 2004). Glutathione-S-transferases metabolize a variety of compounds, including ITCs, in a way that promotes their elimination from the body. Lower GST activity could result in slower elimination and longer exposure to ITCs after *Brassica* vegetable consumption (Seow et al., 1998). Several epidemiological studies have found inverse associations between ITC intake from *Brassica* vegetables and the risk of lung cancer (Lewis et al., 2000; London et al., 2000; Spitz et al., 2000; Zhao et al., 2001) or colon cancer (Slattery et al., 2000; Seow et al., 2002; Turner et al., 2004). These studies imply that protective effects of high intakes of *Brassica* vegetables may be enhanced in individuals that eliminate ITCs more slowly.

Phase I and Phase II detoxifying enzymes play important roles in the metabolism and elimination of a variety of chemicals, including drugs, toxins, and carcinogens. Phase I enzymes catalyze reactions that increase the reactivity of hydrophobic compounds, preparing them for reactions catalyzed by Phase II enzymes. Reactions catalyzed by Phase II enzymes generally increase water solubility and promote the elimination of the compound from the body (Lampe and Peterson, 2002). Most of the attention has been focused on the cancer-preventive potential of these metabolites, primarily as inducers of Phase II enzymes but with potential antiproliferative apoptosis-promoting, redox regulatory and Phase I enzyme inhibiting roles as well (Zhang and Talalay, 1994 and 1998). Sulforphane, for example, has been shown to elevate levels of mammalian Phase II emzymes by ARE (Antioxidant Response Element)-mediated

transcriptional activation (Zhang et al., 1992 and 1994; Talalay et al., 1995; Talalay and Zhang, 1996; Fahey et al., 1997; Prestera et al., 1999). Sulforphane has also reduced the incidence, delayed the appearance of, and reduced the size of tumors in a rat mammary tumor model (Zhang and Talalay, 1994; Fahey et al., 1997).

During human metabolism, ITCs are conjugated to glutathione, an activity that is promoted by a family of enzymes called glutathione-S-transferases (GSTs) and further metabolized to mercapturic acids. These ITCs metabolites can be measured in the urine, and are highly correlated with dietary intake of *Brassica* vegetables (Seow et al., 1998). Evidence also shows that ITCs metabolites contribute to their biological activity (Hecht, 2004; Myzak et al., 2004).

Sulfur

Sulfur in its different oxidation states, 0 (elemental), -2 (sulfide), +4 (sulfite), +6 (sulfate), represents one of the most versatile elements in plant physiology. Sulfur is one of the six macronutrients required by plants and is found in the amino acids Cys and Met, along with S-containing vitamins such as biotin and thiamine, all essential in human nutrition. Sulfur metabolites function among the various S-mediated activities in Fe/S-clusters, structural and regulatory roles via protein disulfide bridges, and catalytic centers (Hell, 1997). Sulfur metabolites also function in secondary compounds ranging from signaling molecules in legumes rhizobia symbioses (Denarie and Cullimore, 1993), the garlic antiseptic alliin (Sendl, 1995), and in ITCs as anti-carcinogens (Zhang et al., 1994).

Sulfur metabolism in plants includes uptake of the macronutrient's sulfate followed by the reduction to sulfides by ATP sulfurylases for assimilation into organic compounds, and channeling into proteins and secondary substances (Hell, 1997; Leustek and Saito, 1999; Saito, 2000). Once reduced to a sulfide form, S is incorporated into Cys, which serves as a precursor for all other S containing organic compounds, including GSs. Plant's uptake of S occurs via sulfate transporters localized in the root plasma membrane. Once inside the plant cells, sulfate is transported by organelle-membrane transport systems within chloroplast envelopes and tonoplast membranes (Saito, 2000). Regulation of S uptake within the plant is controlled by a different set of sulfate transporters. It is well known that sulfate uptake and assimilation activities, such as the production of these transporters, are depressed under conditions of S deficiency via a number of sulfate transporter genes (Saito, 2000; Watanabe-Takahashi, 2000). Control of the sulfate transporter genes is thought to be regulated by S containing compounds, such as Cys and glutathione and other compounds such as O-acetylserine (OAS) (Schmidt and Jager, 1992; Saito, 2000).

O-acetylserine is thought to be a positive regulator of gene expression in sulfate-starved plants. In fact, OAS has been shown to induce expression of genes encoding sulfate transporters in barley and *Arabidopsis* (Smith et al., 1997; Saito, 2000). In contrast to OAS as a positive regulator, glutathione and Cys are thought to be negative regulators of genes whose expression is regulated by S status. Thus, an increase in S within a plant would lead to the down regulation of the genes encoding sulfate transporters (Leustek and Saito, 1999; Saito, 2000).

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Selenium

Physical and chemical similarities of selenium (Se) and S help explain their intimate association in plant metabolism. Selenium, as well as S, is part of the VI-A chalcogen group of elements, with the oxidation states being 0 (elemental Se), -2 (selenide), +4 (selenite), +6 (selenate). Predominate forms of Se available to plants are selenite and selenate. The Se atom is larger than S with a radius of 0.5 Å compared to 0.37 Å respectively. As a consequence, the bond between two Se atoms is approximately one-seventh longer and one-fifth weaker that the disulfide bond (Sors et al., 2005).

Most plant species contain less than 25 μg $Se \cdot g^{-1}$ dry weight and cannot tolerate high levels of Se in the growing environment. Yet several species of the genera *Astragalus, Brassica, Neptunia* and *Stanleya* are able to hyperaccumulate Se at concentrations of up to 10-15 mg $Se \cdot g^{-1}$ dry weight in their shoots while growing on naturally occurring soils containing only 0.2-10 mg $Se \cdot kg^{-1}$ dry weight (Virupaksha and Shrift, 1965; Davis 1972; Davis, 1986). The non-specific integration of Se into the S assimilation pathway enables the plant to metabolize selenoamino acids selenocysteine (SeCys) and selenomethionine (SeMet) into proteins. The process is believed to be the major contributor of Se toxicity in plants (Brown and Shrift, 1981). The ability of hyperaccumulators to accrue and tolerate high concentrations of Se is thought to be associated with a distinct metabolic capacity that enables the plants to convert these selenoamino acids into non-protein amino acids like Se-methylselenocysteine (MeSeCys), γ-glutamyl-Se-methylselenocysteine (GGMeSeCys) and selenocystathionine (Brown and Shrift, 1981), and enzymes like selenocystein methyltransferase (LeDuc et al., 2004) and Se-methyltransferase (Lyi et al., 2005).

Selenium is essential to human nutrition for normal metabolic function and as components of the enzymes glutathione peroxidase, selenoprotein P, and tetraiodothyronine 5'-deiodinase. Selenium has a recommended dietary allowance (RDA) of 15-70 $\mu g \cdot day^{-1}$, depending on age (NIH, 2008). In addition, recent studies have shown supplementation of 100-200 $\mu g\ Se \cdot day^{-1}$ to be associated with a decreased incidence of such cancers as lung and prostate (Lauchi, 1993; Clark et al., 1996; Ip et al., 1991; Ip and Ganther, 1992). However, Se can be toxic if dietary levels exceed 45-400 $\mu g\ Se \cdot day^{-1}$, which depends on age (NIH, 2008). The importance of Se to human health has become noteworthy in recent years due its antioxidant potential and cancer suppression properties. Recent studies have demonstrated that certain Se containing compounds like MeSeCys, GGMeSeCys, and SeMet are effective chemoprotective agents, reducing the incidence of breast, liver, prostate, and colorectal cancers in model systems (Ip and Ganther, 1992; Vadgama et al., 2000; Whanger et al., 2002 and 2004). However, in several parts of the world Se intakes are low enough to cause multiple health problems like heart disease, hypothyroidism, a weakening of the immune system, and selenosis (Combs, 2000).

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Chapter 2

Impact of Selenium Fertilization on Glucosinolate Concentration in *Arabidopsis thaliana* and Rapid Cycling *Brassica oleracea*

Abstract

Brassica vegetables are a significant source of glucosinolates (GSs), which are sulfur (S)-containing phytonutrients that exhibit, upon hydrolysis by endogenous myrosinase, antioxidant and anti-carcinogenic activity. Selenium (Se), an essential micronutrient in mammalian nutrition, exhibits antioxidant activity by inhibiting experimental carcinogenesis in animal models and reducing cancer incidence in human clinical trials. Selenium is readily accumulated in both *Brassica* species and *Arabidopsis thaliana*, a model species with similar S-metabolism. The research objectives for this project were to: 1) compare the impact of Se fertilization on glucosinolate concentrations between *Arabidopsis thaliana* and rapid cycling *Brassica oleracea*; and 2) determine the level of fertilizer Se needed to optimize plant tissue Se concentration to maximize potential human health benefits. Both S and Se concentrations increased in *A. thaliana* and rapid cycling *B. oleracea* tissues in response to increasing Se treatments. Glucosinolate concentrations, expressed as $\mu mol \cdot g^{-1}$ dry mass, for 3-(methylsulfinyl)propyl (1.35 ± 0.22), 4-(methylsufinyl)butyl (6.15 ± 0.75), aliphatic (8.85 ± 1.05), and total GS (9.94 ± 1.21) were statistically significant ($P \leq 0.5$) when comparing both *A. thaliana* and *B. oleracea*. Data demonstrates that anti-carcinogenic GSs can be modified through changes in Se treatment concentrations, yet high levels of anti-carcinogenic GSs can be maintained while increasing Se concentration to 0.8 $mg \cdot L^{-1}$. Thus, it is feasible to increase Se to beneficial dietary levels without compromising GS concentrations.

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Introduction

Brassica vegetables have long been a part of the human diet because of their distinctive pungent flavor, which are caused in part by glucosinolates (GSs). Glucosinolates are sulfur(S)-rich hydrophilic, nonvolatile plant secondary metabolites (Halkier and Gershenzon, 2006). Glucosinolates are hydrolyzed by myrosinase [β-thioglucosidase (E.C. 3.2.3.1], which is physically separated from GSs within intact plant cells. When cells are disrupted by chopping or chewing, myrosinase comes in contact with GSs and catalyzes their hydrolysis. Over the past few decades the importance of GSs has increased following discoveries of their hydrolysis products, isothiocynates (ITCs), as anti-carcinogens, crop-protection compounds, and agricultural biofumigants (Zhang and Talalay, 1994; Fahey et al., 1997; Nestle, 1998; Pereira et al., 2002; Juge et al., 2007).

Dietary consumption of anti-carcinogenic ITCs is highly associated with low incidences of colorectal, liver, lung, and stomach cancers (Hecht, 2004). The most notable ITC, sulforaphane, a hydrolysis product of 4-(methylsulfinyl)butenyl GS, is one of the most powerful natural inducers of phase II detoxifying enzymes, such as glutathione transferases and NAD(P)H: quinine reductase (Fahey and Talalay, 1999). Several studies show that topical applications of sulforaphane extracts protect against UV-light induced skin carcinogenesis in high risk mice (Dinkova-Kostova et al., 2005; Dinkova-Kostova et al., 2006; Dinkova-Kostova et al., 2007). Furthermore, ITCs are suppressive to a number of plant pathogens (Charron and Sams, 1999; Charron et al., 2002; Harvey et al., 2002) and have been tested for feasibility to control soil-born pathogens by incorporation of *Brassica* residues into soils (Chan and Close, 1987; Muehlchen et al., 1990; Subbarao and Hubbard, 1997; Larkin and Griffin, 2007; Njoroge et al., 2008).

Glucosinolate production is closely influenced by genetic and environmental factors. Kopsell et al. (2007) found that differing nitrogen (N) and S ratios can impact concentrations of 2-phenethyl GS in watercress (*Nasturtium officinale* R. Br.). Other studies have shown seasonal and genotypic GS variations in *B. oleracea* (Charron et al., 2005; Farnham et al., 2005) and differing light and temperature regimes in cabbage seedlings (Rosa and Rodrigues, 1998). A specific interest in S and selenium (Se) nutrition has transpired as a result of their prominent regulation of glucosinolates metabolism (Toler et al., 2007). Selenium is similar to S in both size and chemistry and, thus, often substitutes for S in physiological and metabolic processes (Anderson and Scarf, 1983). Selenium readily accumulates in *Brassica* species through the S assimilation pathway and has been shown to affect GS production in rapid-cycling B. oleracea (Charron et al., 2002) by varying Se fertilizer regimes. *Brassica* species have the ability to accrue and tolerate high concentrations of Se through the metabolic capacity to convert selenoamino acids into non-protein amino acids such as Se-methylselenocysteine (MeSeCys), γ-glutamyl-Se-methylselenocysteine (GGMeSeCys) and selenocystathionine and enzymes like selenocystein methyltransferase (LuDuc et al., 2004) and Se-methyltransferase (Lyi et al., 2005).

Selenium, an essential micronutrient in mammalian nutrition, exhibits antioxidant activity through inhibiting experimental carcinogenesis in animal models and reducing cancer incidence in human clinical trials (Clark et al., 1996; Combs and Gray, 1998). Selenium has a recommended dietary allowance of 15-70 µg per day, depending on age and medical history (National Institute of Health, 2008). In addition, recent studies have shown excess supplementation of 100-200 µg Se per day to be associated with a decreased incidence of cancers such as lung and prostate (Clark et al., 1996; Lauchli, 1994; Ip et al., 1991; Ip and Ganther, 1992). Selenization of food crops and herbs has recently become more common in the functional

food era with incorporation into *Brassica*'s (Charron et al., 2002; Finley, 2005), soybeans (*Glycine max*) (Ferretti and Levander, 1976; Marks and Mason, 1993; Wang et al., 1996), and herbs (Kopsell et al., 2009). Recent studies have also demonstrated that certain Se containing compounds like MeSeCys, GGMeSeCys, and selenomethionine (SeMet) are effective chemoprotective agents, reducing the incidence of breast, liver, prostate, and colorectal cancers in model systems (Ip et al., 1991; Vadgama et al., 2000; Whanger, 2002; Whanger, 2004).

Understanding of genetic elements that contribute to Se assimilation and its effects on S and GS metabolism will impact future phytonutrient improvements in *B. oleracea* and other important agronomic crops. *Brassica* species share a close taxonomic and molecular relationship with *A. thaliana*. However, there are several subtle but distinct metabolic characteristics in *A. thaliana* that differ from *Brassica* crop species. To address these, the use of *B. oleracea* on *A. thaliana* microarrays has given insight to homology of key genes, proteins, and enzymes in biochemical experiments (Kang et al., 2008). Therefore, microarrays can be tested with specific RNA targets from embryos, germinating seeds, or leaf tissues to indicate expression patterns for genes unique to *Brassica* species (Li et al., 2003). The justification of this project was to study the effects of Se fertilization on S assimilation and GS metabolism in *A. thaliana* while comparing this to the food crop *B. oleracea.* The similarity of S and Se assimilation and GS metabolism in *A. thaliana* and *B. oleracea* allows the study of these mechanisms across the *Brassicaceae* family. The extensive genetic and biochemical analysis of *A. thaliana* gives our experiment a foundation to study the regulation of S, Se, and GS metabolism in *B. oleracea*. In the current study, we have compared the impact of Se fertilization on GS concentrations between *A. thaliana* cv. Columbia and a rapid cycling base population of B. *oleracea* to determine the level of Se needed in fertilizer solutions that optimize plant tissue Se and GS concentrations to

maximize potential human health benefits. This is, to our knowledge, the first study to look at the effects of increasing Se concentrations on GS concentration in *A. thaliana* and to compare the results with *B. oleracea.*

Methods and Materials

A rapid cycling base population of *B. oleracea* and *A. thaliana* cv. Columbia plants were grown in 10 cm diameter pots filled with a soilless medium (BM-1, Berger Horticulture, Saint-Modeste, Quebec, Canada) and fertilized with a modified full strength Hoagland's nutrient solution. Plants were grown in a controlled environment chamber (Conviron, Pembina, ND) at 18 °C/23 °C under a 16 hr photoperiod. Light intensity was measured at 876 $\mu mol \cdot m^{-2} \cdot s^{-1} \pm 7\%$ photosynthetically active radiation (PAR). Rapid-cycling *B. oleracea* seeds were obtained from the University of Wisconsin, Department of Plant Pathology, and *A. thaliana* cv. Columbia seeds were acquired from The Ohio State University, Arabidopsis Biological Resource Center. Seeds were sown in soilless media, thinned to one plant per pot, and watered or fertilized with 60 ml once daily.

Two experiments were conducted and set by randomized complete block design in a factorial arrangement. One week after germination and the appearance of the first true leaves, treatments were initiated. There were four blocks containing four replications of each treatment for both species, with individual pots representing an experimental unit. The treatments consisted of the control (full strength nutrient solution, containing no Se) and six Se treatments (0.1, 0.2, 0.4, 0.8, 1.6, and 3.2 mg $Se \cdot L^{-1}$).

Twenty-eight to 31 days after seeds were sown, just before anthesis, all plants were harvested. At the time of harvest, plant fresh weights were taken for quantification of biomass. Leaves and stems were immediately bagged after fresh weights where taken and frozen in a -20

°C freezer. When frozen, all tissue was lyophilized (Labconco, Kansas City, Mo.) to remove water content for dry biomass determination and to prevent glucosinolate degradation.

For GS analysis, 200.0 ± 0.1 mg of lyophilized leaf tissue samples were combined with 1 ml of benzylglucosinolate solution (1mM) as an internal standard, 2.0 ml of methanol and 0.3 ml of barium lead acetate (0.6mM) in a 16 mm x 100 mm culture tube and shaken at 60 rpm for 1 hr. Each tube was centrifuged at 2000 g_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) containing 0.3 ml of DEAE Sephadex A-25 (Sigma-Aldrich, St. Louis, MO.) and desulfated by the procedure of Raney and McGregor (1990).

Extracted desulfoglucosinolates were separated in a high performance liquid chromatography (HPLC) unit with a photodiode array detector (1100 series, Agilent Technologies, Santa Clara, CA) using a reverse-phase 250 mm x 4.6 mm i.d., 5-µm Luna C_{18} column (Phenomenex, Inc., Torrance, CA) at a wavelength of 229 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.

Nutrient analysis for Se and S was performed with 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 (Model: Ethos, Milestone Inc., Shelton, CT). The microwave temperature was ramped to 140 °C for 5 min. at 1000W and 2000 kPa, followed by an increase to 210 °C for 10 min at 1000W and 3000 kPa. Next, microwave temperature was held at 210 °C for 10 min at 1000W and 4000 kPa, then cooled for 10 min at 0W 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 a matrix that consisted of 2% HNO_3 and 0.5% HCl (v/v) and measured by inductively coupled plasma mass spectroscopy (ICP-MS; Agilent Technologies, Inc., Wilmington, DE) equipped with a ASX-510 (CETAC, Omaha, NE) autosampler.

Statistical analysis of data was performed using SAS (Version 9.1.3 for Windows, SAS Institute, Cary, N.C.).

Results and Discussion

Extracted GSs from *A. thaliana* and *B. oleracea* were identified by HPLC as desulfoglucosinolates. Common GSs identified between both species were 3-(methylsulfinyl) propyl, 4-(methylsulfinyl)butenyl, 2-propenyl, 3-butenyl, indol-3-lymethyl, 4-methoxyindol-3-lymethyl, 2-phenylethyl, and 1-methoxyindol-3-ylmethyl (Appendix A; Table 1). These GSs have been identified in both *A. thaliana* (Petersen et al., 2002), rapid cycling *B. oleracea* (Toler et al., 2007; Charron and Sams 2004; Kopsell et al., 2000), and in numerous *B. oleracea* vegetables (Bellostas et al., 2007).

In contrast to the chemopreventive quantities of GSs measured from leaf tissue harvested just before anthesis in this study, leaf tissue concentrations in *A. thaliana* and *B. oleracea* of 2-phenethyl and 4-(methylthio)butyl GSs were each present at concentrations <0.2 % of total GSs compared to 62% of 4-(methylsulfinyl)butenyl GS. The overall comparison between *A. thaliana* and *B. oleracea* total GS concentrations were higher in the Se treatments ranging from 0.0 to 0.8 mg $Se \cdot L^{-1}$, yet decreasing by ~ 59% at 1.6 and 3.2 mg $Se \cdot L^{-1}$. These results are consistent with other similar studies in *B. oleracea* (Toler et al., 2007; Charron and Sams 2004; Charron et al., 2002). Comparisons of aliphatic GSs significantly decreased with increasing Se concentrations from 0.8 mg $Se \cdot L^{-1}$ to 1.6 mg $Se \cdot L^{-1}$ ($P<0.001$). Individual GSs, 3-(methylsulfinyl)propyl and 4-

(methylsulfinyl)butenyl, were significantly influenced by increasing Se concentrations yet decreased by 56% and 45%, when comparing the 0.0 mg·L^{-1} to 3.2 mg Se·L^{-1} in both *A. thaliana* and *B. oleracea*, respectively.

Brassica oleracea did not see significant decreases in GSs with increasing Se concentration when analyzed separately (Appendix A; Table 2). This may have been caused by leaching of Se from the soilless medium or due to biomass dilution. Similarly, aliphatic (2.57 ± 1.05 mg Se·L^{-1}), indole (0.12 ± 0.04 mg Se·L^{-1}), and total (2.70 ± 0.58 mg Se·L^{-1}) GSs were statistically similar across all Se treatments in *B. oleracea.* However, increasing Se concentrations in the soilless medium increased Se content in *B. oleracea* (Appendix B; Figure 1) while increasing S concentration in leaf tissue (Appendix B; Figure 2). Consequently, increasing concentrations of Se treatments in the nutrient solution affected total GSs while increasing S (Appendix B; Figure 3) and Se (Appendix B; Figure 4). Research associated with increasing Se concentration in food crops is becoming more prevalent with greater interest in functional food. Functional foods with elevated phytonutrients facilitate improved human health, by fighting against oxidative stress and cancers, through the diet.

In *A. thaliana*, 4-(methylsulfinyl)butenyl GS was highest at 0.0 mg Se·L^{-1} and decreased by 52% at 1.6 mg Se·L^{-1}. The swift decline in 4-(methylsulfinyl)butenyl GS with increasing Se concentrations is of interest, since sulforaphane, a breakdown product of 4-(methylsulfinyl)butenyl GS, is the most potent natural inducer of Phase II detoxification enzymes in human metabolism (Fahey et al., 1997; Fahay and Talalay, 1999). Indol-3-lymethyl GS did not decrease with increasing Se treatments; however, it was significant ($P \leq 0.05$) in a linear contrast analysis. Interestingly, 4-(meththio)butenyl GS increased by 33% from 0.14 ±

0.07 to 0.42 ± 0.07 μmol·g^{-1} of DW when comparing 0.0 mg·L^{-1} to 3.2 mg Se·L^{-1} (Appendix A; Table 3).

Increasing Se concentrations in the soilless medium decreased total GSs in *A. thaliana*, while increasing Se and S concentration in the leaf tissue (Appendix B; Figure 2). Consequently, increasing concentrations of Se treatments in the nutrient solution affected total GSs while increasing S (Appendix B; Figure 5) and Se (Appendix B; Figure 6). Selenium concentration in the leaf tissue approached a maximum of about 550 μg·g^{-1}, and S content reached approximately 22,000 μg·g^{-1}, far exceeding values found in *B. oleracea*. The significant increase in Se and S leaf tissue content in *A. thaliana* suggests a greater ability to accumulate Se through the S metabolic pathway at the highest levels of Se fertilization. Increased accumulation may be caused by shunting reduced Se into non-protein amino acids through the cysteine pathway or forming O-sulfated compounds from oxidized Se through the 3'-phosphoadenosine-5'-phosphosulfate (PAPS) pathway (LaDuc et al., 2004; Lyi et al., 2005).

Based on data from these experiments, Se fertilization increased S uptake at low Se/S fertilization ratios, and the production of Se-amino acids, selenocysteine and selenomethionine, and assimilation of these Se-amino acids into proteins may unfavorably affect the production and metabolism of GSs. However, high levels of anti-carcinogenic GSs, such as 3-(methylsulfinyl)propyl, 4-(methylsulfinyl)butenyl, 3-(methylsulfinyl)propyl , 2-propenyl, 3-butenyl, indol-3-lymethyl, and 4-(methylthio)butyl, in *A. thaliana* and *B. oleracea* can be maintained while increasing Se concentration to 0.8 mg Se·L^{-1} (Appendix A; Table 1). Thus, it is feasible to increase Se to beneficial dietary levels without losing the benefits of high GSs.

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Chapter 3

The Effect of Selenium Fertilization on Glucosinolate Conversion to Isothiocyanates in *Arabidopsis thaliana* and Rapid-Cycling *Brassica oleracea*

Abstract

Evidence indicates that a diet high in *Brassica* vegetables reduces the risk of developing certain cancers and that dietary consumption has been highly associated with lower incidences of cancers such as colorectal, lung, and prostate. The chemopreventative properties of *Brassica* vegetables are often linked to glucosinolate (GS) degradation products. Isothiocyanates (ITCs) are extremely potent and have shown remarkable ability to act on the process of carcinogenesis by affecting all three phases: tumor initiation, promotion and progression phases, and also by suppressing the final steps of carcinogenesis. The most notable ITC, sulforaphane, a hydrolysis product of 4-(methylsulfinyl)butenyl GS, is a powerful natural inducer of phase II detoxifying enzymes. Selenium (Se) is an essential micronutrient in mammalian nutrition which exhibits antioxidant activity through inhibiting experimental carcinogenesis in animal models and reducing cancer incidence in human clinical trials. Plants of *Arabidopsis thaliana* and rapid-cycling *Brassica oleracea* were grown hydroponically in four blocks containing four replications of each treatment for both species, with individual reservoirs representing an experimental unit. Each reservoir contained 6 plants. The treatments consisted of the control (half strength nutrient solution, containing no Se) and four Se treatments (0.4, 0.8, 1.6, and 3.2 mg $Se \cdot L^{-1}$), one Se and elevated S combination treatment (0.8 mg $Se \cdot L^{-1}$ and 37 mg $SO_4 \cdot L^{-1}$ given in addition to the base 96 mg $SO_4 \cdot L^{-1}$ in the nutrient solution), and one elevated S treatment (37 mg $SO_4 \cdot L^{-1}$). Total GSs and ITCs decreased with increasing Se treatments in both *A. thaliana* and *B. oleracea*. Elevated sulfate treatment significantly increased GS and ITC concentrations in both *A. thaliana* and *B. oleracea* when compared to 1.6 and 3.2 mg $Se \cdot L^{-1}$ treatments. Overall Se treatment affected GSs and ITCs in *A. thaliana* and *B. oleracea.*

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Introduction

Evidence indicates that a diet high in *Brassica* vegetables reduces the risk of developing certain cancers and that dietary consumption has been highly associated with lower incidences of colorectal (Lin et al., 1998; Seow et al., 2002), lung (London et al., 2000; Spitz et al., 2000; Zhao et al., 2001; Wang et al., 2004b), stomach (Hanssonet et al., 1993) breast (Fowke et al., 2003; Ambrosone et al., 2004), bladder (Zhao et al. 2007) and prostate cancers (Cohen et al., 2000; Giovannucci et al., 2003; Kirshet et al., 2007). The chemopreventative properties of *Brassica* vegetables are often linked to glucosinolate (GS) degradation products. Glucosinolates are hydrophilic, non-volatile plant secondary metabolites containing β-D-thioglucose moiety, a sulfate attached through a sulfonated oxime (C=N bond), and a variable side chain (Halkier, 2006). Glucosinolates are a diverse group of metabolites that exhibit various forms and functions for nutritional and agricultural uses such as crop-protection compounds and agricultural biofumigants (Zhang and Talalay, 1994; Fahay, 1997; Nestle, 1998; Pereira et al., 2002; Juge et al., 2003). Approximately 20 GSs are found in commercial *Brassica* crops, which include *B. oleracea*, *B. napus*, *B. rapa*, *and B. juncea* (Mithen et al., 1987).

Glucosinolates are hydrolyzed by myrosinase [β-thioglucosidase (E.C. 3.2.3.1] which is physically separated within the intact plant cells. When cells are disrupted by chopping or chewing, myrosinase comes in contact with GSs and catalyzes their hydrolysis. Isothiocyanates (ITCs), thiocyanates, and nitriles are generally produced, even though epithionitriles, oxazolidinethiones, and amines may result depending on substrate, pH, and the availability of ferrous ions (Rosa et al., 1997).

The epidemiological evidence for cancer protection associated with consumption of cruciferous vegetables is attributed to GSs; however, GSs are not bioactive as demonstrated in

cancer cell toxicity experiments (Musk et al., 1995). In contrast, their degradation products, ITCs, are extremely potent and have shown remarkable ability to act on the process of carcinogenesis by affecting all three phases: tumor initiation, promotion and progression phases, and also by suppressing the final steps of carcinogenesis, i.e. angiogenesis and metastasis (Fahey and Talalay, 1999). Data also suggest a strong association between ITCs and induction of Phase II detoxifying enzymes such as glutathione-S-transferase(GST), UDP-glucuronosyl transferase (UGT), NAP(P)H:Quinone Oxidoreductase (NQO1), thioredoxinreductase 1 (TR1) and heme oxygenase 1(HO-1). Isothiocyanates are powerful electrophiles and readily react with S, N, and oxygen-based nucleophiles (Fahey and Talalay, 1999). The most notable ITC, sulforaphane, a hydrolysis product of 4-(methylsulfinyl)butenyl GS, is a powerful natural inducer of phase II detoxifying enzymes (Fahey et al., 1997). Other studies have shown that ITCs, such as benzyl, allyl, phenylethyl, and 4-(methylsulfinyl)butenyl induced apoptosis in colorectal adenocarcinoma cells (HT29) by rapidly blocking proliferating cancer cells (Lund et al., 2001).

Selenium (Se) is an essential micronutrient in mammalian nutrition which exhibits antioxidant activity through inhibiting experimental carcinogenesis in animal models and reducing cancer incidence in human clinical trials (Clark et al., 1996; Combs and Gray, 1998). A specific interest in S and Se nutrition has transpired as a result of their regulation of GS metabolism (Toler et al., 2007) and function in nutritional metabolites. Selenium is similar to S in both size and chemistry and, thus, often substitutes for S in physiological and metabolic processes (Anderson and Scarf, 1983). *Brassica* species have been shown to accumulate large quantities of Se through the S assimilation pathway and has been shown to affect GS production in rapid-cycling *B. oleracea* (Charron et al., 2002) by varying Se fertilizer regimes. Since Se readily accumulates in *Brassica* species, the analysis of these metabolites is necessary to evaluate

the mechanisms by which GSs and ITC are affected metabolically and how they participate in cancerous control strategies. Thus, additions of both Se and GS-containing *Brassica* vegetables in an individual's diet may offer sizeable health benefits.

Understanding of genetic elements that contribute to Se assimilation and its effects on S and GS metabolism can impact future phytonutrient improvements in *B. oleracea* and other important agronomic crops. *Brassica* species share a close taxonomic and molecular relationship with *A. thaliana*. However, there are several subtle but distinct metabolic characteristics in *A. thaliana* that differ from *Brassica* crop species. To address these, the use of *B. oleracea* on *A. thaliana* microarrays has given insight to homology of key genes, proteins, and enzymes in biochemical experiments (Kang et al., 2008). Therefore, microarrays can be tested with specific RNA targets from embryos, germinating seeds, or leaf tissues to indicate expression patterns for genes unique to *Brassica* species (Li et al., 2003). The justification of this project was to study the effects of Se fertilization on S assimilation and GS metabolism in *A. thaliana* while comparing this to the food crop *B. oleracea.* The similarity of S and Se assimilation and GS metabolism in *A. thaliana* and *B. oleracea* allows the study of these mechanisms across the *Brassicaceae* family. The extensive genetic and biochemical analysis of *A. thaliana* gives our experiment a foundation to study the regulation of S, Se, and GS metabolism in *B. oleracea*. In the current study, we have compared the impact of Se fertilization on ITC concentrations between *A. thaliana* cv. Columbia and a rapid cycling base population of B. *oleracea* to determine the level of Se needed in fertilizer solutions to optimize plant tissue Se and GS concentrations and maximize potential human health benefits.

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Materials and Methods

Experiments were conducted and set by randomized complete block design 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* cv. Columbia (Arabidopsis Biological Resource Center, The Ohio State University, Columbus, OH) were sown into Oasis® cubes (Smithers-Oasis North America, Kent, OH) and germinated in a growth chamber (Conviron Controlled Environments Inc., Pimbenia, ND). One week after germination and the appearance of the first true leaves, treatments were initiated. *Brassica oleracea* and *A. thaliana* plants were grown hydroponically with a half strength Hoagland's nutrient solution in 11 L reservoirs. There were four blocks containing four replications of each treatment for both species, with individual reservoirs representing an experimental unit. Each reservoir contained 6 plants. The treatments consisted of the control (half strength nutrient solution, containing no Se) and four Se treatments (0.4, 0.8, 1.6, and 3.2 mg $Se \cdot L^{-1}$), one Se and S combination treatment (0.8 mg $Se \cdot L^{-1}$ and elevated 37 mg $SO_4 \cdot L^{-1}$), and one S treatment (elevated 37 mg $SO_4 \cdot L^{-1}$). The elevated sulfate treatments were added to the 96 $mg \cdot L^{-1}$ SO_4 already in a half strength Hoagland's nutrient solution to give a total of 133 $mg \cdot L^{-1}$ SO_4. Plants of *A. thaliana* were grown in a controlled environment growth chamber at 18 °C under an 8 hr photoperiod. Light intensity was measured at 354 $\mu mol \cdot m^{-2} \cdot s^{-1} \pm 4\%$ photosynthetically active radiation (PAR). Plants of *B. oleracea* were grown in a greenhouse at 23 °C under a 16 hr photoperiod and 18°C under an 8 hr dark period. Average light intensity measured 854 $\mu mol \cdot m^{-2} \cdot s^{-1} \pm 4\%$ PAR.

Twenty-eight to 31 days after seeds were sown, just before anthesis, all plants were harvested. Leaves and stems were immediately separated into equal halves and frozen in an ultra low -80 °C freezer (Isotemp, Fisher Scientific, Waltham, MA). When frozen, half of the tissue was lyophilized (Labconco, Kansas City, Mo.) to remove water content and prevent GS degradation.

For glucosinolate analysis, 200 ± 0.1 mg of lyophilized leaf tissue samples were combined with 1 mL of benzylglucosinolate solution (1mM) as an internal standard, 2.0 ml of methanol, and 0.3 ml of barium lead acetate (0.6mM) in a 16 mm x 100 mm culture tube and vortexed at 60 rpm for 1 hr. Each tube was centrifuged at 2000 g_n for 10 min. An aliquot of 0.5 ml of supernatant was added to a 1 ml column (Visiprep^{TM} solid phase extraction Vacuum Manafold, Supelco, St. Louis, MO) containing 0.3 ml of DEAE Sephadex A-25 (Sigma-Aldrich, St. Louis, MO) and desulfated by the procedure of Raney and McGregor (1990).

Extracted desulfoglucosinolates were separated in a high performance liquid chromatography (HPLC) unit with a photodiode array detector (1100 series, Agilent Technologies, Santa Clara, CA) using a reverse-phase 250 mm x 4.6 mm i.d., 5-µm Luna C_{18} column (Phenomenex, Inc., Torrance, CA) 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.

Isothiocyanates were extracted according to the procedure of Brown and Morra (1994) with modifications. 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 2000 g_n for 10 min. The hexane

was discarded, and 10 ml of methylene chloride (MeCl), 5 ml RO water (< 18.0 MΩ) and 2 ml of 100 µmol methyl isothiocyanates (MITC) as an internal standard were added and vortexed. The mixture was added to an orbital shaker for 2 hr at 150 rpm then centrifuged. The organic fraction was placed into a new test tube, and the pellet was re-extracted with 2 ml MeCl and 1 ml of RO water according to the steps above. Two grams of anhydrous sodium sulfate was added to the combined organic fractions and allowed to set for 1 hr. 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 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 (< 18.0 MΩ). The 20 mm cap (rubber/Teflon septa) was immediately crimped to minimize volatile compounds loss. The sample was vortexed for 30 sec then placed on the carousel for analysis with a G1888 Headspace analyzer (Agilent Technologies Inc., Wilmington, DE). Hydrated plant material was incubated for 30 min at 45 ºC, and vials were pressurized at 0.965 bar.

Sample analysis was conducted using a Hewlett-Packard 5890 Series II gas chromatograph (GC), 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 x 0.25 mm x 0.25 µm. The following conditions were used with the FID: injection, 1 µl; inlet, 250 °C; He flow rate, 37 $cm \cdot sec^{-1}$; detector, 260 °C; purge time after injection, 0.5 min.; initial oven temperature 35 °C for 2 min., increasing 10 $°C \cdot min^{-1}$ 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) with the same column, coupled to a 5973N quadrupole mass selective

(MS) detector, was used for identification of the compounds. The MS parameters were as followed: injection, 1 µl; inlet, 250°C; MS source, 230; MS quad, 150; Aux-2 temp, 280. For headspace analysis the GC oven ranged from 35°C for 2 min increasing 20°C per min. to 220°C. Total run time was approximately 18 min.

Nutrient analysis for Se and S was performed using a 100 mg subsample of lyophilized plant tissue was combined with 10 ml of 70% HNO_3 and digested in a microwave digestion unit (Model: Ethos, Milestone Inc., Shelton, CT). The microwave temperature was ramped to 140 °C for 5 min at 1000W and 2000 kPa, followed by an increase to 210 °C for 10 min at 1000W and 3000 kPa. Next, microwave temperature was held at 210 °C for 10 min. at 1000W and 4000 kPa and cooled for 10 min at 0W 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 that consisted of 2% HNO_3 and 0.5% HCl (v/v) and measured by inductively coupled plasma mass spectroscopy (ICP-MS; Agilent Technologies, Inc., Wilmington, DE) equipped with a CETAC (ASX-510, CETAC, Omaha, NE) autosampler.

Statistical analysis of data was performed using SAS (Version 9.1.3 for Windows, SAS Institute, Cary, N.C.).

Results and Discussion

Glucosinolates extracted from *B. oleracea* leaf tissue and identified by HPLC as desulfoglucosinolates were glucoiberin (3-(methylsufinyl)propyl), progoiterin (2(R)-2-hydroxy-3-butenyl), glucoraphanin (4-(methylsufinyl)butyl), sinigrin (2-propenyl), glucosinalbin (4-hydroxybenzyl), gluconapin (3-butenyl), glucobrassicin (Indole-3-methyl), 4-methoxyglucobrassicin (4-methoxyindol-3-ylmethyl), and neoglucobrassicin (1-methoxyindol-3-ylmethyl) (Appendix A; Table 6). Glucosinolates in this study have been identified in other *B.*

oleracea vegetables which include cabbage, Brussels sprouts, kale, cauliflower, and kohlrabi (Ciska et al., 2000; Bellostas, 2007). Although concentrations of gluconasturtiin (2-phenylethyl) have been identified in *B. oleracea* plants (Kushad et al., 1999; Ciska et al., 2000; Bellostas, 2007), we were unable to detect and quantify it accurately.

Isothiocyanates from *B. oleracea* leaf tissuewere identified by GC-FID and GC-MS as either volatile headspace samples or extracted compounds in MeCl. Isothiocyanates identified by GC-FID included allyl, 3-butenyl, iberin, sulforphane, goiterin, and 2-phenylethyl (Appendix A; Table 7) with low/trace amounts of 4-hydroxybenzyl and indole-3-carbinol. Other ITCs from their respective GSs were not detected or identified due to their unstable nature as ITCs.

Glucosinolates extracted from *A. thaliana* leaf tissue and identified by HPLC as desulfoglucosinolates were glucoiberin (3-(methylsufinyl)propyl), glucoraphanin (4-(methylsufinyl)butyl), sinigrin (2-propenyl), glucosinalbin (4-hydroxybenzyl), gluconapin (3-butenyl), 4-hydroxyglucobrassicin (4-hydroxyindol-3-ylmethyl), glucoerucin (4-(methylthio)butyl), glucobrassicin (Indole-3-methyl), 4-methoxyglucobrassicin (4-methoxyindol-3-ylmethyl), and neoglucobrassicin (1-methoxyindol-3-ylmethyl) (Appendix A; Table 8). Glucosinolates in this study have also been identified in other studies with *A. thaliana* (Peterson et al., 2004). Although concentrations of gluconasturtiin (2-phenylethyl) have been identified in *A. thaliana* plants (Peterson et al., 2004), we were unable to detect and quantify these accurately.

Isothiocyanates identified from *A.thaliana* were identified by GC-FID and GC-MS as extracted compounds in MeCl. Isothiocyanates identified by GC-FID included 3-butenyl, iberin, sulforphane, erucin, indole-3-carbinol, and 2-phenylethyl (Appendix A; Table 9). Other ITCs identified by GC-MS were in trace/low amounts were allyl and 4-hydroxybenzyl. These ITCs

were not consistently detected and difficult to quantify. Other ITCs from their respective GSs were not detected or identified due to their unstable nature as ITCs.

Total GS concentration in *B. oleracea* decreased while leaf Se concentration increased with the most prominent decrease of 63.6% from 1.6 to 3.2 mg $Se \cdot L^{-1}$. Diminishing GS concentrations when exposed to increasing concentrations of Se has been observed in *B. oleracea* within a number of studies. Charron et al. (2002) found a significant decline in GSs when exposed to Se concentrations up to 9.0 $mg \cdot L^{-1}$ $NaSeO_4$ and Toler et al. (2007) found significant decreases in GS concentrations when plants were exposed to Se fertilization at 1.5 $mg \cdot L^{-1}$ SeO_4.

The highest concentration of total GSs were found in the elevated sulfate treatment. However, total GSs in the elevated sulfate treatment was not significant when compared to the control, although there was a 16.4% difference. There was a significant increase ($P < 0.05$) in total GSs from the elevated sulfate treatment compared to the combination of Se and sulfate treatment. Under increasing S fertilization treatments, Mailer (1989) and Toler et al. (2007) found elevated concentration of GSs, similar to the results of the current study. Aliphatic GSs had similar trends with a 21.5% and a 37.3% decrease in the control and combination of Se and sulfate treatments compared to the elevated sulfate treatment, respectively.

Independently, the production of glucoiberin decreased with increasing Se concentration treatments ($P < 0.05$). Plants that were exposed to 3.2 mg $Se \cdot L^{-1}$ treatment revealed a reduction of 74.1 % in the production of glucoiberin. In the elevated sulfate treatment, glucoiberin exhibited a 51.7% increase compared to the control and an 87.1% increase compared to the 3.2 mg $Se \cdot L^{-1}$ treatment. Interestingly, there was a considerable decrease of 60.1% in glucoiberin concentration when 0.8 mg $Se \cdot L^{-1}$ was combined with the elevated sulfate when compared with the elevated sulfate treatment alone. Glucoraphanin was not significantly affected by increasing

Se treatments; however, a significant decrease ($P < 0.05$) occurred when the elevated sulfate treatment was compared in a t-test to 3.2 mg $Se \cdot L^{-1}$ treatment. Glucoiberin and glucoraphanin are the only two GSs detected in *B. oleracea* in this study that contain an alkythioalkyl side chain in which S is retained from methionine in the side chain modification step of GS biosynthesis. Models for side chain modification from Mithen et al. (1995) and Mithen (1996) has shown oxidation of the methylthio group in glucoiberin and glucoraphanin

The production of sinigrin and progoitrin was significantly affected ($P < 0.05$) by the presence of increasing concentrations of Se treatments. Sinigrin exhibited a 64.2% and progoitrin a 94.5% decrease from 0.0 to 3.2 mg $Se \cdot L^{-1}$. Notably, both sinigrin and progoitrin showed increases in concentration of 19% and 23.1% in the elevated sulfate treatment compared to the control. Sinigrin and progoitrin are derived from methionine side-chain modification and are both aliphatic olefins. Side-chain modification of sinigrin consists of a desaturation of the alkyl group and progoitrin's alkyl group going through a hydroxylation modification. Other notable GSs are sinalbin, glucobrassicin, neoglucobrassicin, and 4-methoxyglucobrassicin; however, these were not significantly affected by increasing Se concentration in the nutrient solution or by the presence of elevated sulfate.

Isothiocyantes extracted from the *B. oleracea* plants and identified by GC-FID and GC-MS were allyl, sulforphane, 3-butenyl, iberin, goitrin, sec-butyl, neopentyl, and 4-methylpentyl. Total ITCs decreased significantly ($P < 0.05$) from 0.0 to 3.2 mg $Se \cdot L^{-1}$with a 63.6% drop. Sulforphane was significantly affected ($P < 0.05$) by increasing concentrations of Se treatments, with a 94.7% decrease from 0.0 to 3.2 mg $Se \cdot L^{-1}$. Interestingly, sulforphane was not affected by the elevated sulfate treatment when comparing it to the control. Although there were no

significant differences in iberin and goitrin, these ITCs showed a 37.5% and an 86% increase in concentration in the elevated sulfate treatment, respectively.

Isothiocyanates allyl, 3-butenyl, sec-butenyl, neopentyl, and 4-methylpentyl were identified by GC-HS due to the volatility of the compounds, and these isothiocyantes had no significant differences among treatments. However, allyl and 3-butenyl showed increases in concentrations, when comparing the control and elevated sulfate treatments, of 50.6% and 54.8%, respectively. When comparing the control to 3.2 mg $Se \cdot L^{-1}$ treatment there was a 43.4% decrease in allyl and a 63.7% decrease in 3-butenyl. Treatment comparison distinguished significant ($P < 0.05$) increases for allyl, 3-butenyl, neopentyl, and total ITCs between 3.2 mg $Se \cdot L^{-1}$ and the elevated sulfate treatments. Consequently, these ITCs contribute mainly to flavor and aromas due to the volatile nature of the compounds instead of being orally digested and contributing to the overall anticarcinogenic properties with other ITCs (Traka and Mitten, 2009)

In *A. thaliana,* total GSs decreased 46.2% from 1.6 to 3.2 mg $Se \cdot L^{-1}$, while a decrease of 72.2% was observed from Se treatments 0.0 to 3.2 mg $Se \cdot L^{-1}$. These significant decreases in GS concentrations were comparable to the decline in GSs in *B. oleracea*. This is one of the first observations of the effect of increasing Se concentration in the fertilizer solution on GS concentrations in *A. thaliana*. Recent studies in our lab 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 $Se \cdot L^{-1}$ applied to a soilless medium (data not shown). Aliphatic GSs also demonstrate a significant decrease ($P < 0.01$) with an 81.6% difference from 0.0 to 3.2 mg $Se \cdot L^{-1}$. Overall, aliphatic GS concentrations in *A. thaliana* were higher in the elevated sulfate treatment. This is consistent with increases in aliphatic GS concentrations observed in *B. oleracea* exposed to the same elevated sulfate treatment.

The production of glucoiberin and glucoraphanin was negatively impacted with increasing Se concentration in the nutrient solution ($P<0.01$). Plants exposed to 3.2 mg $Se \cdot L^{-1}$ treatment exhibited 86.8% and 72.8% reductions in the production of glucoiberin and glucoraphanin, respectively, when compared to plants in the control treatment. Further, plants exposed to elevated sulfate levels in the nutrient solution exhibited a significant increase in glucoiberin production, with a concentration of 18.4% higher than the control treatment. Plants in the combination treatment of 0.8 mg $Se \cdot L^{-1}$ and elevated sulfate had glucoiberin and glucoraphanin equal to plants in the control treatment. Plants exposed to elevated sulfate levels in the nutrient solution exhibited a significant increase in glucoerucin production, with a concentration 34.8% higher than the control treatment ($P<0.05$).

Indole GS concentrations in leaf tissues were negatively impacted by increasing Se in the nutrient solution ($P<0.05$). Plants exposed to 3.2 mg $Se \cdot L^{-1}$ treatment had a 60% reduction overall in indole GSs when compared to the control. 4-hydroxyglucobrassicin exhibited the most dramatic reduction in concentration with a 90% difference when comparing the control to the 3.2 mg $Se \cdot L^{-1}$ treatment. Other indole GSs, such as glucobrassicin, 4-methoxyglucobrassicin, and neoglucobrassicin showed similar trends with 62.3%, 27.7%, and 81.6% reductions in concentrations, respectively.

Isothiocyantes extracted from the *A. thaliana* plants and identified by GC-FID and GC-MS were 3-butenyl, iberin, sulforphane, erucin, phenethyl, and trace amounts of allyl and the indole compound indole-3-carbinol. Erucin and phenethyl ITCs did not vary with increasing concentration of Se treatments in the nutrient solution, although there were 66.7% and 95.1% reductions in quantity when comparing 0.0 to 3.2 mg $Se \cdot L^{-1}$ treatments, respectively. Sulforphane, iberin, and 3-butenyl were the predominant ITCs in *A. thaliana* leaf tissue.

Sulforphane was highest with 71.6% of the total ITCs present in the control treatment, with iberin and 3-butenyl making up 12.7% and 4.9%, respectively. Sulforphane 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 $Se \cdot L^{-1}$. Iberin exhibited an 87.3% decrease in production and 3-butenyl a 90.6% decrease when comparing 0.0 to 3.2 mg $Se \cdot L^{-1}$ treatments. In the elevated sulfate treatment there was an overall increase of 22.2% in total ITCs when compared to the control treatment. The combination of 0.8 mg $Se \cdot L^{-1}$ and the elevated sulfate treatment showed a similar trend with a 21.7% increase in production of total ITCs. This suggests that combining a Se treatment not only increases the amount of Se, but also causes high concentrations of ITCs in the leaf tissue.

Conversions of GSs to ITCs were calculated based on the mean values at each treatment. In general, the highest percent conversion occurred at the control, combination of Se and elevated sulfate, and the elevated sulfate treatments. Lower percent conversions occurred at the highest two Se treatments of 1.6 and 3.2 mg $Se \cdot L^{-1}$. In *A. thaliana*, erucin had the highest conversion efficiency at 41.9%. Indole-3-carbinol exhibited the lowest conversion efficiency with 1.8%; however, this may be due to its unstable nature and conversion to other low molecular weight indoles (Appendix A; Table 10). In *B.oleracea*, sulforphane had the largest conversion efficiency at 59.5% and allyl ITC the lowest at 19.8% (Appendix A; Table 11). Generally, the conversion efficiency of GSs to ITCs decreased with increasing Se treatments, thus the decrease in conversion efficiency may be due to the increasing concentrations of Se in the leaf tissue (Appendix B; Figure 6). Further studies are needed to determine Se affect on myrosinase activity and its effect on the conversion efficiency.

As Se concentrations increased in the nutrient solution, *B. oleracaea* leaf tissue Se concentration increased to a mean level of 2056.69 µg $Se \cdot g^{-1}$ dry weight (DW). Means ranged from 0.0 to 2056.69 µg $Se \cdot g^{-1}$ DW in the 0.0 to 3.2 $mg \cdot L^{-1}$ Se treatments, respectively. Leaf Se increased significantly from 1.6 to 3.2 mg $Se \cdot L^{-1}$ (Appendix B; Figure 8). Consequently, there was a significant increase of S in the leaf tissue leading to a 74.3% change from 1.6 to 3.2 mg $Se \cdot L^{-1}$ (Appendix B; Figure 7). This trend was also demonstrated by Kopsell et al. (1999) and Toler et al. (2007), in which increasing concentrations of Se in the nutrient solution increased S concentrations in the leaf tissue.

In *A. thaliana*, Se concentration in the leaf tissue increased significantly ($P<0.001$) as Se treatment concentrations increased in the nutrient solution. Leaf tissue concentrations of Se accumulated to a mean value of 3724.31 µg $Se \cdot g^{-1}$ DW (Appendix B; Figure 8) and S concentration to 90141 µg $Se \cdot g^{-1}$ DW (Appendix B; Figure 7). The dramatic increase of Se and S in the leaf tissue occurred in the 1.6 and 3.2 mg $Se \cdot L^{-1}$ treatments leading to a decrease in the production of GSs and ITCs. This is, to our knowledge, the first report on the effects of increasing Se treatment concentrations in the nutrient solution on S accumulation in *A. thaliana* leaf tissue.

In contrast to the GSs measured from the leaf tissue harvested in *A. thaliana* and *B. oleracea* just before anthesis, 2-phenethyl was not detected, perhaps due to concentrations below the detection limit of the HPLC detector. However, analysis of ITCs resulted in detectable levels of 2-phenethyl ITCs. The result of the ITC detection may be due to the greater sensitivity of the GC-FID and GC-MS.

Concepts such as diets high in phytonutrients aimed at providing cancer protection to large segments of the population that are not at an increased risk for is rapidly growing. Because

of the intrinsic requirements of these concepts, a wide distribution method and expeditious way of delivering these protective agents is through diets high in fruits and vegetables that contain phytonutrients. Incidentally, a driving force for chemoprevention can be traced to the mounting epidemiological and experimental data that strongly suggest the beneficial effects of various fruit and vegetable constituents for human consumption.

Chemopreventive qualities of Se and degradation products of glucoraphanin, glucoiberin, glucoerucin, sinigrin, and indole-3-ylmethyl GSs have been extensively examined (Tanaka et al., 1990; Tanaka et al., 1992; Bradfield et al., 1987; Gamet-Payrastre et al., 1998; Lin et al, 1993; Clark et al, 1996, Mayland et al., 1989; Wang et al, 2005). Since glucoraphanin, glucoiberin, glucoerucin, sinigrin, and indole-3-ylmethyl GSs were detected in both *B. oleracea* and *A. thaliana* at all Se and elevated sulfate treatments, it is possible to simultaneously deliver dietary Se and chemopreventative ITCs in *Brassica* vegetable crops at concentrations 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, since sulforphane, iberin, and erucin are some of the most powerful natural inducers of phase II detoxification enzymes (Fahey and Talalay, 1999; Fahey et al., 1997; Wang et al., 2005). In addition to stimulating detoxification enzymes, these compounds protect against oxidative stresses by enhancing synthesis of glutathione, an antioxidant, and by inducing enzymes with antioxidant functions (Fahey et al., 1997). Whether significant decreases in GSs and subsequent declines in ITCs resulting from Se treatment fertilization is acceptable needs to be tested for efficacy 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 and how these compounds react together as alternative chemopreventative therapies.

The increase in S concentration with increasing Se treatments compared to S concentrations in the control treatment with no Se provided has been observed previously in rapid-cycling *B. oleracea* (Kopsell and Randle, 2000; Charron et al., 2001; Toler et al., 2007). At high ratios of Se/S fertilization, S and Se absorption and translocation are antagonistic, but at ratios of 1:125 to 1:500, Se fertilizations enhanced S uptake in onions (*Allium cepa* L.) (Kopsell and Randle, 1997). Nevertheless, the increased S uptake observed in this experiment resulted in lower GS concentrations. Selenium is incorporated into Se-amino acids such as Se-methylselenocystein, seleno-cystathione, and Se-methylselenomethionine and can replace S-amino acids as components of proteins (Anderson and Scarf, 1983). Although Se fertilization increased S uptake at low Se/S fertilization ratios, the synthesis of Se-amino acids and incorporation of these amino acids into proteins may adversely affect GS synthesis and metabolism.

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APPENDICES

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Appendix A
Tables

Table 1. Glucosinolate (GS) Concentrations (Mean ± SE) in *Arabidopsis thaliana* and rapid-cycling *Brassica oleracea* Leaf Tissue Grown at Different Selenium (Se) Concentrations

Glucosinolate concentrations (μmol·g^{-1} of dry weight)										
Se trt	GI	GR	SN	GN	GBN	4-MOBN	NGBN	aliphatic	indole	total
0.0	1.35 ± 0.22	6.15 ± 0.75	0.19 ± 0.10	0.48 ± 0.19	1.06 ± 0.15	0.16 ± 0.03	0.13 ± 0.04	8.52 ± 1.05	1.40 ± 0.20	9.94 ± 1.21
0.1	1.14 ± 0.22	4.93 ± 0.75	0.52 ± 0.10	0.75 ± 0.19	0.78 ± 0.15	0.12 ± 0.03	0.21 ± 0.04	7.77 ± 1.05	1.12 ± 0.20	8.92 ± 1.21
0.2	1.33 ± 0.22	5.49 ± 0.75	0.27 ± 0.10	0.48 ± 0.19	1.10 ± 0.15	0.17 ± 0.03	0.14 ± 0.04	7.96 ± 1.05	1.45 ± 0.20	9.45 ± 1.21
0.4	1.21 ± 0.22	5.64 ± 0.75	0.26 ± 0.10	0.63 ± 0.19	0.93 ± 0.15	0.12 ± 0.03	0.12 ± 0.04	8.20 ± 1.05	1.22 ± 0.20	9.47 ± 1.21
0.8	1.00 ± 0.22	5.41 ± 0.75	0.25 ± 0.10	0.40 ± 0.19	1.10 ± 0.15	0.14 ± 0.03	0.18 ± 0.04	7.53 ± 1.05	1.47 ± 0.20	9.05 ± 1.21
1.6	0.73 ± 0.22	3.00 ± 0.75	0.29 ± 0.10	0.43 ± 0.19	0.78 ± 0.15	0.15 ± 0.03	0.12 ± 0.04	4.71 ± 1.05	1.08 ± 0.20	5.83 ± 1.21
3.2	0.75 ± 0.22	2.76 ± 0.75	0.28 ± 0.10	0.77 ± 0.19	0.78 ± 0.15	0.12 ± 0.03	0.13 ± 0.04	4.98 ± 1.05	1.07 ± 0.20	6.08 ± 1.21
F-Test										
Species	***	***	***	***	***	***	***	***	***	***
Trt	*	**	NS	NS	NS	NS	NS	NS	NS	*
Species*Trt	*	**	NS	NS	NS	NS	NS	NS	NS	**
Linear	**	***	NS	NS	NS	NS	NS	***	NS	***
Quadratic	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

NS, *, **, *** Nonsignificant or Significant at P≤0.05, 0.01 or 0.001 Significance

GI-Glucoiberin; GR-Glucoraphanin; SN-Sinigrin; GN-Gluconapin; GBN-Glucobrassicin; 4-MOGB-4-methoxyglucobrassicin; NGBN-Neoglucobrassicin

Table 2. Glucosinolate (GS) Concentrations (Mean ± SE) in rapid-cycling *Brassica oleracea* Grown at Different Selenium Concentrations

Glucosinolate concentrations (μmol·g^{-1} of dry weight)										
Se trt	GI	GR	SN	GN	GBN	4-MOBN	NGBN	aliphatic	indole	total
0.0	0.36 ± 0.13	0.60 ± 0.19	0.46 ± 0.23	1.00 ± 0.40	0.08 ± 0.03	0.02 ± 0.01	0.02 ± 0.01	2.57 ± 0.60	0.12 ± 0.04	2.70 ± 0.58
0.1	0.33 ± 0.13	0.45 ± 0.19	0.99 ± 0.23	1.40 ± 0.40	0.10 ± 0.03	0.03 ± 0.01	0.03 ± 0.01	3.40 ± 0.60	0.16 ± 0.04	3.57 ± 0.58
0.2	0.41 ± 0.13	0.54 ± 0.19	0.51 ± 0.23	0.77 ± 0.40	0.13 ± 0.03	0.02 ± 0.01	0.01 ± 0.01	2.29 ± 0.60	0.16 ± 0.04	2.46 ± 0.58
0.4	0.32 ± 0.13	0.92 ± 0.19	0.49 ± 0.23	1.17 ± 0.40	0.09 ± 0.03	0.03 ± 0.01	0.02 ± 0.01	3.12 ± 0.60	0.14 ± 0.04	3.28 ± 0.58
0.8	0.12 ± 0.13	0.45 ± 0.19	0.46 ± 0.23	0.69 ± 0.40	0.11 ± 0.03	0.03 ± 0.01	0.03 ± 0.01	1.95 ± 0.60	0.16 ± 0.04	2.15 ± 0.58
1.6	0.27 ± 0.13	0.31 ± 0.19	0.52 ± 0.23	0.76 ± 0.40	0.10 ± 0.03	0.03 ± 0.01	0.02 ± 0.01	2.02 ± 0.60	0.14 ± 0.04	2.18 ± 0.58
3.2	0.47 ± 0.13	0.50 ± 0.19	0.50 ± 0.23	0.99 ± 0.40	0.16 ± 0.03	0.03 ± 0.01	0.02 ± 0.01	2.75 ± 0.60	0.20 ± 0.04	2.98 ± 0.58
F-Test	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Linear	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Quadratic	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

NS, *, **, *** Nonsignificant or Significant at $P \leq 0.05$, 0.01, or 0.001 Significance

GI-Glucoiberin; GR-Glucoraphanin; SN-Sinigrin; GN-Gluconapin; GBN-Glucobrassicin; 4-MOGB-4-methoxyglucobrassicin; NGBN-Neoglucobrassicin

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

Glucosinolate concentrations (μmol·g^{-1} of dry weight)													
Se trt	GI	GR	SN	SB	GN	4-HGB	GE	GBN	4-MOGB	NGB	aliphatic	indole	total
0.0	2.37 ± 0.42	11.74 ± 1.79	0.03 ± 0.01	0.01 ± 0.01	0.12 ± 0.05	0.09 ± 0.03	0.14 ± 0.07	2.05 ± 0.31	0.30 ± 0.07	0.25 ± 0.07	14.81 ± 2.34	2.69 ± 0.38	17.53 ± 2.66
0.1	1.93 ± 0.43	9.38 ± 1.85	0.03 ± 0.01	0.01 ± 0.01	0.11 ± 0.05	0.09 ± 0.03	0.18 ± 0.07	1.63 ± 0.33	0.28 ± 0.07	0.34 ± 0.07	12.00 ± 2.41	2.33 ± 0.40	14.38 ± 2.74
0.2	2.09 ± 0.42	10.12 ± 1.79	0.03 ± 0.01	0.01 ± 0.01	0.08 ± 0.05	0.10 ± 0.03	0.22 ± 0.07	2.04 ± 0.31	0.31 ± 0.07	0.23 ± 0.07	12.91 ± 2.34	2.68 ± 0.38	15.63 ± 2.66
0.4	2.10 ± 0.42	10.36 ± 1.79	0.03 ± 0.01	0.02 ± 0.01	0.10 ± 0.05	0.12 ± 0.03	0.28 ± 0.07	1.77 ± 0.31	0.21 ± 0.07	0.21 ± 0.07	13.29 ± 2.34	2.31 ± 0.38	15.65 ± 2.66
0.8	1.88 ± 0.42	10.39 ± 1.79	0.03 ± 0.01	0.02 ± 0.01	0.11 ± 0.05	0.10 ± 0.03	0.30 ± 0.07	2.10 ± 0.31	0.25 ± 0.07	0.33 ± 0.07	13.01 ± 2.34	2.78 ± 0.38	15.95 ± 2.66
1.6	1.19 ± 0.42	5.68 ± 1.79	0.04 ± 0.01	0.01 ± 0.01	0.10 ± 0.05	0.08 ± 0.03	0.22 ± 0.07	1.45 ± 0.31	0.27 ± 0.07	0.22 ± 0.07	7.41 ± 2.34	2.02 ± 0.38	9.47 ± 2.66
3.2	1.04 ± 0.42	4.90 ± 1.79	0.04 ± 0.01	0.00 ± 0.01	0.10 ± 0.05	0.09 ± 0.03	0.42 ± 0.07	1.42 ± 0.31	0.21 ± 0.07	0.21 ± 0.07	6.65 ± 2.34	1.95 ± 0.38	8.64 ± 2.66
F-Test	**	***	NS	NS	NS	NS	***	NS	NS	NS	***	NS	**
Linear	***	***	NS	NS	NS	NS	***	*	NS	NS	***	NS	***
Quadratic	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

NS, *, **, *** Nonsignificant or Significant at P≤0.05, 0.01 or 0.001 Significance

GI-Glucoiberin; GR-Glucoraphanin; SN-Sinigrin; SB-Sinalbin; GN-Gluconapin; 4-HGB-4-Hydroxyglucobrassicin; GE-Glucoerucin; GBN-Glucobrassicin; 4-MOGB-4-methoxyglucobrassicin; NGBN-Neoglucobrassicin

Table 4. Glucosinolate (GS) Concentrations (Mean ± SE) in *Arabidopsis thaliana* and rapid-cycling *Brassica oleracea* Leaf Tissue Grown at Different Selenium (Se) Concentrations

Glucosinolate concentrations (μmol·g^{-1} of dry weight)															
Se mg·L^{-1}	GI	GTN	GR	SN	SB	GN	4-HGB	GE	GB	4-MOGB	NGB	Aliphatic	Aromatic	Indoles	Total
0.0	1.32 ± 0.12	1.64 ± 0.40	9.23 ± 0.90	1.78 ± 0.25	0.75 ± 0.23	1.63 ± 0.42	0.15 ± 0.02	0.67 ± 0.12	7.23 ± 0.70	1.91 ± 0.17	2.14 ± 0.35	17.00 ± 1.11	0.75 ± 0.23	11.42 ± 1.08	29.18 ± 1.85
0.4	0.90 ± 0.12	0.37 ± 0.40	5.45 ± 0.90	1.81 ± 0.25	0.58 ± 0.23	3.36 ± 0.42	0.14 ± 0.02	0.82 ± 0.12	5.87 ± 0.70	1.31 ± 0.17	1.54 ± 0.35	13.33 ± 1.11	0.58 ± 0.23	8.86 ± 1.08	22.80 ± 1.85
0.8	0.77 ± 0.12	1.08 ± 0.40	4.10 ± 0.90	1.22 ± 0.25	0.43 ± 0.23	0.86 ± 0.42	0.12 ± 0.02	0.87 ± 0.12	5.51 ± 0.70	0.19 ± 0.17	0.83 ± 0.35	9.63 ± 1.11	0.43 ± 0.23	7.65 ± 1.08	17.71 ± 1.85
1.6	0.56 ± 0.12	0.30 ± 0.40	2.52 ± 0.90	1.00 ± 0.25	0.26 ± 0.23	1.42 ± 0.42	0.05 ± 0.02	1.37 ± 0.12	5.17 ± 0.70	1.14 ± 0.17	0.94 ± 0.35	7.57 ± 1.11	0.26 ± 0.23	7.31 ± 1.08	15.14 ± 1.85
3.2	0.26 ± 0.12	0.09 ± 0.40	0.83 ± 0.90	0.90 ± 0.25	0.23 ± 0.23	0.77 ± 0.42	0.02 ± 0.02	0.98 ± 0.12	3.59 ± 0.70	1.77 ± 0.17	0.66 ± 0.35	4.50 ± 1.11	0.23 ± 0.23	6.04 ± 1.08	10.75 ± 1.85
0.8/37	1.30 ± 0.12	0.11 ± 0.40	8.43 ± 0.90	1.40 ± 0.25	0.27 ± 0.23	2.56 ± 0.42	0.16 ± 0.02	0.75 ± 0.12	6.35 ± 0.70	2.31 ± 0.17	1.55 ± 0.35	15.25 ± 1.11	0.27 ± 0.23	10.35 ± 1.08	25.86 ± 1.85
37	1.72 ± 0.12	2.14 ± 0.40	8.86 ± 0.90	2.23 ± 0.25	0.82 ± 0.23	1.76 ± 0.42	0.13 ± 0.02	1.11 ± 0.12	7.18 ± 0.70	2.40 ± 0.17	1.09 ± 0.35	18.53 ± 1.11	0.82 ± 0.23	10.80 ± 1.08	30.15 ± 1.85
F-Test															
Species	***	***	***	***	NS	***	***	***	***	***	***	***	NS	***	***
Trt	***	**	***	**	NS	**	***	**	**	***	NS	***	NS	**	***
Species x Trt	***	**	***	**	NS	**	***	**	NS	***	NS	***	NS	NS	***

NS, *, **, *** Nonsignificant or Significant at P≤0.05, 0.01 or 0.001 Significance

GI-Glucoiberin; PGN GR-Glucoraphanin; SN-Sinigrin; SB-Sinalbin; GN-Gluconapin; 4-HGB-4-Hydroxyglucobrassicin; GE-Glucoerucin; GBN-Glucobrassicin; 4-MOGB-4-methoxyglucobrassicin; NGBN-Neoglucobrassicin

Table 5. Isothiocyanate (ITC) Concentrations (Mean ± SE) in *Arabidopsis thaliana* and rapid-cycling *Brassica oleracea* Leaf Tissue Grown at Different Selenium (Se) Concentrations

Isothiocyanate (ITC) Concentrations (μmol·g^{-1} of dry weight)										
Se mg·L^{-1}	AITC	3-Butenyl	Erucin	Goiterin	Iberin	PEITC	SF	IC	Aliphatic	Total
0.0	0.57 ± 0.02	0.72 ± 0.05	0.47 ± 0.38	0.43 ± 0.03	0.17 ± 0.02	0.29 ± 0.06	2.66 ± 0.38	0.42 ± 0.04	4.59 ± 0.46	5.73 ± 0.49
0.4	0.24 ± 0.02	0.29 ± 0.05	0.20 ± 0.38	0.36 ± 0.03	0.09 ± 0.02	0.10 ± 0.06	0.61 ± 0.38	0.27 ± 0.04	1.44 ± 0.46	2.17 ± 0.49
0.8	0.24 ± 0.02	0.35 ± 0.05	0.43 ± 0.38	0.27 ± 0.03	0.09 ± 0.02	0.11 ± 0.06	0.81 ± 0.38	0.27 ± 0.04	1.97 ± 0.46	2.62 ± 0.49
1.6	0.05 ± 0.02	0.25 ± 0.05	0.38 ± 0.38	0.06 ± 0.03	0.07 ± 0.02	0.08 ± 0.06	0.34 ± 0.38	0.19 ± 0.04	1.08 ± 0.46	1.40 ± 0.49
3.2	0.04 ± 0.02	0.18 ± 0.05	0.01 ± 0.38	0.05 ± 0.03	0.05 ± 0.02	0.06 ± 0.06	0.09 ± 0.38	0.22 ± 0.04	0.34 ± 0.46	0.68 ± 0.49
0.8/37	0.42 ± 0.02	0.75 ± 0.05	0.53 ± 0.38	0.06 ± 0.03	0.30 ± 0.02	0.12 ± 0.06	3.39 ± 0.38	0.29 ± 0.04	5.54 ± 0.46	6.02 ± 0.49
37	0.58 ± 0.02	0.94 ± 0.05	0.58 ± 0.38	0.53 ± 0.03	0.30 ± 0.02	0.14 ± 0.06	3.51 ± 0.38	0.42 ± 0.04	5.89 ± 0.46	6.96 ± 0.49
F-Test										
Species	***	***	***	***	***	NS	***	***	***	***
Trt	***	***	*	***	***	NS	***	***	***	***
Species x Trt	***	***	*	***	***	NS	***	*	***	***

NS, *, **, *** Nonsignificant or Significant at P≤0.05, 0.01 or 0.001 Significance

AITC-Allyl isothiocyanates; PEITC- 2-phenethyl isothiocyanates; SF- Sulforphane; IC- Indole-3-carbinol

Table 6. Glucosinolate (GS) Concentrations (Mean ± SE) in rapid-cycling *Brassica oleracea* Leaf Tissue Grown at Different Selenium (Se) Concentrations

Glucosinolate (GS) Concentrations (μmol·g^{-1} of dry weight)													
Se mg·L^{-1}	GI	PGN	GR	SN	SB	GN	GBN	4-MOGB	NGBN	aliphatic	aromatic	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	0.45 ± 0.11	1.03 ± 0.35	9.73 ± 1.47	0.42 ± 0.13	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	0.33 ± 0.11	0.87 ± 0.35	7.97 ± 1.47	0.33 ± 0.13	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	0.32 ± 0.11	0.25 ± 0.35	5.86 ± 1.47	0.39 ± 0.13	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	0.30 ± 0.11	0.54 ± 0.35	5.31 ± 1.47	0.24 ± 0.13	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	0.30 ± 0.11	0.51 ± 0.35	2.92 ± 1.47	0.24 ± 0.13	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	0.49 ± 0.11	0.32 ± 0.35	7.78 ± 1.47	0.27 ± 0.13	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	0.46 ± 0.11	0.27 ± 0.35	12.40 ± 1.47	0.57 ± 0.13	3.26 ± 0.13	16.22 ± 1.73
F-Test	**	**	NS	***	NS	*	NS	NS	NS	***	NS	NS	***

NS, *, **, *** Nonsignificant or Significant at P≤0.05, 0.01 or 0.001 Significance
GI-Glucoiberin; PGN-Progoiterin; GR-Glucoraphanin; SN-Sinigrin; SB-Sinalbin; GN-Gluconapin; GBN-Glucobrassicin; 4-MOGB-4-methoxyglucobrassicin; NGBN-Neoglucobrassicin

Table 7. Isothiocyanate (ITC) Concentrations (Mean ± SE) in rapid-cycling *Brassica oleracea* Leaf Tissue Grown at Different Selenium (Se) Concentrations

Isothiocyanate (ITC) Concentrations (μmol·g^{-1} of dry weight)									
Se mg·L^{-1}	AITC	3-Butenyl	Goiterin	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	***	***	***	***	***	***	**	***	***

NS, *, **, *** Nonsignificant or Significant at P≤0.05, 0.01 or 0.001 Significance

AITC-Allyl isothiocyanates; PEITC- 2-phenethyl isothiocyanates; SF- Sulforphane; IC- Indole-3-carbinol

Table 8. Glucosinolate (GS) Concentrations (Mean ± SE) in *Arabidopsis thaliana* Leaf Tissue Grown at Different Selenium (Se) Concentrations

Glucosinolate (GS) Concentrations (µmol·g^{-1} of dry weight)														
Se mg·L^{-1}	GI	GR	SN	SB	GN	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	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	0.1 ± 0.0	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	0.0 ± 0.0	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	ND	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	ND	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	0.1 ± 0.0	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	0.0 ± 0.0	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	***	***	NS	NS	*	***	**	*	***	**	***	NS	*	***

NS, *, **, *** Nonsignificant or Significant at P≤0.05, 0.01 or 0.001 Significance
GI-Glucoiberin; GR-Glucoraphanin; SN-Sinigrin; SB-Sinalbin; GN-Gluconapin; 4-HGB-4-Hydroxyglucobrassicin; GE-Glucoerucin; GBN-Glucobrassicin; 4-MOGB-4-methoxyglucobrassicin; NGBN-Neoglucobrassicin

Table 9. Isothiocyanate (ITC) Concentrations (Mean ± SE) in *Arabidopsis thaliana* Leaf Tissue Grown at Different Selenium and Sulfur Concentrations

Isothiocyanate (ITC) Concentrations (µmol·g^{-1} of dry weight)									
Se 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	***	***	NS	NS	***	***	*	***	***

NS, *, **, *** Nonsignificant or Significant at P≤0.05, 0.01 or 0.001 Significance
AITC-Allyl isothiocyanates; PEITC- 2-phenethyl isothiocyanates; SF- Sulforphane;
IC- Indole-3-carbinol

Table 10. Conversion efficiencies of GSs to ITCs in rapid-cycling *Brassica oleracea*

Glucosinolate and Isothiocyanate Concentrations (μmol·g^{-1} of dry weight)																		
Se mg·L^{-1}	SN	AITC	% conversion	GN	3-Butenyl	% conversion	PGN	GT	% conversion	GI	Iberin	% conversion	GBN	IC	% conversion	GR	SF	% conversion
0.0	3.07	1.12	36.5%	2.91	1.07	36.8%	3.29	0.86	26.1%	0.28	0.08	28.6%	1.93	0.68	35.2%	0.19	0.06	31.6%
0.4	2.13	0.47	22.1%	4.92	0.39	7.9%	0.55	0.72	130.9%	0.18	0.06	33.3%	1.18	0.41	34.7%	0.2	0.03	15.0%
0.8	1.79	0.08	4.5%	1.65	0.4	24.2%	2.17	0.53	24.4%	0.2	0.07	35.0%	1.25	0.41	32.8%	0.04	0.02	50.0%
1.6	1.61	0.08	5.0%	2.85	0.39	13.7%	0.6	0.12	20.0%	0.18	0.06	33.3%	0.8	0.32	40.0%	0.07	0.02	28.6%
3.2	1.1	0.07	6.4%	1.55	0.33	21.3%	0.18	0.12	66.7%	0.08	0.05	62.5%	0.98	0.32	32.7%	0.01	0.02	200.0%
0.8/37	2.29	0.8	34.9%	4.97	0.7	14.1%	0.23	0.1	43.5%	0.22	0.09	40.9%	1.59	0.41	25.8%	0.07	0.03	42.9%
37	3.79	1.12	29.6%	3.5	1.08	30.9%	4.28	1.05	24.5%	0.56	0.09	16.1%	2.53	0.69	27.3%	0.27	0.13	48.1%
Mean	2.25	0.53	19.8%	3.19	0.62	21.3%	1.61	0.50	48.0%	0.24	0.07	35.7%	1.47	0.46	32.6%	0.12	0.04	59.5%

SN-Sinigrin; AITC-Allyl isothiocyanates; GN-Gluconapin; PGN-Progoiterin; GT-Goiterin; GI-Glucoiberin; GBN-Glucobrassicin; IC- Indole-3-carbinol GR-Glucoraphanin; SF- Sulforphane

Table 11. Conversion efficiencies from GSs to ITCs in *Arabidopsis thaliana*

Glucosinolate and Isothiocyanate Concentrations (μmol·g^{-1} of dry weight)															
Se mg·L$^{-}_{1}$	GE	Erucin	% Conversion	GI	Iberin	% Conversion	GR	SF	% Conversion	SN	AITC	% Conversion	GB	IC	% Conversion
0.0	1.35	0.933	69.1%	2.35	0.264	11.2%	18.27	5.255	28.8%	0.49	0.018	3.7%	12.53	0.159	1.3%
0.4	1.65	0.407	24.7%	1.53	0.101	6.6%	10.31	1.408	13.7%	0.80	0.009	1.1%	9.92	0.147	1.5%
0.8	1.74	0.853	49.0%	1.33	0.174	13.1%	8.17	1.831	22.4%	0.65	0.011	1.7%	9.78	0.129	1.3%
1.6	2.75	0.756	27.5%	0.94	0.082	8.7%	4.97	0.478	9.6%	0.39	0.006	1.5%	9.55	0.039	0.4%
3.2	1.93	0.016	0.8%	0.44	0.043	9.8%	2.01	0.149	7.4%	0.65	0.006	0.9%	6.17	0.111	1.8%
0.8/37	1.51	1.050	69.5%	2.37	0.508	21.4%	16.80	6.744	40.1%	0.50	0.034	6.8%	11.11	0.158	1.4%
37	2.19	1.150	52.5%	2.88	0.499	17.3%	17.46	6.767	38.8%	0.67	0.030	4.5%	11.82	0.130	1.1%
Mean	1.87	0.74	41.9%	1.69	0.24	12.6%	11.14	3.23	23.0%	0.59	0.02	2.9%	10.13	0.12	1.3%

GE-Glucoerucin; GI-Glucoiberin; GR-Glucoraphanin; SF- Sulforphane; SN-Sinigrin; AITC-Allyl isothiocyanates; GBN-Glucobrassicin; IC- Indole-3-carbinol

Appendix B
Figures

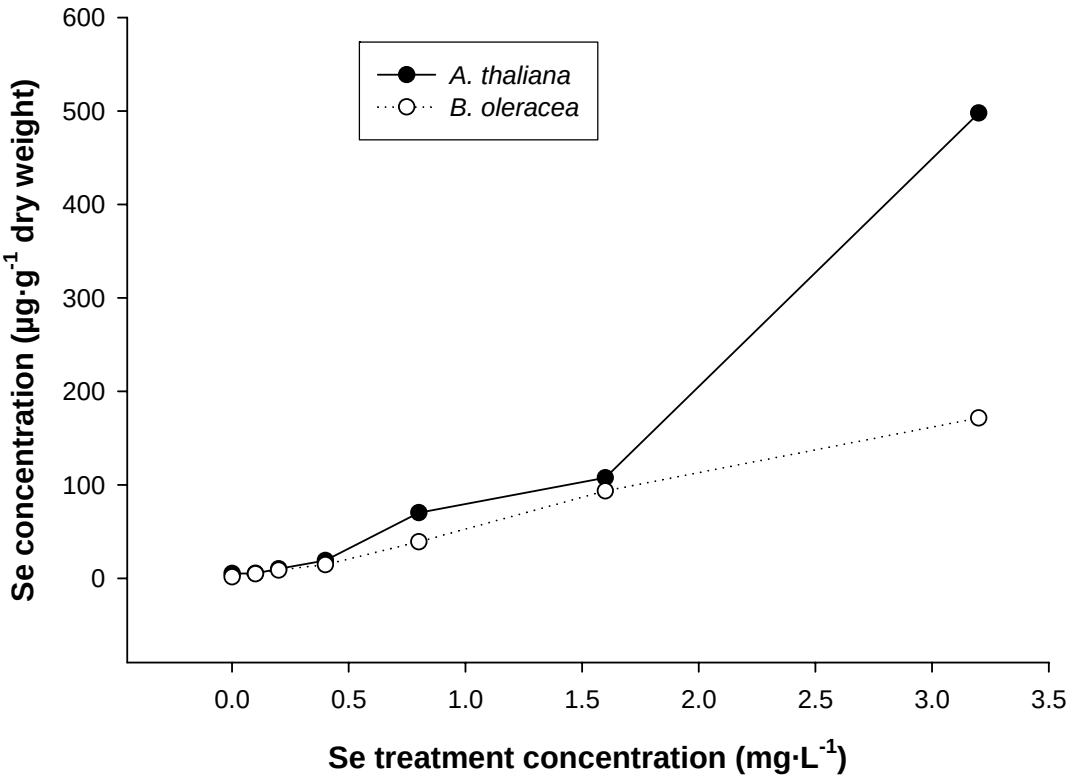

Figure 1. Selenium ($\mu g \cdot g^{-1}$ dry weight) concentrations in leaf tissue of *Arabidopsis thaliana* cv. Columbia and rapid-cycling *Brassica oleracea* grown in soilless medium fertilized with a modified full strength Hoagland's nutrient solution. Plants were exposed to Se concentrations ranging from 0.0 to 3.2 $mg \cdot L^{-1}$ weekly. Selenium was measured with an ICP-MS at The University of Tennessee-Knoxville, Plant Sciences Department.

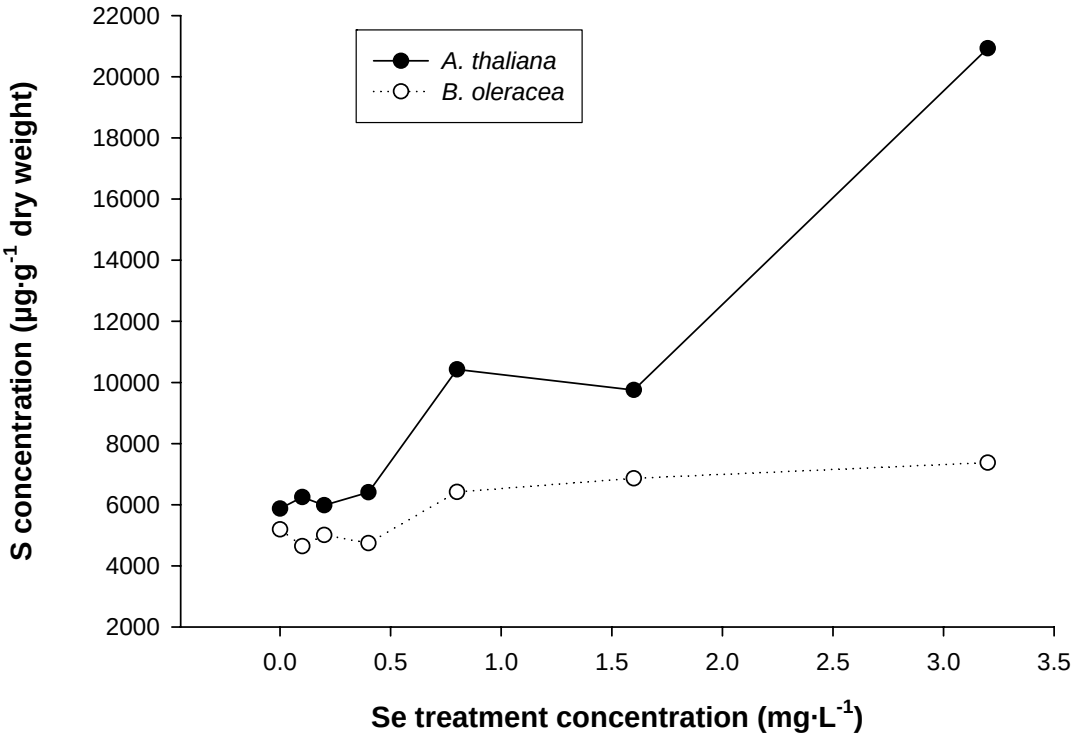

Figure 2. Sulfur ($\mu g \cdot g^{-1}$ dry weight) concentrations in leaf tissue of *Arabidopsis thaliana* cv. Columbia and rapid-cycling *Brassica oleracea* grown in soilless medium fertilized with a modified full strength Hoagland's nutrient solution. Plants were exposed to Se concentrations ranging from 0.0 to 3.2 $mg \cdot L^{-1}$ weekly. Sulfur was measured with an ICP-MS at The University of Tennessee-Knoxville, Plant Sciences Department.

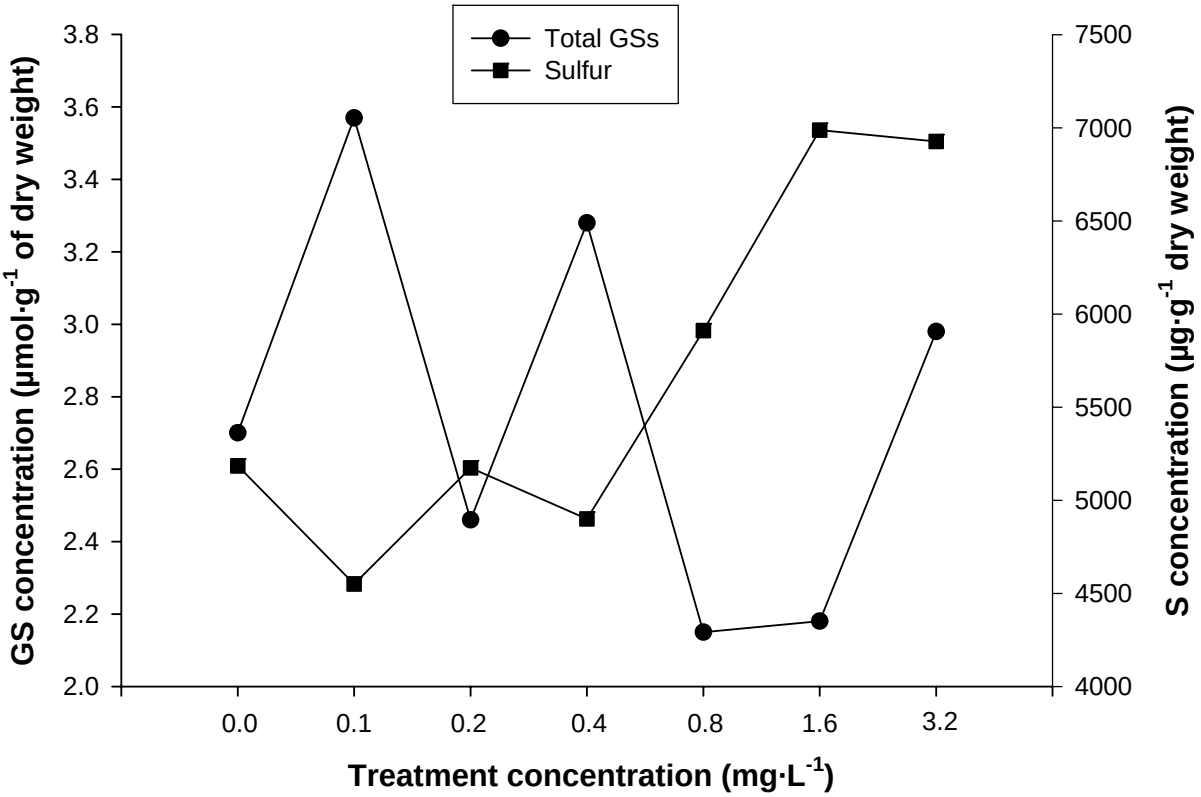

Figure 3. Total GS (µmol·g^{-1} dry weight) concentrations vs. total S in leaf tissue of rapid-cycling *Brassica oleracea* grown hydroponically with a half strength Hoagland's nutrient solution. Plants were exposed to Se concentrations ranging from 0.0 to 3.2 mg·L^{-1}, a combination of 0.8 mg·L^{-1} Se and +37 mg·L^{-1} elevated SO_4, and a +37 mg·L^{-1} elevated SO_4 treatments. The elevated SO_4 was added to the 96 mg·L^{-1} SO_4 already in the half strength Hoagland's nutrient solution.

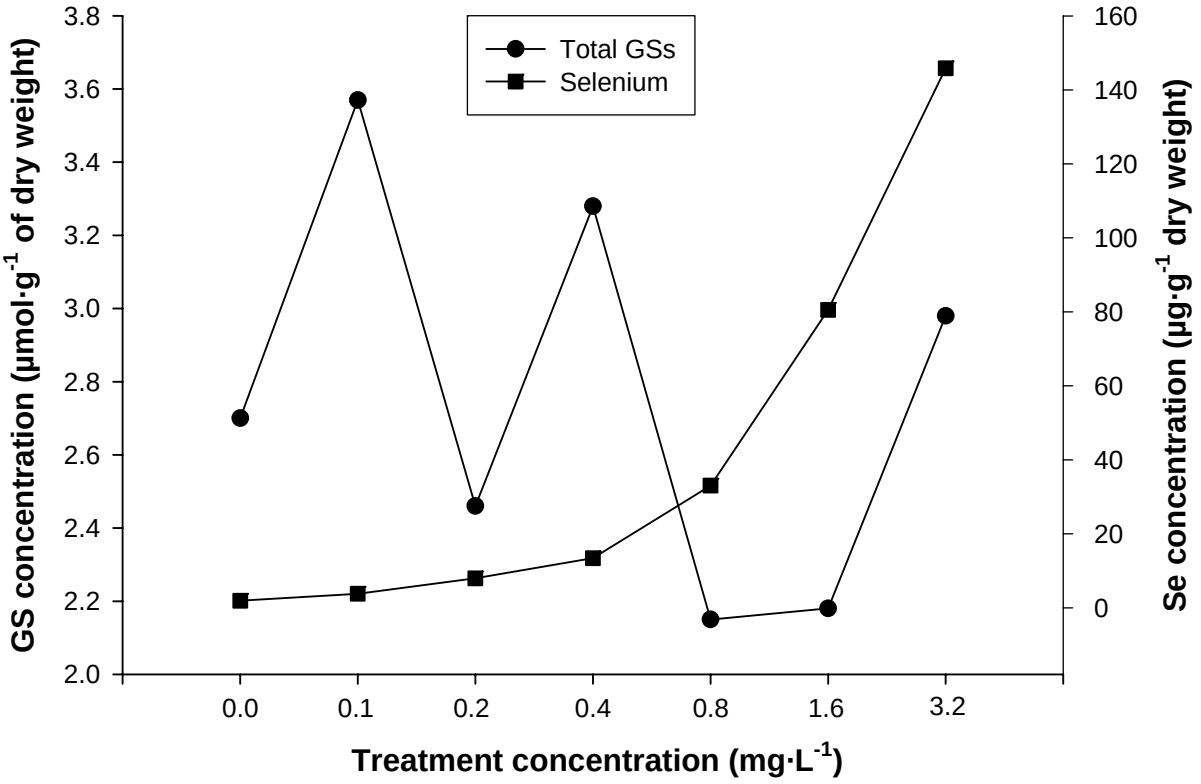

Figure 4. Total GS (µmol·g^{-1} dry weight) concentrations vs. total Se in leaf tissue of rapid-cycling *Brassica oleracea* grown hydroponically with a half strength Hoagland's nutrient solution. Plants were exposed to Se concentrations ranging from 0.0 to 3.2 mg·L^{-1}, a combination of 0.8 mg·L^{-1} Se and +37 mg·L^{-1} elevated SO_4, and a +37 mg·L^{-1} elevated SO_4 treatments. The elevated SO_4 was added to the 96 mg·L^{-1} SO_4 already in the half strength Hoagland's nutrient solution.

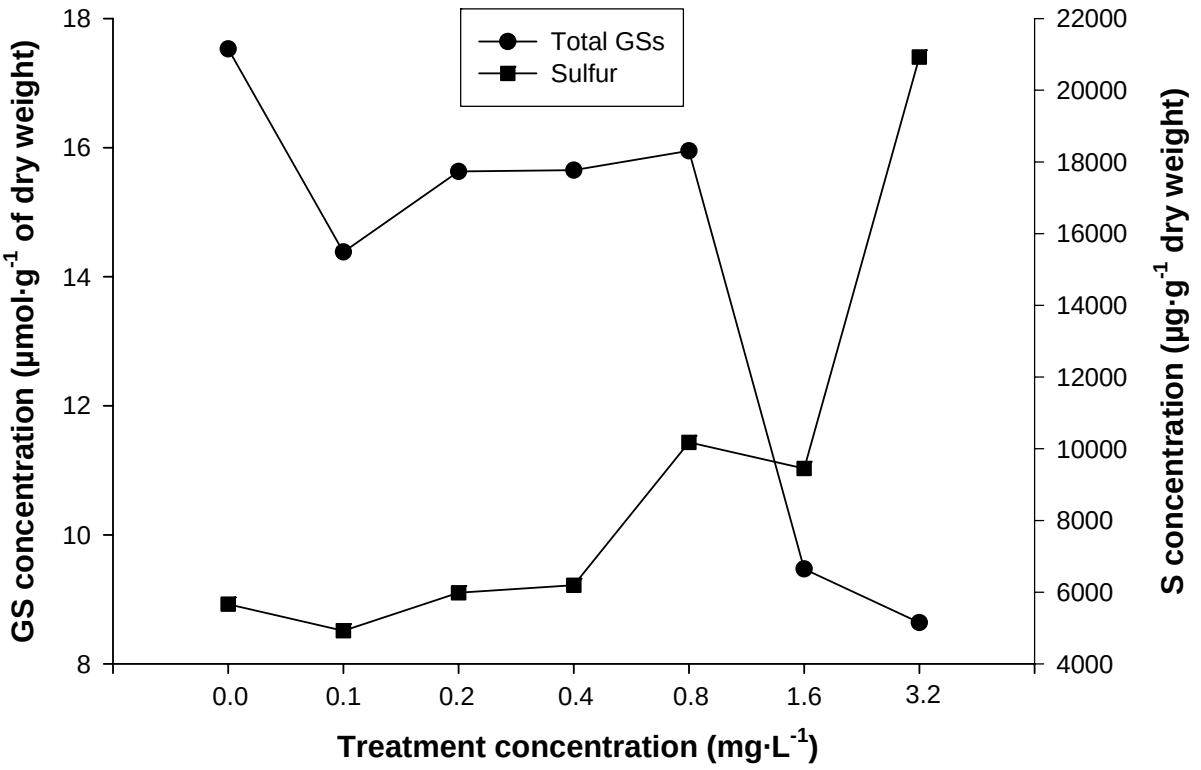

Figure 5. Total GS (µmol·g^{-1} dry weight) concentrations vs. total S in leaf tissue of *Arabidopsis thaliana* grown hydroponically with a half strength Hoagland's nutrient solution. Plants were exposed to Se concentrations ranging from 0.0 to 3.2 mg·L^{-1}, a combination of 0.8 mg·L^{-1} Se and +37 mg·L^{-1} elevated SO_4, and a +37 mg·L^{-1} elevated SO_4 treatments. The elevated SO_4 was added to the 96 mg·L^{-1} SO_4 already in the half strength Hoagland's nutrient solution.

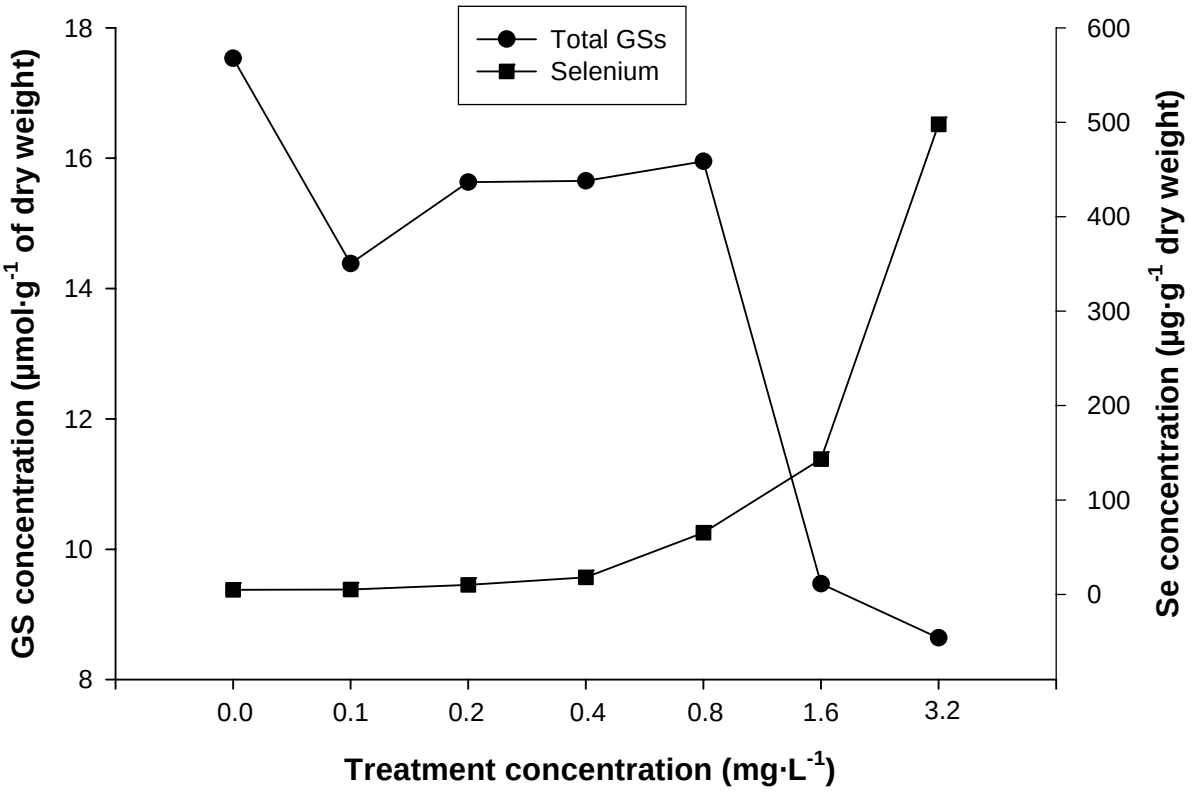

Figure 6. Total GS (µmol·g^{-1} dry weight) concentrations vs. total Se in leaf tissue of *Arabidopsis thaliana* grown hydroponically with a half strength Hoagland's nutrient solution. Plants were exposed to Se concentrations ranging from 0.0 to 3.2 mg·L^{-1}, a combination of 0.8 mg·L^{-1} Se and +37 mg·L^{-1} elevated SO_4, and a +37 mg·L^{-1} elevated SO_4 treatments. The elevated SO_4 was added to the 96 mg·L^{-1} SO_4 already in the half strength Hoagland's nutrient solution.

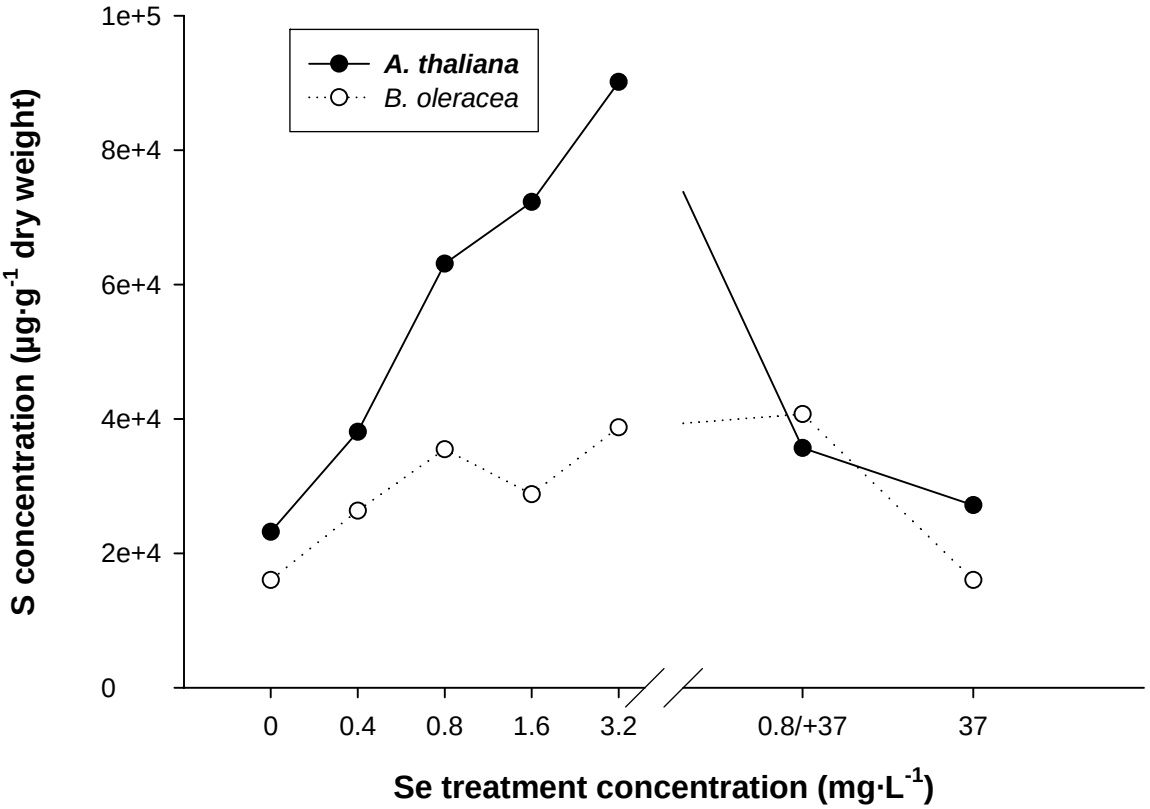

Figure 7. Sulfur ($\mu g \cdot g^{-1}$ dry weight) concentrations in leaf tissue of *Arabidopsis thaliana* cv. Columbia and rapid-cycling *Brassica oleracea* grown in soilless medium fertilized with a modified full strength Hoagland's nutrient solution. Plants were exposed to Se concentrations ranging from 0.0 to 3.2 $mg \cdot L^{-1}$ weekly. Sulfur was measured with an ICP-MS at The University of Tennessee-Knoxville, Plant Sciences Department.

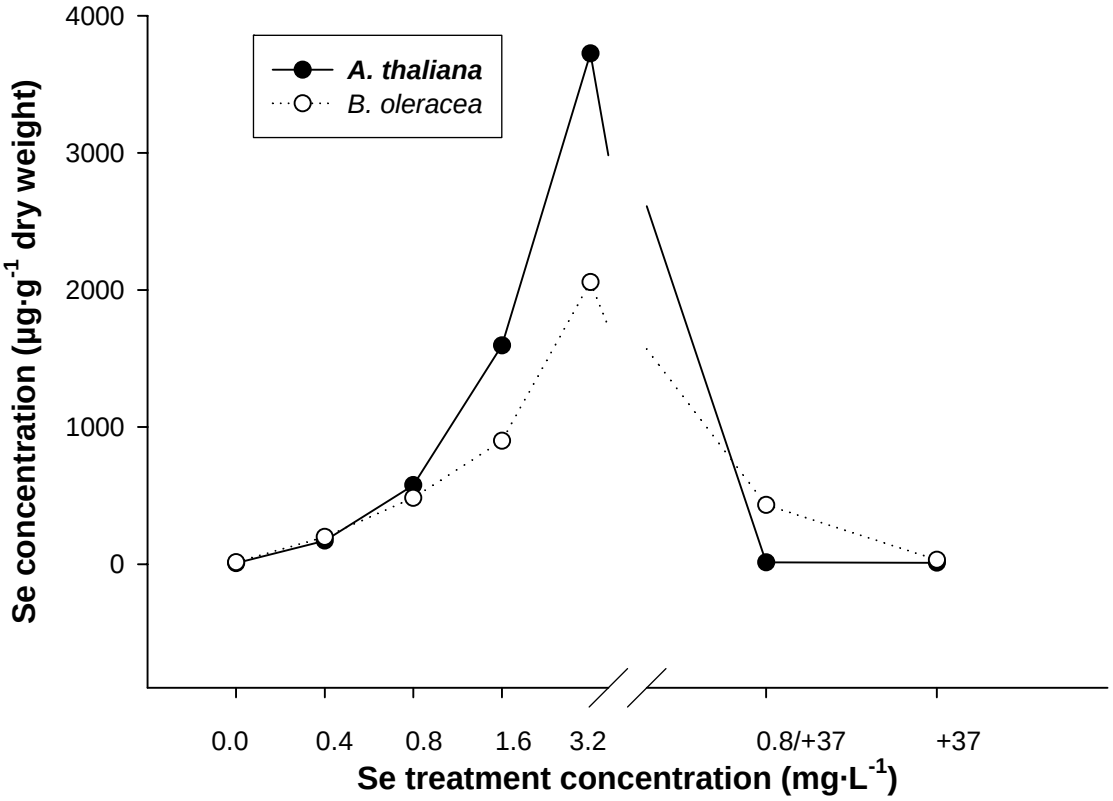

Figure 8. Selenium ($\mu g \cdot g^{-1}$ dry weight) concentrations in leaf tissue of *Arabidopsis thaliana* cv. Columbia and rapid-cycling *Brassica oleracea* grown in soilless medium fertilized with a modified full strength Hoagland's nutrient solution. Plants were exposed to Se concentrations ranging from 0.0 to 3.2 $mg \cdot L^{-1}$ weekly. Selenium was measured with an ICP-MS at The University of Tennessee-Knoxville, Plant Sciences Department.

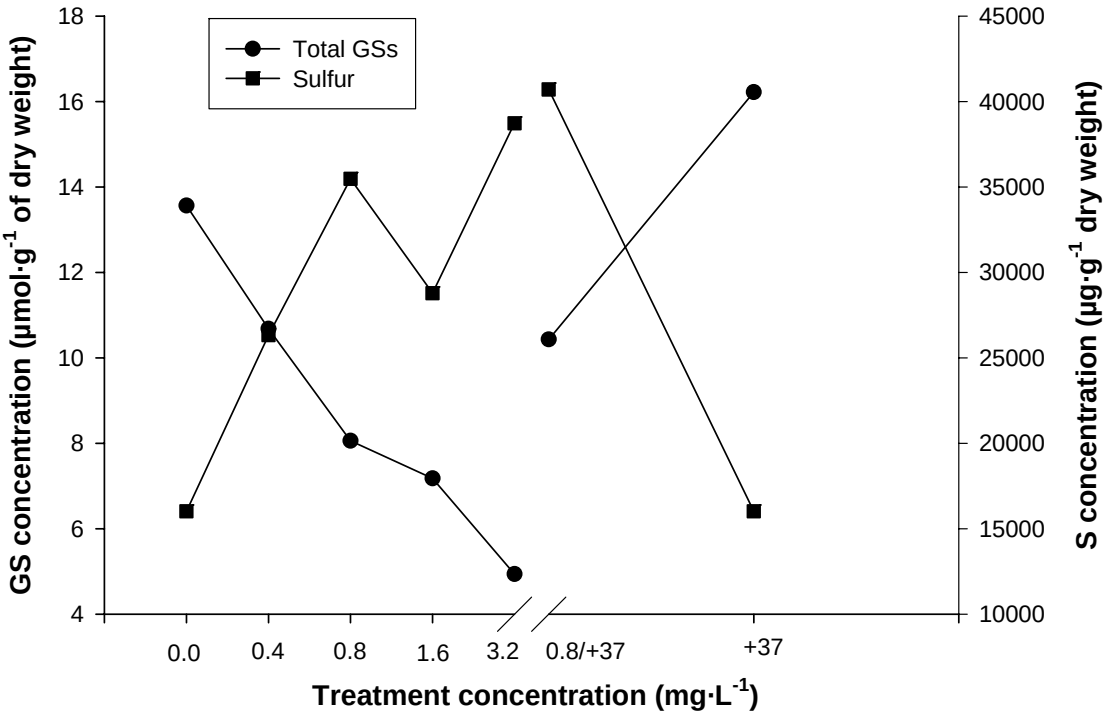

Figure 9. Total GS (µmol·g^{-1} dry weight) concentrations vs. total S in leaf tissue of rapid-cycling *Brassica oleracea* grown hydroponically with a half strength Hoagland's nutrient solution. Plants were exposed to Se concentrations ranging from 0.0 to 3.2 mg·L^{-1}, a combination of 0.8 mg·L^{-1} Se and +37 mg·L^{-1} elevated SO_4, and a +37 mg·L^{-1} elevated SO_4 treatments. The elevated SO_4 was added to the 96 mg·L^{-1} SO_4 already in the half strength Hoagland's nutrient solution.

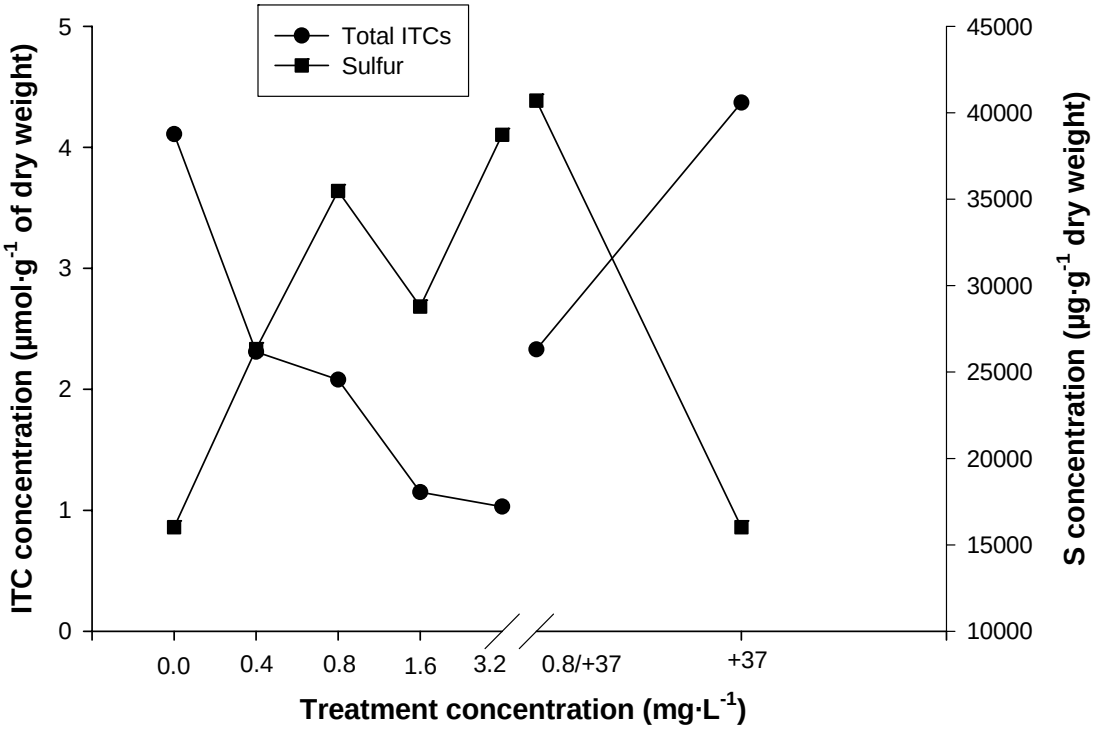

Figure 10. Total ITC (µmol·g^{-1} dry weight) concentrations vs. total S in leaf tissue of rapid-cycling *Brassica oleracea* grown hydroponically with a half strength Hoagland's nutrient solution. Plants were exposed to Se concentrations ranging from 0.0 to 3.2 mg·L^{-1}, a combination of 0.8 mg·L^{-1} Se and +37 mg·L^{-1} elevated SO_4, and a +37 mg·L^{-1} elevated SO_4 treatments. The elevated SO_4 was added to the 96 mg·L^{-1} SO_4 already in the half strength Hoagland's nutrient solution.

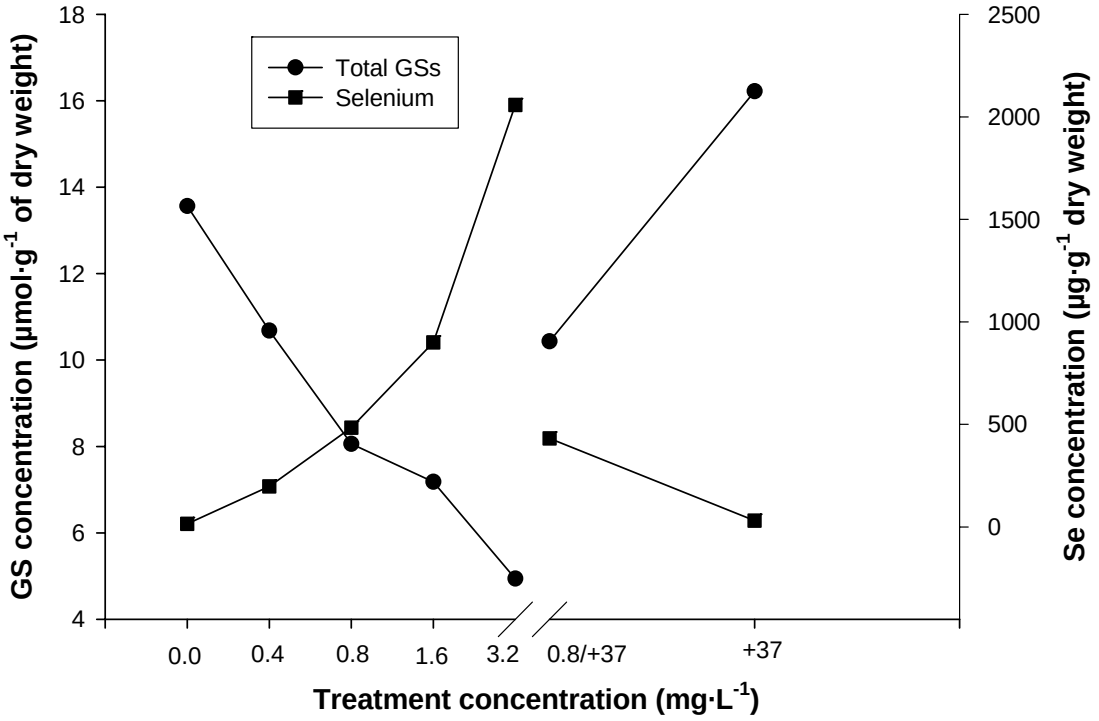

Figure 11. Total GS (µmol·g^{-1} dry weight) concentrations vs. total Se in leaf tissue of rapid-cycling *Brassica oleracea* grown hydroponically with a half strength Hoagland's nutrient solution. Plants were exposed to Se concentrations ranging from 0.0 to 3.2 mg·L^{-1}, a combination of 0.8 mg·L^{-1} Se and +37 mg·L^{-1} elevated SO_4, and a +37 mg·L^{-1} elevated SO_4 treatments. The elevated SO_4 was added to the 96 mg·L^{-1} SO_4 already in the half strength Hoagland's nutrient solution.

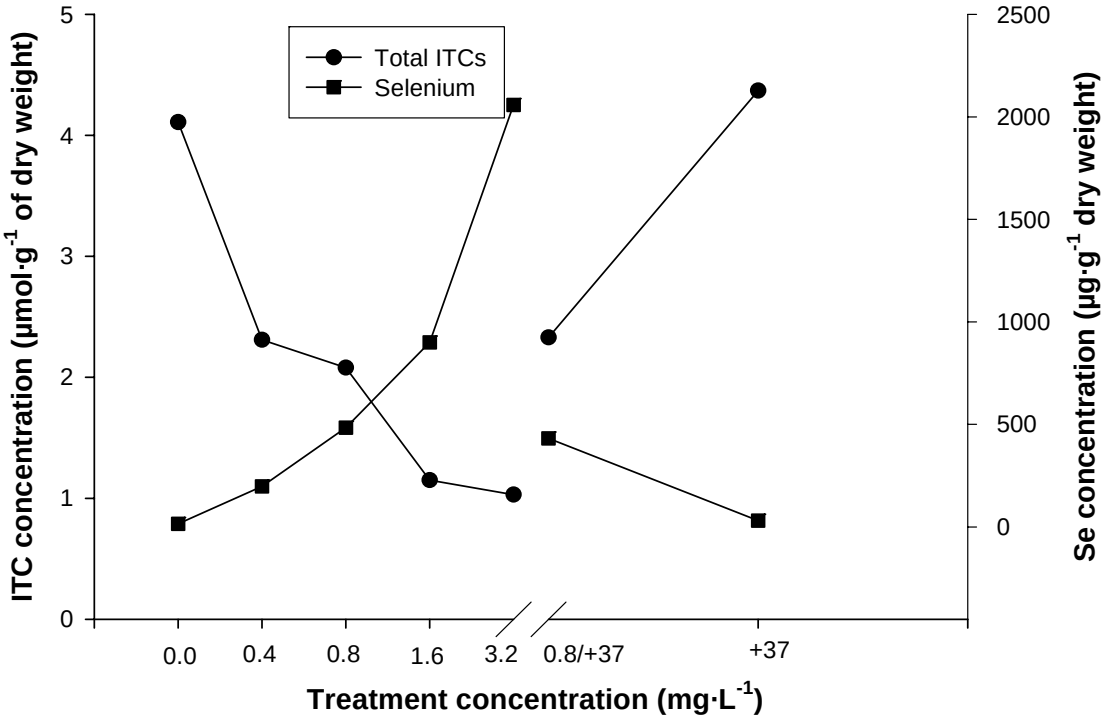

Figure 12. Total ITC (µmol·g^{-1} dry weight) concentrations vs. total Se in leaf tissue of rapid-cycling *Brassica oleracea* grown hydroponically with a half strength Hoagland's nutrient solution. Plants were exposed to Se concentrations ranging from 0.0 to 3.2 mg·L^{-1}, a combination of 0.8 mg·L^{-1} Se and +37 mg·L^{-1} elevated SO_4, and a +37 mg·L^{-1} elevated SO_4 treatments. The elevated SO_4 was added to the 96 mg·L^{-1} SO_4 already in the half strength Hoagland's nutrient solution.

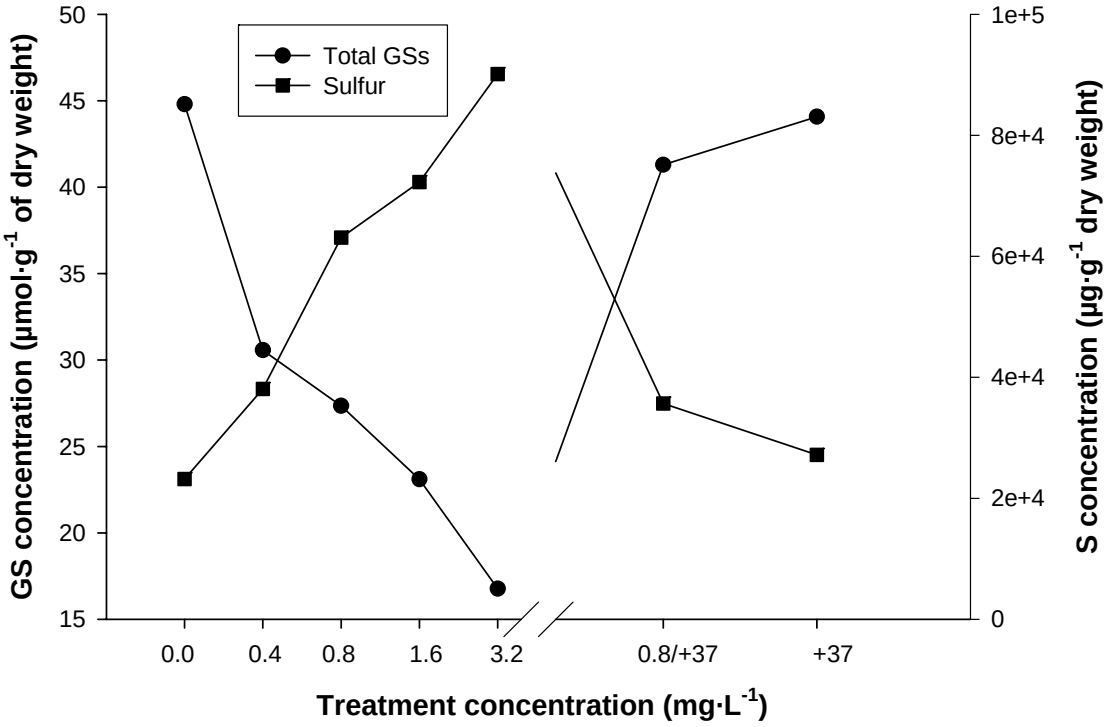

Figure 13. Total GS (µmol·g^{-1} dry weight) concentrations vs. total S in leaf tissue of *Arabidopsis thaliana* grown hydroponically with a half strength Hoagland's nutrient solution. Plants were exposed to Se concentrations ranging from 0.0 to 3.2 mg·L^{-1}, a combination of 0.8 mg·L^{-1} Se and +37 mg·L^{-1} elevated SO_4, and a +37 mg·L^{-1} elevated SO_4 treatments. The elevated SO_4 was added to the 96 mg·L^{-1} SO_4 already in the half strength Hoagland's nutrient solution.

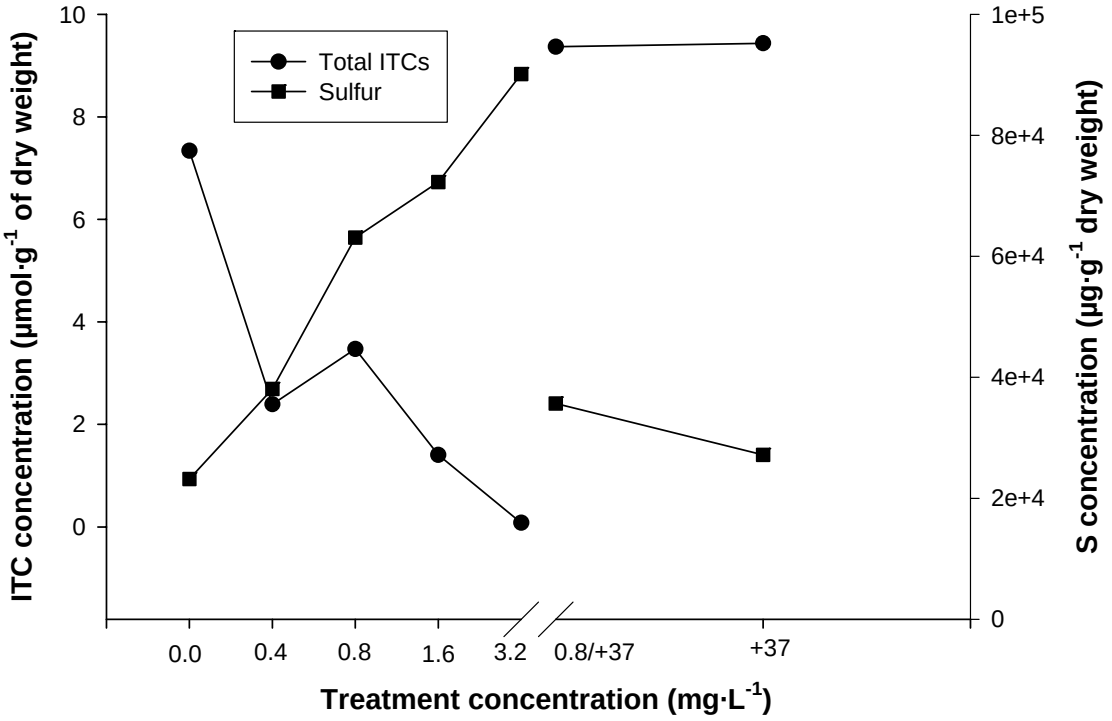

Figure 14. Total ITC (µmol·g^{-1} dry weight) concentrations vs. total S in leaf tissue of *Arabidopsis thaliana* grown hydroponically with a half strength Hoagland's nutrient solution. Plants were exposed to Se concentrations ranging from 0.0 to 3.2 mg·L^{-1}, a combination of 0.8 mg·L^{-1} Se and +37 mg·L^{-1} elevated SO_4, and a +37 mg·L^{-1} elevated SO_4 treatments. The elevated SO_4 was added to the 96 mg·L^{-1} SO_4 already in the half strength Hoagland's nutrient solution.

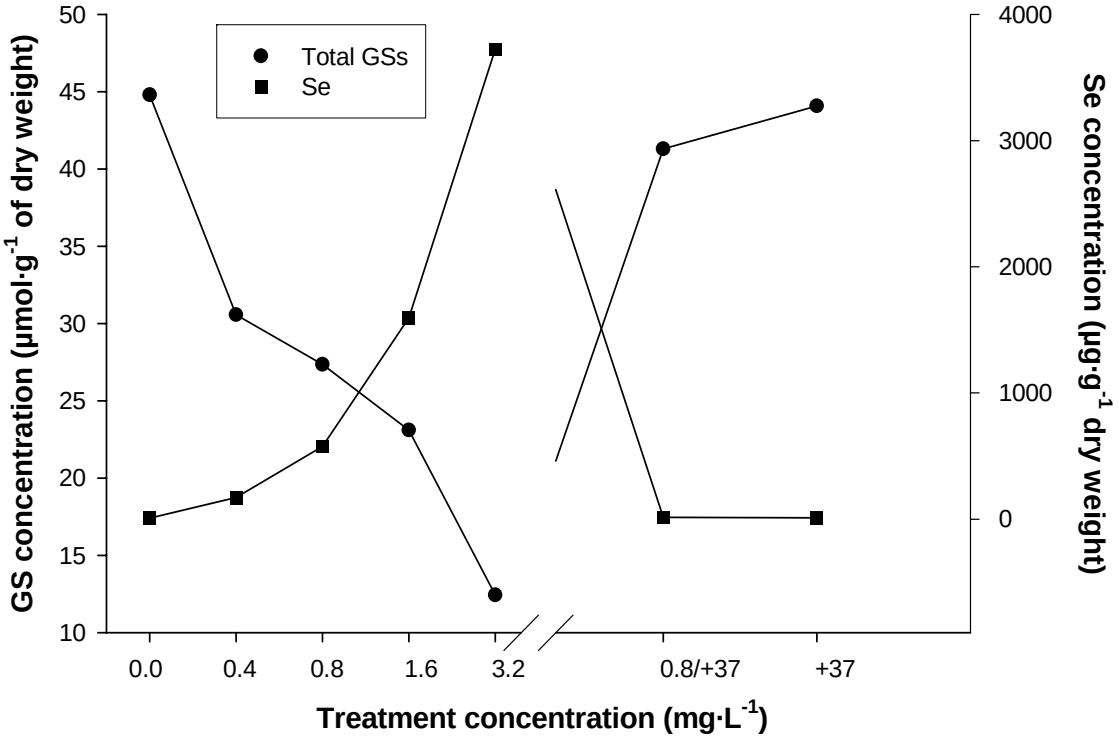

Figure 15. Total GS (µmol·g^{-1} dry weight) concentrations vs. total Se in leaf tissue of *Arabidopsis thaliana* grown hydroponically with a half strength Hoagland's nutrient solution. Plants were exposed to Se concentrations ranging from 0.0 to 3.2 mg·L^{-1}, a combination of 0.8 mg·L^{-1} Se and +37 mg·L^{-1} elevated SO_4, and a +37 mg·L^{-1} elevated SO_4 treatments. The elevated SO_4 was added to the 96 mg·L^{-1} SO_4 already in the half strength Hoagland's nutrient solution.

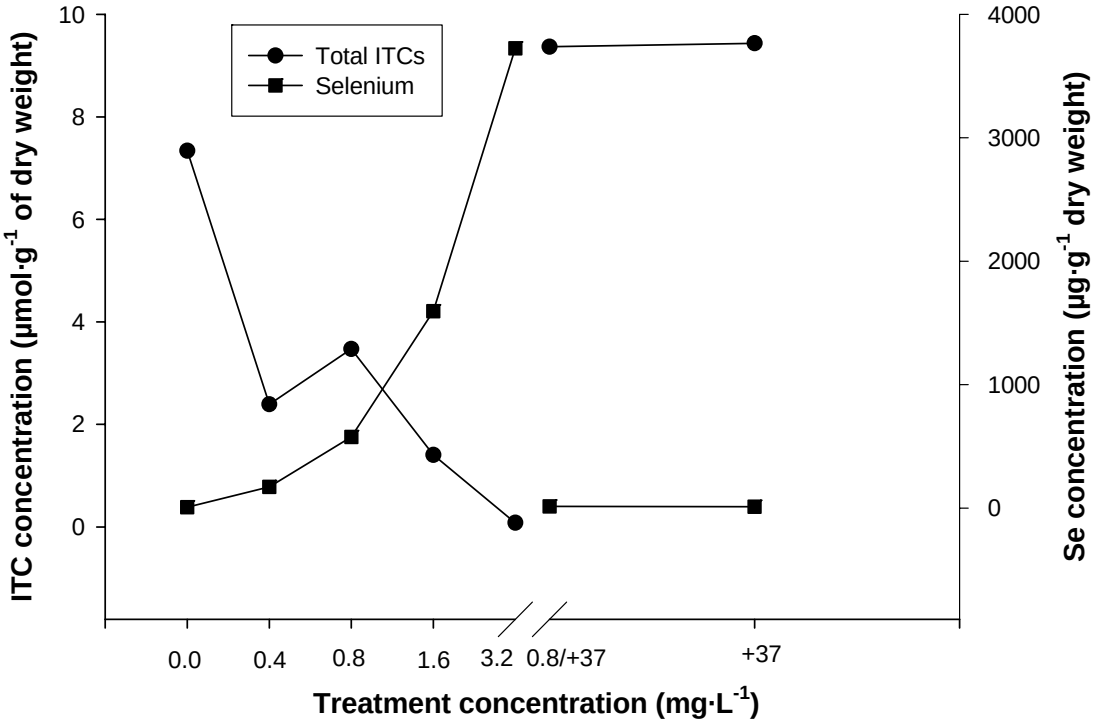

Figure 16. Total ITC (µmol·g^{-1} dry weight) concentrations vs. total Se in leaf tissue of *Arabidopsis thaliana* grown hydroponically with a half strength Hoagland's nutrient solution. Plants were exposed to Se concentrations ranging from 0.0 to 3.2 mg·L^{-1}, a combination of 0.8 mg·L^{-1} Se and +37 mg·L^{-1} elevated SO_4, and a +37 mg·L^{-1} elevated SO_4 treatments. The elevated SO_4 was added to the 96 mg·L^{-1} SO_4 already in the half strength Hoagland's nutrient solution.

Appendix C

SAS Output Statements

Chapter 2

SAS Contrast Statement for Glucosinolate Concentrations in *A. thaliana* and *B. oleracea*

```
data one;
input iberin      glucoraph   sinigrin    gluconapin  indolyl3    meth4
      nasturtiin  meth1 aliphatic   aromatic    indole      total;
datalines;


%include 'c:\danda.sas';
%orthpoly (0.0 0.1 0.2 0.4 0.8 1.6 3.2)
%mmaov (one,iberin      glucoraph   sinigrin    gluconapin  indolyl3    meth4
      nasturtiin  meth1 aliphatic   aromatic    indole      total,
class=species trt rep, fixed=species trt species*trt, random=rep,
contrast=%str(

                          Contrast 'Linear'                    Trt
            -9           -8           -7           -5           -1
7           23 ;


                          Contrast 'Quadratic'                 Trt
    4.163200564 2.7317589002 1.3940782517           -1 -4.663024321 -
7.488544237 4.8625308424 ;


                          Contrast 'Cubic'                     Trt
   -4.162893666 -1.266014425 1.0407170814 4.0492014384 4.8045073291 -
5.465517758            1 ;));
```

Chapter 2

SAS Contrast Statement for Selenium and Sulfur Concentrations in

A. thaliana and *B. oleracea*

```
data one;
input  S Se;
datalines;


include 'c:\danda.sas';
%orthpoly (0.0 0.1 0.2 0.4 0.8 1.6 3.2)
%mmaov (one, S    Se, class=species trt rep, fixed= species trt species*trt,
random= rep,contrasts=%str(
 Contrast 'Linear'                    Trt
             -9           -8           -7           -5           -1
7           23 ;
                          Contrast 'Quadratic'                Trt
    4.163200564 2.7317589002 1.3940782517           -1 -4.663024321 -
7.488544237 4.8625308424 ;));
```

Chapter 3

SAS Contrast Statement for Glucosinolate Concentrations in *A. thaliana* and *B. oleracea*

```
data one;
input iberin      progoitrin  glucoraph   sinigrin    sinalbin    gluconapin
      hydroxy     erucin      indolyl     meth4 nasturtiin  meth1 aliphatic
      aromatic    indole      total;
datalines;


%include 'c:\danda.sas';
%mmaov (one,iberin      progoitrin  glucoraph   sinigrin    sinalbin
      gluconapin  hydroxy     erucin      indolyl     meth4 nasturtiin  meth1
      aliphatic   aromatic    indole      total,
class=species trt rep, fixed=species trt species*trt, random=rep, sort=yes);
```

Chapter 3

SAS Contrast Statement for Isothiocyante Concentrations in *A. thaliana* and *B. oleracea*

```
data one;
input AITC Butenyl Erucin Goiterin PEITC Iberin Sulforphane Indole Aliphatic
Total;
datalines;


%include 'c:\danda.sas';
%mmaov(one, AITC Butenyl      Erucin      Goiterin    PEITC       Iberin
      Sulforphane Indole      Aliphatic Total
, class= species trt rep, fixed=species trt species*trt, random=rep,

sort=yes);
```

Chapter 3

SAS Contrast Statement for Selenium and Sulfur Concentrations in *A. thaliana* and *B. oleracea*

```
data one;
input S Se;
datalines;


%include 'c:\danda.sas';
%mmaov (one, S Se, class=trt sub rep, fixed= trt sub trt*sub, random=

rep,sort=yes);
```

Vita

T. Casey Barickman was born in Quincy, IL on June 23, 1978. He was raised in Canton, MO until moving to New Sharon, IA in 1992, where he graduated from North Mahaska High School in 1997. He went on to graduate college from Iowa State University of Science and Technology with a B.S. degree in Horticultural Science and Plant Health and Protection in the spring 2009. Currently, he is a Research Associate II at The University of Tennessee, Knoxville, were he manages a horticultural crop physiology and biochemistry laboratory while pursuing a Ph.D in Plant, Soil, and Insects from the Plant Sciences Department.