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Kale Carotenoids Are Unaffected by, whereas Biomass Production, Elemental Concentrations, and Selenium Accumulation Respond to, Changes in Selenium Fertility

MARK G. LEFSRUD AND DEAN A. KOPSELL*

Plant Sciences Department, The University of Tennessee, Knoxville, Tennessee 37996

DAVID E. KOPSELL

School of Agriculture, The University of Wisconsin—Platteville, Platteville, Wisconsin 53818

WILLIAM M. RANDLE

Department of Horticulture, The University of Georgia, Athens, Georgia 30602

Selenium (Se) is a micronutrient in mammalian nutrition and is accumulated in kale (*Brassica oleracea* L. var. *acephala*), which has high levels of lutein and β-carotene. Selenium, lutein, and β-carotene have important human health benefits and possess strong antioxidant properties. The objectives of this study were to determine the influence of different Se [as sodium selenate (Na_2SeO_4) and sodium selenite (Na_2SeO_3)] fertility levels on (1) biomass accumulation, (2) the accumulation patterns of carotenoid pigments, and (3) elemental accumulation in the leaves of kale. Winterbor kale was greenhouse-grown using nutrient solution culture with Se treatment concentrations of 0.0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, and 3.5 mg Se/L as Na_2SeO_4 and 0.0, 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 mg Se/L as Na_2SeO_3. Increases in either selenate (SeO_4^{-2}) or selenite (SeO_3^{-2}) resulted in decreases in kale leaf tissue biomass. Neither of the Se treatments had an effect on the accumulation of lutein or β-carotene in leaf tissues. Increasing SeO_4^{-2} significantly increased the accumulation of kale leaf Se; however, leaf tissue Se did not significantly change over the SeO_3^{-2} treatments. Increases in SeO_4^{-2} affected the leaf tissue concentrations of P, K, Ca, Mg, S, B, Cu, Mn, and Mo, whereas SeO_3^{-2} only affected B and S. Growing kale in the presence of SeO_4^{-2} would result in the accumulation of high levels of tissue Se without affecting carotenoid concentrations.

KEYWORDS: ***β*-Carotene; *Brassica oleracea*; lutein; nutrition; selenium; selenate; selenite; HPLC**

INTRODUCTION

Selenium (Se) is an essential trace element in mammalian nutrition but is not yet classified as an essential plant micronutrient (*1*). Selenium is absorbed by plants in three forms: selenite (SeO_3^{-2}), selenate (SeO_4^{-2}), or as organic Se complexes, such as selenocysteine and selenomethionine (*2*, *3*). Plants preferentially accumulate SeO_4^{-2}, as compared to SeO_3^{-2}, under hydroponic or soil conditions (*4*). Selenate competes with sulfate (SO_4^{-2}) for uptake by the roots, with increased levels of SO_4^{-2} resulting in decreases in SeO_4^{-2} uptake (*4*). Selenate enters the root through normal sulfur (S) transporters in the plasma membrane (*3*). After long-distance transport to the leaves, the conversion of SeO_4^{-2} into organic Se compounds is believed to occur in the chloroplasts, where it enters into normal S metabolic pathways (*3*, *5*). Selenate is converted to SeO_3^{-2} by ATP sulfurylase before incorporation into various selenoether amino acids.

In most plant species, selenium amino acids replace corresponding sulfur amino acids in metabolic pathways (*6*). Phytotoxicity occurs mainly from Se interferences with normal S metabolism (*2*), resulting in chlorosis and decreases in protein and dry matter synthesis (*7*). Plants are classified by their ability to accumulate Se, with nonconcentrator species (<25 mg Se/kg dry mass), secondary absorbers (25−100 mg Se/kg dry mass), and primary indicators (100−1000 mg Se/kg dry mass). Members of the brassica family readily accumulate Se and have been characterized as primary indicators (*1*, *8*, *9*). It is this characteristic which makes brassica vegetables candidate crops to deliver Se to human diets.

The distribution of Se in soils is highly variable, with toxic and deficient plant levels being reported worldwide. In areas of the world with low soil Se levels, fertilization is practiced to avoid potential mammalian deficiencies (*10*). Consumption of

* To whom correspondence should be addressed. Phone: (865) 974-1145. Fax: (865) 974-1947. E-mail: dkopsell@utk.edu.

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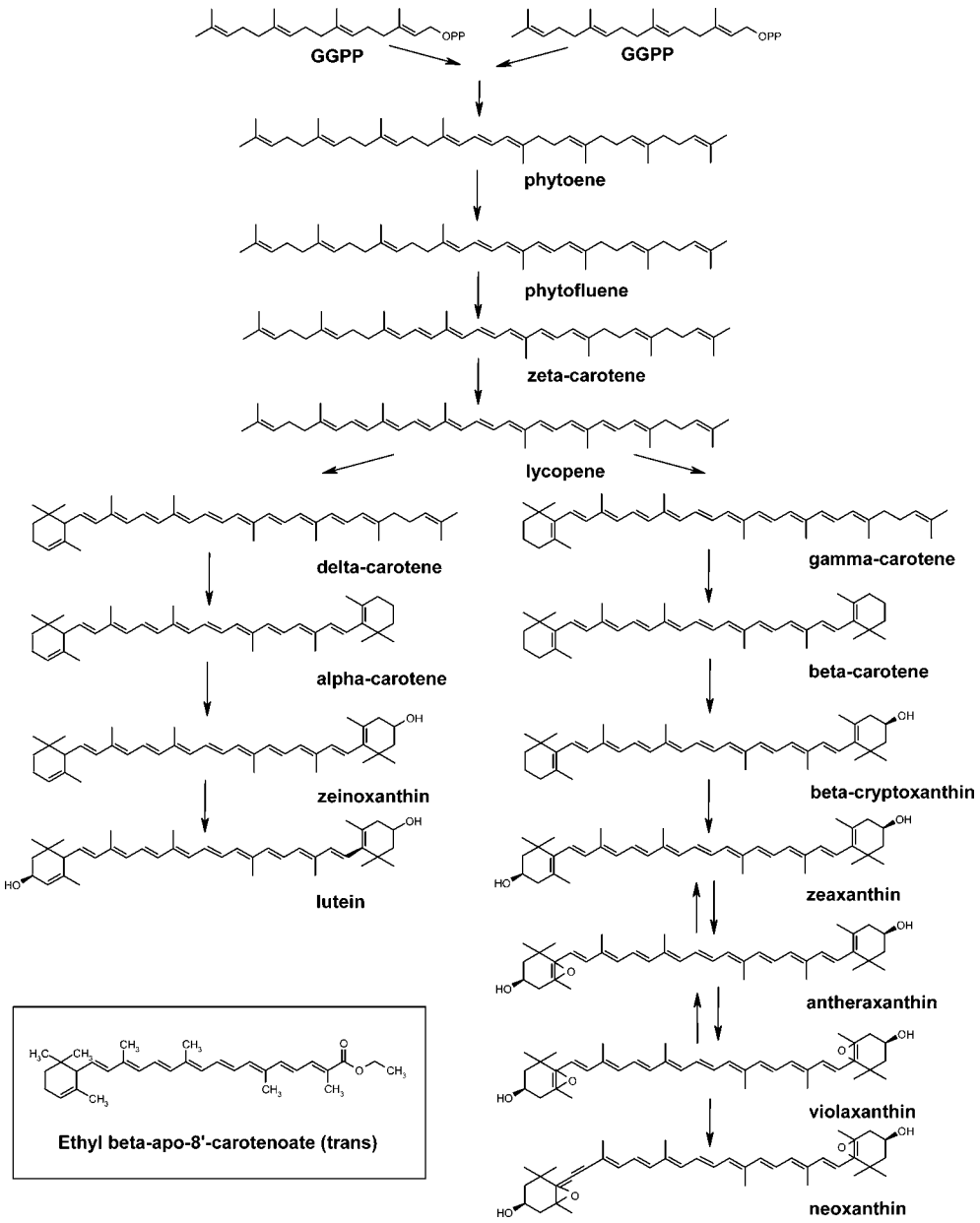

Figure 1. Simplified version of the carotenoid biosynthetic pathway in plants. GGPP is geranylgeranyl pyrophosphate. Ethyl β-apo-8′-carotenoate was used as internal standard.

selenium-fertilized vegetables is a good way to ensure the necessary level of Se in the human diet (*11*). With the importance of brassica plants as vegetable crops, increasing tissue Se concentration through Se applications would improve the overall Se contribution to human diets (*12*). Health benefits associated with increased Se intake include immune system enhancement (*13*), prevention of cardiovascular diseases (*14*), and prevention of several forms of cancer (*11*, *15*).

Carotenoids are C_{40} isoprenoid polyene secondary compounds that form yellow, orange, and red lipid-soluble pigments in higher plants, algae, and bacteria (**Figure 1**). In plants, carotenoids are used as antenna pigments to funnel light energy to the photosynthetic reaction center. These carotenoids are closely associated with the chlorophyll molecules to prevent excess energy from entering the photosynthetic system (*4*, *16*−*18*). Lutein [(3*R*,3′*R*,6′*R*)-β,ϵ-carotene-3,3′-diol] and β-carotene (β,β-carotene) possess important human health properties. Mammalian systems are unable to synthesize these compounds, so plants are one of the primary sources of dietary lutein and β-carotene. Moreover, intake of foods rich in lutein and β-carotene has been associated with reduced risk of lung cancer, cataracts, and age-related macular degeneration (*19*−*21*). The USDA ranks kale (*Brassica oleracea* L. var. *acephala*) as the highest source of lutein and β-carotene among the vegetable crops, making it an excellent source of dietary carotenoids (*22*−*24*). However, kale has low consumption rates, with per capita fresh intake at less than 0.33 kg/year (*25*).

Dietary lutein is selectively deposited in the human retina and is responsible for the yellow pigmentation referred to as macular pigment (*26*, *27*). Macular pigment is postulated to participate in photoprotection of the eye (*28*, *29*), and increases can be achieved through dietary modification (*30*) and supplementation (*31*). However, studies have indicated that consumption of a variety of vegetables, providing a mixture of carotenoids, was more strongly associated with reduced eye disease and cancer risk than individual carotenoid supplements (*21*, *32*). The ability of plants to accumulate and incorporate Se into bioactive compounds can also influence human health

and nutrition. Most notable is the anticarcinogenic activity of organic Se forms against certain types of cancers (*33*−*35*). One of the most effective anticarcinogenic organic Se compounds is methylselenocysteine, which occurs in members of the brassica genus (*36*).

Brassica vegetables have high nutritional and medicinal value imparted from essential dietary minerals and secondary compounds in their tissues. Kale has the highest concentrations of lutein and β-carotene, providing antioxidant activity when consumed in the diet. Brassica vegetables also have the ability to accumulate high concentrations of Se. Therefore, the objectives of this study were to determine the influence of different Se (as SeO_4^{-2} or SeO_3^{-2}) fertility levels on (1) biomass accumulation, (2) the accumulation patterns of carotenoid pigments, and (3) elemental accumulation in the leaves of Winterbor kale, a member of the Brassica family. Our research hypothesis contends that it will be possible to increase the concentration of Se in the leaf tissues of kale, while maintaining high concentrations of nutritionally valuable lutein and β-carotene.

MATERIALS AND METHODS

Plant Culture. Winterbor kale (*B. oleracea* L. var. *acephala*) seeds (Johnny's Selected Seed, Winslow, ME) were sown into rockwool growing cubes (Grodan A/S, Dk-2640, Hedehusene, Denmark) for germination on February 24, 2004 and grown in a greenhouse (22 °C day/14 °C night) under natural lighting conditions (latitude 43° 09′ N, Durham, NH). Peter's 20N−6.9P−16.6K water-soluble fertilizer (Scotts Company, Marysville, OH) was applied every 5 days at a rate of 200 mg/L. After 2.5 weeks, the plants were transferred to 10 L plastic containers (Rubbermaid Inc., Wooster, OH). Six plants were placed into 2 cm round holes set at 10.6 cm × 9.5 cm spacing on each container lid.

The plants were grown in 9 L of a modified nutrient solution (*37*). Elemental concentrations of the nutrient solutions were (mg/L): N (105), P (15.3), K (117.3), Ca (80.2), Mg (24.6), S (32.0), Fe (0.5), B (0.25), Mo (0.005), Cu (0.01), Mn (0.25), and Zn (0.025). Plants were grown in separate studies under increasing Se treatment concentrations at 0.0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, and 3.5 mg Se/L as Na_2SeO_4 and at 0.0, 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 mg Se/L as Na_2SeO_3. The EC of the starting nutrient solution was 1.0 dS/m, and pH was measured at 5.6. Solutions were aerated with an air blower (model 25E133W222; Spencer; Winsor, CT) connected to air stones. Water was added daily to maintain initial solution volumes in each container. Nutrient solutions were replaced every week throughout the experiment to refresh the solution to the initial nutrient concentrations. The experimental design was a randomized complete block for each study, with four replications of eight SeO_4^{-2} treatments and seven SeO_3^{-2} treatments, respectively.

Plants were harvested on April 4, 2004. At harvest, shoot and root tissues were separated and weighed. Shoot tissues were washed with soap (Aquet, Bel-art Products, Pequannock, NJ), rinsed, and blotted dry with paper towels. The third fully expanded leaf from each of the 6 plants was selected, and a 4 cm^2 piece of the leaf was removed. This treatment sample was stored at −20 °C prior to lyophilization. The remaining shoot material was dried at 60 °C for no less than 72 h.

Elemental Determination. A sample mill grinder (model 1093, Cyclotec-Tector, Höganäs, Sweden) with a 0.5 mm screen was used to grind dried shoot tissue. A 0.3 g tissue sample was mixed with 10 mL of 70% concentrated nitric acid (HNO_3) and digested in a microwave accelerated reaction system (MARS5, CEM Corp., Matthews, NC). The digested solution was cooled to room temperature, and deionized water was added to result in a final volume of 40 mL. Elemental analysis was determined by ICP-AES (inductively coupled argon plasma atomic emission spectrometry; model Vista AX, Varian Inc., Palo Alto, CA) (*38*).

A wet-acid digest was used for Se analysis (*39*). Ground tissues were placed into a 125 mL flask with 10 mL of concentrated nitric acid (70% NHO_3) and placed on a hot plate (Thermolynem, model 2200, Dubuque, IA) under reflux for 4 h at 165 °C. Flasks were allowed to cool to room temperature and brought to a final volume of 50 mL with deionized water. The solutions were filtered through Whatman no. 1 filter paper (Maidstone, England) before total Se was measured by graphite furnace atomic absorption spectrophotometry (GFAA; Perkin-Elmer Corporation, model 4100ZL, Norwalk, CT) (*39*). The detection limit for Se by GFAA was 4.0 μg/L.

Carotenoid Determination. *Tissue Extraction.* The frozen plant samples were lyophilized for a minimum of 72 h (model 6L FreeZone, LabConCo, Kansas City, MO). The dried tissues samples were ground with dry ice in a kitchen grinder (Handy Chopper Plus, HC 3000, Household Products Inc., Shelton, CT). Pigments were extracted and separated according to previously published methods (*40*, *41*). A 0.1 g subsample was placed into a Potter-Elvehjem tissue grinder tube (Kontes, Vineland, NJ) and hydrated with 0.8 mL of deionized water. The sample was placed in a 40 °C water bath for 20 min. After hydration, 0.8 mL of the internal standard, ethyl-β-8′-apo-carotenoate (Sigma Chemical Co., St. Louis, Mo.), and 2.5 mL of tetrahydrofuran (THF), stabilized with 25 mg/L 2,6-di-*tert*-butyl-4-methoxyphenol (BHT), were added. The sample was homogenized in the tube with ∼25 insertions with a Potter-Elvehjem tissue grinder pestle attached to a drill press (model Craftsman 15 in. drill press; Sears, Roebuck and Co., Hoffman Estates, IL) at 540 rpm. The sample tube was kept immersed in ice. The tube was placed into a clinical centrifuge for 3 min at 500*g*. The supernate was removed with a Pasteur pipet, placed into a conical 15 mL test tube, capped, and held on ice. The sediment was resuspended in 2.0 mL of THF and homogenized with ∼25 insertions of the grinding pestle. The tube was centrifuged for 3 min at 500*g*, and the supernate was collected and combined with the first extracted supernate. The extraction procedure was repeated twice more until the supernate was colorless. The sediment was discarded, and the combined four supernates were placed in a 40 °C water bath and reduced to 0.5 mL under a stream of nitrogen (model N-EVAP 111, Organomatic Inc., Berlin, MA). Added to the 0.5 mL sample was 2.5 mL of MeOH and 2.0 mL of THF, with the solution vortexed. The sample was filtered through a 0.2 μm poly(tetrafluoroethylene) (PTFE) filter (model Econofilter PTFE 25/20, Agilent Technologies, Wilmington, DE) using a 5 mL syringe (Becton, Dickinson and Company, Franklin Lakes, NJ).

HPLC Analysis. An HPLC unit with photodiode array detector (Agilent 1100, Agilent Technologies, Palo Alto, CA) was used for pigment separation. All samples were analyzed for carotenoid compounds using a Vydac RP-18 5.0 μm 250 mm × 4.6 mm column (model 201TP54, Phenomenex, Torrance, CA) fitted with a 4 mm × 3.0 mm, 7.0 μm guard column compartment (*40*, *41*). The column was maintained at 16 °C using a thermostatic column compartment. The eluents were as follows: A, 75% acetronitrile, 20% methanol, 5% hexane, 0.05% BHT, and 0.013% triethyamine (TEA) in water (v/v); and B, 50% acetonitrile, 25% THF, 25% hexane and 0.013% TEA in water (v/v). The flow rate was 0.7 mL/min, and the gradient was 100% eluent A for 30 min; 50% A and 50% B for 2 min; 100% B for 2 min; and 50% A and 50% B for 2 min. The eluent was returned to 100% A for 10 min prior to the next injection. Eluted carotenoids and chlorophyll compounds from a 20 μL injection were detected at 452, 652, and 665 nm, with data collected and integrated using 1100 HPLC ChemStation Software (Agilent Technologies, Palo Alto, CA). Peak assignment was performed by comparing retention times and line spectra obtained from photodiode array detection with authentic standards (lutein from Carotenature, Lupsingen, Switzerland; β-carotene, chlorophyll *a*, chlorophyll *b* from Sigma Chemical Co.). Recovery rates of the internal standard during extractions were >90%.

Statistical Analysis. Data were analyzed by the ANOVA procedure using SPSS (Chicago, IL). The relationship between experimental dependent variables and selenium treatments were determined by regression analysis. Orthogonal polynomials were used to study changes associated with increasing SeO_4^{-2} and SeO_3^{-2} treatment levels by partitioning the sum of squares into components associated with linear and quadratic terms (*42*).

Table 1. Mean Biomass of Leaf Tissues of Winterbor Kale (*B. oleracea* L. Var. *acephala* Group) Grown under Increasing Selenium (as Selenate or Selenite) Concentrations in Nutrient Solution Culture[a]

	plant biomass (g)	
mg of Se/L	fresh mass	dry mass
	Selenate	
0.0	53.7 ± 1.4	3.9 ± 0.2
0.5	53.1 ± 2.0	3.9 ± 0.2
1.0	47.2 ± 3.2	3.4 ± 0.2
1.5	52.3 ± 1.8	3.8 ± 0.1
2.0	48.5 ± 1.7	3.6 ± 0.1
2.5	46.2 ± 3.8	3.4 ± 0.3
3.0	40.3 ± 2.8	3.0 ± 0.2
3.5	39.1 ± 2.3	2.9 ± 0.2
contrasts		
linear	$P \leq 0.001$	$P \leq 0.001$
quadratic	$P \leq 0.001$	$P \leq 0.001$
	Selenite	
0.0	50.7 ± 3.3	3.8 ± 0.3
0.5	49.7 ± 2.1	3.7 ± 0.2
1.0	51.3 ± 4.4	3.8 ± 0.3
1.5	49.3 ± 2.4	3.7 ± 0.1
2.0	44.5 ± 5.1	3.4 ± 0.3
2.5	45.3 ± 3.3	3.4 ± 0.2
3.0	43.7 ± 1.3	3.4 ± 0.1
contrasts		
linear	$P = 0.035$	ns[b]
quadratic	ns[b]	ns[b]

[a] Mean composition of sampled leaf tissue of four replications, six plants each ± standard deviation. [b] Nonsignificant.

Table 2. Mean Carotenoid and Chlorophyll Pigment Concentrations Expressed on a Fresh Mass Basis (mg per 100 g Fresh Mass) for Leaf Tissues of Winterbor Kale (*B. oleracea* L. Var. *acephala* Group) Grown under Increasing Selenium (as Selenate or Selenite) Concentrations in Nutrient Solution Culture[a]

	pigment concentration (mg/100 g fresh mass)			
mg of Se/L	lutein	β-carotene	chlorophyll *a*	chlorophyll *b*
		Selenate		
0.0	10.2 ± 0.4	8.9 ± 0.5	177.0 ± 6.8	45.0 ± 2.2
0.5	9.5 ± 0.3	8.2 ± 0.2	166.1 ± 3.5	41.1 ± 2.0
1.0	9.5 ± 0.2	8.2 ± 0.2	144.1 ± 17.4	41.5 ± 2.3
1.5	9.6 ± 0.7	8.1 ± 0.6	161.0 ± 11.3	38.2 ± 3.9
2.0	9.8 ± 0.3	8.4 ± 0.3	168.7 ± 5.8	41.6 ± 1.7
2.5	9.7 ± 0.3	8.1 ± 0.1	169.0 ± 6.1	40.4 ± 1.5
3.0	9.9 ± 0.6	8.0 ± 0.3	169.9 ± 9.3	41.4 ± 3.0
3.5	8.7 ± 0.9	8.5 ± 0.2	170.0 ± 9.9	40.2 ± 1.6
contrasts				
linear	ns[b]	ns[b]	ns[b]	ns[b]
quadratic	ns[b]	ns[b]	ns[b]	ns[b]
		Selenite		
0.0	10.2 ± 0.5	8.9 ± 0.5	178.9 ± 5.5	45.0 ± 2.1
0.5	9.9 ± 0.5	8.7 ± 0.5	175.6 ± 8.0	43.7 ± 3.2
1.0	9.3 ± 0.7	8.6 ± 0.2	175.1 ± 7.3	43.2 ± 0.8
1.5	10.3 ± 0.5	9.3 ± 0.3	188.0 ± 7.4	46.0 ± 1.7
2.0	10.6 ± 0.4	8.9 ± 0.3	191.3 ± 11.0	44.9 ± 3.0
2.5	9.4 ± 0.6	8.5 ± 0.4	166.8 ± 6.1	40.5 ± 2.4
3.0	9.7 ± 0.6	8.9 ± 0.3	188.5 ± 7.1	44.1 ± 1.7
contrasts				
linear	ns[b]	ns[b]	ns[b]	ns[b]
quadratic	ns[b]	ns[b]	ns[b]	ns[b]

[a] Mean composition of sampled leaf tissue of four replications, six plants each ± standard deviation. [b] Nonsignificant.

RESULTS AND DISCUSSION

Plant Growth. Kale leaf tissue fresh mass (FM) and leaf tissue dry mass (DM) responded to the increases in SeO_4^{-2} ($P \leq 0.001$ and $P \leq 0.001$, respectively) but not to increases in SeO_3^{-2} ($P = 0.523$ and $P = 0.633$, respectively). Leaf tissue FM responded quadratically [FM $SeO_4 = 53.1 - 0.70(\text{trt}) - 0.98(\text{trt})^2$] and ranged from 53.7 g/plant under 0.0 mg Se/L as SeO_4^{-2} to 39.1 g/plant under 3.5 mg Se/L as SeO_4^{-2} (**Table 1**). Leaf tissue DM also responded quadratically [DM $SeO_4 = 4.0 - 0.02(\text{trt}) - 0.08(\text{trt})^2$], and ranged from 3.9 g/plant under 0.0 mg Se/L as SeO_4^{-2} to 2.9 g/plant under 3.5 mg Se/L as SeO_4^{-2} (**Table 1**). The largest FM and DM were observed with the 0.0 mg Se/L SeO_4^{-2} treatment, whereas the largest biomass accumulations for kale grown under SeO_3^{-2} occurred at the 1.0 mg Se/L treatment. Decreases in biomass in response to increasing SeO_4^{-2} concentrations in the current study follow previously reported trends. Kopsell and Randle (*12*) reported decreases in shoot FM and DM in rapid-cycling *B. oleracea* in nutrient solution from 0.0 to 9.0 mg Na_2SeO_4/L (0.0−3.8 mg Se/L). *Brassica juncea* L. land races showed decreases in DM yields of 12−23% as the concentration of Se in solution culture increased from 0 to 4 mg Se/L, added as Na_2SeO_4 (*43*). Similarly, Bañuelos et al. (*44*) showed a decrease in shoot DM in canola (*Brassica napus* L. cv. Westar) when comparing plants grown in a low-Se soil (0.1 mg Se/kg) with those grown in a high-Se soil (40 mg Se/kg). Yield considerations in brassicas may be noteworthy when supplying Se in concentrations provided in this study.

Carotenoid and Chlorophyll Compounds. Kale leaf tissues were measured for lutein, β-carotene, chlorophyll *a*, and chlorophyll *b* concentrations (**Table 2**). None of the pigments responded to increases in SeO_4^{-2} or SeO_3^{-2} concentrations. Values recorded for lutein and β-carotene concentrations were within previously reported ranges for Winterbor kale (*40*). For kale grown under increasing SeO_4^{-2} concentrations, maximum lutein accumulation was 10.2 mg/100 g FM and occurred under the 0.0 mg Se/L treatment. For kale grown under increasing SeO_3^{-2}, maximum lutein accumulation was 10.6 mg/100 g FM and occurred under the 2.0 mg Se/L treatment. Maximum β-carotene accumulation for kale grown under increasing SeO_4^{-2} treatments was 8.9 mg/100 g FM and occurred under 0.0 mg Se/L, whereas maximum β-carotene accumulation for kale grown under increasing SeO_3^{-2} treatments was 9.3 mg/100 g FM and occurred under 1.5 mg Se/L (**Table 2**). To the best of our knowledge, this is the first attempt to measure the influence of Se fertility on the accumulation of lutein and β-carotene in the leaf tissues of kale. One previous report in the literature described the influences of Na_2SeO_4 and Na_2SeO_3 on total carotenoid accumulation in the aquatic plant *Lemna minor* L. (*45*). There were no changes in total carotenoid values in *L. minor* as plants were grown in concentrations of Na_2SeO_4 and Na_2SeO_3 up to 32 μM (equivalent to the highest Se treatments in the current study). Decreases in total carotenoid pigments were found only when *L. minor* was grown in Na_2SeO_4 and Na_2SeO_3 concentrations of 128 μM, values that would be phytotoxic for brassica plants (*12*). Data from the current study demonstrates that kale leaf tissue lutein and β-carotene concentrations are not affected by increases in either SeO_4^{-2} or SeO_3^{-2} within the ranges provided in this study.

Neither of the Se treatments affected the concentrations of chlorophyll *a* or *b* in the kale leaf tissues (**Table 2**). Previous research on the influences of Se on chlorophyll pigments has reported mixed results. Decreases in chlorophyll pigments in response to increases in both SeO_4^{-2} and SeO_3^{-2} occurred in maize (*Zea maize* L.) (*46*), mung bean (*Vigna mungo* L.) (*47*), and ryegrass (*Lolium multiflorum* L.) (*48*), whereas increases in chlorophyll pigments were reported for potato (*Solanum tuberosum* L.) (*49*) and ryegrass (*48*). Data from the current study demonstrates that kale leaf tissue chlorophyll concentra-

Table 3. Mean Percent Dry Matter and Carotenoid Pigment Concentrations Expressed on a Dry Mass Basis (mg per g Dry Mass) for Leaf Tissues of Winterbor Kale (*B. oleracea* L. Var. *acephala* Group) Grown under Increasing Selenium (as Selenate or Selenite) Concentrations in Nutrient Solution Culture[a]

		pigment (mg/g dry mass)	
mg of Se/L	% dry matter	lutein	β-carotene
	Selenate		
0.0	7.3 ± 0.2	1.09 ± 0.03	0.94 ± 0.04
0.5	7.3 ± 0.2	1.09 ± 0.05	0.91 ± 0.04
1.0	7.3 ± 0.1	1.06 ± 0.07	0.90 ± 0.02
1.5	7.3 ± 0.1	1.07 ± 0.02	0.89 ± 0.02
2.0	7.4 ± 0.1	0.94 ± 0.05	0.90 ± 0.03
2.5	7.4 ± 0.2	1.02 ± 0.02	0.89 ± 0.02
3.0	7.3 ± 0.2	0.96 ± 0.07	0.83 ± 0.06
3.5	7.3 ± 0.1	1.10 ± 0.04	0.90 ± 0.03
contrasts			
linear	ns[b]	ns[b]	ns[b]
quadratic	ns[b]	ns[b]	ns[b]
	Selenite		
0.0	7.5 ± 0.1	1.09 ± 0.03	0.98 ± 0.01
0.5	7.4 ± 0.2	1.01 ± 0.05	0.91 ± 0.03
1.0	7.4 ± 0.1	1.07 ± 0.03	0.92 ± 0.02
1.5	7.5 ± 0.1	1.00 ± 0.06	0.90 ± 0.04
2.0	7.6 ± 0.1	1.00 ± 0.07	0.94 ± 0.01
2.5	7.5 ± 0.2	0.93 ± 0.05	0.80 ± 0.04
3.0	7.9 ± 0.2	1.02 ± 0.02	0.89 ± 0.02
contrasts			
linear	$P = 0.044$	ns[b]	$P = 0.010$
quadratic	$P = 0.044$	ns[b]	$P = 0.034$

[a] Mean composition of sampled leaf tissue of four replications, six plants each ± standard deviation. [b] Nonsignificant.

tions are not affected by increases in either SeO_4^{-2} or SeO_3^{-2} within the ranges provided in this study.

Vegetable tissues can be dried and encapsulated for use as lutein and β-carotene dietary supplements (*50*, *51*). Therefore, concentrations of lutein and β-carotene expressed on a dry mass (DM) basis were calculated for kale tissues in the current study (**Table 3**). The % DM of the leaf tissues of kale was not affected by increasing concentrations of SeO_4^{-2}; however, a slight increase in % DM occurred in kale grown under increasing SeO_3^{-2} (**Table 3**). The accumulation of lutein in kale leaf tissues expressed on a DM basis was not affected by increasing concentration of either SeO_4^{-2} or SeO_3^{-2}. The accumulation of β-carotene in kale leaf tissues was not affected by increases in SeO_4^{-2}; however kale tissue β-carotene expressed on a DM basis was influenced by increases in SeO_3^{-2} (**Table 3**). β-Carotene expressed on a DM basis responded quadratically [β-carotene DM $SeO_4 = 0.97 - 0.05(trt) + 0.01(trt)^2$] to increases in SeO_3^{-2} concentrations.

At low concentrations (<10 mg/kg), Se acts as an antioxidant, but higher levels can result in pro-oxidant activity (*48*). Selenium performs a similar role as lutein and β-carotene, which scavenge singlet oxygen and excited chlorophyll molecules (*49*). The dual role of Se seems to depend on the Se concentration in the tissue. Selenate has a much greater influence on plant metabolism than does SeO_3^{-2}. Although not significant, there were decreases in lutein and β-carotene concentrations, when expressed on a FM basis, in the kale leaf tissues grown under increasing SeO_4^{-2} concentrations. Therefore, it may be possible that under higher levels of Se than those used in the current study, reduced concentrations of lutein and β-carotene may result. However, Se applied in concentrations within the range of the current study would not be expected to significantly lower carotenoid concentrations in kale.

Table 4. Mean Values of Macronutrients for Leaf Tissues of Winterbor Kale (*B. oleracea* L. Var. *acephala* Group) Grown under Increasing Selenium (as Selenate or Selenite) Concentrations in Nutrient Solution Culture[a]

	macronutrients				
mg of Se/L	% P	% K	% Ca	% Mg	% S
	Selenate				
0.0	0.73 ± 0.02	4.04 ± 0.16	4.13 ± 0.08	0.66 ± 0.01	1.40 ± 0.05
0.5	0.65 ± 0.01	4.02 ± 0.09	4.25 ± 0.08	0.69 ± 0.02	1.88 ± 0.06
1.0	0.63 ± 0.02	4.28 ± 0.03	4.42 ± 0.10	0.71 ± 0.01	2.56 ± 0.07
1.5	0.61 ± 0.01	4.35 ± 0.07	4.40 ± 0.17	0.74 ± 0.04	2.90 ± 0.13
2.0	0.60 ± 0.02	4.43 ± 0.11	4.41 ± 0.14	0.75 ± 0.02	3.00 ± 0.07
2.5	0.58 ± 0.01	4.39 ± 0.14	4.41 ± 0.12	0.74 ± 0.02	3.11 ± 0.10
3.0	0.58 ± 0.01	4.45 ± 0.15	4.40 ± 0.09	0.74 ± 0.02	3.12 ± 0.13
3.5	0.57 ± 0.01	4.53 ± 0.11	4.43 ± 0.07	0.77 ± 0.01	3.15 ± 0.08
contrasts					
linear	$P \leq 0.001$	$P \leq 0.001$	$P = 0.013$	$P \leq 0.001$	$P \leq 0.001$
quadratic	$P \leq 0.001$	$P \leq 0.001$	$P = 0.031$	$P \leq 0.001$	$P \leq 0.001$
	Selenite				
0.0	0.71 ± 0.02	4.10 ± 0.13	4.01 ± 0.06	0.65 ± 0.01	1.35 ± 0.03
0.5	0.68 ± 0.02	4.27 ± 0.02	4.02 ± 0.06	0.65 ± 0.01	1.28 ± 0.02
1.0	0.70 ± 0.01	4.01 ± 0.06	3.90 ± 0.04	0.65 ± 0.01	1.28 ± 0.06
1.5	0.69 ± 0.02	4.24 ± 0.09	3.83 ± 0.05	0.64 ± 0.01	1.19 ± 0.01
2.0	0.67 ± 0.01	4.25 ± 0.09	3.95 ± 0.09	0.65 ± 0.02	1.11 ± 0.04
2.5	0.69 ± 0.02	4.30 ± 0.08	4.01 ± 0.11	0.63 ± 0.02	1.06 ± 0.04
3.0	0.68 ± 0.01	4.26 ± 0.05	3.97 ± 0.04	0.64 ± 0.01	1.06 ± 0.03
contrasts					
linear	ns[b]	ns[b]	ns[b]	ns[b]	$P \leq 0.001$
quadratic	ns[b]	ns[b]	ns[b]	ns[b]	$P \leq 0.001$

[a] Mean composition of sampled leaf tissue of four replications, six plants each ± standard deviation. [b] Nonsignificant.

Elemental Determinations. Elemental nutrient levels in the kale leaves were within reported ranges for mature, greenhouse-grown plants (*52*) (**Tables 4** and **5**). Phosphorus levels in the leaves were influenced by increases in SeO_4^{-2} treatments ($P \leq 0.001$). Phosphorus concentrations responded quadratically to increases in SeO_4^{-2} [P $SeO_4 = 0.71 - 0.09(trt) + 0.02(trt)^2$] treatment concentrations and ranged from 0.57% under 3.5 mg Se/L to 0.73% under 0.0 mg Se/L. Leaf tissue K was affected by increases in SeO_4^{-2} treatments ($P = 0.032$). Potassium increased, then decreased in a quadratic response [K $SeO_4 = 4.0 + 0.27(trt) - 0.04(trt)^2$] as the SeO_4^{-2} concentrations increased in the nutrient solutions, and ranged from 4.02% under 0.5 mg Se/L to 4.53% under 3.5 mg Se/L. Leaf tissue Mg responded to increasing SeO_4^{-2} treatments ($P = 0.013$). Tissue Ca concentrations were influenced by SeO_4^{-2} treatments [Ca $SeO_4 = 4.2 + 0.09(trt)$] and ranged from 4.13% under 0.0 mg Se/L to 4.43% under 3.5 mg Se/L. Tissue Mg concentrations responded quadratically to increases in SeO_4^{-2} [Mg $SeO_4 = 0.66 + 0.05(trt) - 0.01(trt)^2$] and ranged from 0.66% under 0.0 mg Se/L to 0.77% under 3.5 mg Se/L. Leaf tissue B was affected by both SeO_4^{-2} ($P \leq 0.001$) and SeO_3^{-2} ($P \leq 0.001$) treatments. Boron responded quadratically to increases in both SeO_4^{-2} [B $SeO_4 = 61.5 - 12.3(trt) + 2.1(trt)^2$] and SeO_3^{-2} [B $SeO_3 = 60.3 - 10.3(trt) + 0.74(trt)^2$]. Leaf tissue Cu was affected by SeO_4^{-2} ($P = 0.006$), and responded quadratically to increases in SeO_4^{-2} [Cu $SeO_4 = 3.9 - 0.28(trt) + 0.16(trt)^2$] treatments. Leaf tissue Mo was affected by SeO_4^{-2} ($P = 0.018$), and the trend was quadratic [Mo $SeO_4 = 2.48 - 1.07(trt) + 0.23(trt)^2$], decreasing then increasing with increasing treatment concentrations. Kopsell et al. (*38*) reported nutrient accumulation in rapid-cycling *B. oleracea* plants in response to increases in SeO_4^{-2} treatment concentrations. Data from their study revealed increases in K, and decreases in P, Fe, and B, in the leaf tissues as the SeO_4^{-2} treatment concentrations increased from 0.0 to 3.8 mg Se/L. There was no response previously reported for the interaction between SeO_4^{-2} treatments and tissue

Table 5. Mean Values of Micronutrients (μg per g) for Leaf Tissues of Winterbor Kale (*B. oleracea* L. Var. *acephala* Group) Grown under Increasing Selenium (as Selenate or Selenite) Concentrations in Nutrient Solution Culture[a]

	micronutrients (μg/g)						
mg of Se/L	Se	B	Cu	Fe	Mn	Mo	Zn
				Selenate			
0.0	nd[b]	64.2 ± 5.5	3.6 ± 0.1	38.9 ± 3.8	147.1 ± 4.1	2.1 ± 0.6	24.5 ± 3.6
0.5	212.8 ± 22.5	52.7 ± 1.1	3.9 ± 0.2	42.3 ± 5.9	147.2 ± 3.9	2.7 ± 0.2	24.2 ± 3.0
1.0	280.0 ± 58.0	49.5 ± 0.8	4.1 ± 0.2	43.0 ± 4.5	142.5 ± 9.0	1.8 ± 0.2	21.5 ± 1.4
1.5	467.8 ± 27.8	48.2 ± 2.3	4.3 ± 0.5	44.1 ± 8.3	140.2 ± 6.0	0.8 ± 0.4	21.6 ± 1.0
2.0	603.3 ± 44.0	46.1 ± 1.6	3.8 ± 0.1	47.0 ± 3.7	134.8 ± 1.9	1.2 ± 0.3	23.1 ± 2.4
2.5	718.0 ± 50.1	45.5 ± 1.9	3.8 ± 0.1	31.4 ± 14.7	136.4 ± 2.4	1.4 ± 0.4	27.2 ± 3.6
3.0	988.3 ± 42.6	44.2 ± 1.2	4.3 ± 0.3	43.7 ± 4.5	134.3 ± 3.1	1.3 ± 0.1	24.9 ± 3.4
3.5	1056.7 ± 18.8	42.6 ± 1.5	5.3 ± 0.3	37.2 ± 4.6	138.4 ± 9.3	1.5 ± 0.2	22.0 ± 2.2
contrasts							
linear	$P \leq 0.001$	$P \leq 0.001$	$P = 0.004$	ns[c]	$P = 0.035$	$P = 0.024$	ns[c]
quadratic	$P \leq 0.001$	$P \leq 0.001$	$P = 0.004$	ns[c]	ns[c]	$P = 0.011$	ns[c]
				Selenite			
0.0	nd[b]	61.9 ± 5.9	3.5 ± 0.1	32.8 ± 6.3	139.8 ± 4.3	2.2 ± 0.4	23.8 ± 3.7
0.5	195.0 ± 4.7	51.5 ± 2.4	3.4 ± 0.4	38.0 ± 7.0	127.8 ± 3.6	2.6 ± 0.7	22.6 ± 3.7
1.0	209.0 ± 25.3	53.2 ± 2.9	3.6 ± 0.1	26.2 ± 6.7	139.9 ± 4.7	2.5 ± 0.7	20.4 ± 0.4
1.5	199.3 ± 22.0	46.7 ± 1.0	3.7 ± 0.3	37.4 ± 18.1	129.3 ± 6.1	2.9 ± 0.6	19.0 ± 0.7
2.0	235.0 ± 34.5	43.4 ± 2.6	3.5 ± 0.3	46.4 ± 3.4	127.9 ± 5.1	3.6 ± 0.1	21.2 ± 0.8
2.5	216.8 ± 12.7	38.1 ± 1.6	3.2 ± 0.1	38.3 ± 9.1	127.7 ± 4.1	3.1 ± 0.7	21.1 ± 1.2
3.0	198.0 ± 8.3	36.5 ± 1.7	3.1 ± 0.2	43.2 ± 3.2	127.2 ± 3.1	3.6 ± 0.1	22.0 ± 1.4
contrasts							
linear	ns[c]	$P \leq 0.001$	ns[c]	ns[c]	$P = 0.048$	$P = 0.029$	ns[c]
quadratic	ns[c]	$P \leq 0.001$	ns[c]	ns[c]	ns[c]	ns[c]	ns[c]

[a] Mean composition of sampled leaf tissue of four replications, six plants each ± standard deviation. [b] Nondetectable. [c] Nonsignificant.

Ca accumulation (*44*). Decreases in leaf tissue B in brassica land races has been reported under increases in SeO_3^{-2} treatment concentrations (*44*). Results from the current study utilizing kale, a member of the brassicas, confirms the earlier results with the rapid-cycling and land races of brassicas.

Sulfur and Selenium Determination. Leaf tissue S responded to increases in either SeO_4^{-2} ($P \leq 0.001$) or SeO_3^{-2} ($P \leq 0.001$) treatments (**Table 4**). The trends were a quadratic increase in response to increases in SeO_4^{-2} [S $SeO_4 = 1.4 + 1.3(\text{trt}) - 0.23(\text{trt})^2$] or a decrease in S in response to SeO_3^{-2} [S $SeO_3 = 1.36 - 0.12(\text{trt}) - 0.01(\text{trt})^2$] treatments. Sulfur increased from 1.40% to 3.15% as the SeO_4^{-2} treatment concentrations increased from 0.0 to 3.5 mg Se/L, respectively. However, leaf tissue S decreased from 1.35% to 1.06% as the SeO_3^{-2} treatment concentrations increased from 0.0 to 3.0 mg Se/L. Previous reports have identified increases in leaf tissue S under increasing SeO_4^{-2} treatment concentrations (*12*, *39*). It has been postulated that either SeO_4^{-2} or Se metabolites antagonize the repression of SO_4^{-2} transporters by SO_4^{-2} and other S metabolites, thereby increasing S uptake in the presence of elevated media Se (*3*). The reason for the decrease in S as the SeO_3^{-2} treatments increased is not known.

Kale leaf tissue Se concentrations were affected by increases in SeO_4^{-2} treatments ($P \leq 0.001$); however, no changes in leaf tissue Se occurred when kale was grown in the presence of increasing SeO_3^{-2}. No Se was detected in the kale tissues under the 0.0 mg Se/L treatment. Kale leaf tissue Se concentrations ranged from nondetectable to as high as 1056.7 μg Se/g as the SeO_4^{-2} treatment concentrations increased from 0.0 to 3.5 mg Se/L (**Table 5**). As SeO_4^{-2} increased in nutrient solutions, the response in kale leaf tissue Se accumulation was quadratic [$SeO_4 = 168.5 + 53.7(\text{trt}) - 14.1(\text{trt})^2$; **Table 5**]. Bañuelos et al. (*43*) and Kopsell et al. (*38*) reported increases in brassica leaf tissue Se with increasing SeO_4^{-2} treatment concentrations.

Tissue Se results from the current study are within the ranges of reported Se accumulation in the leaves of rapid-cycling *B. oleracea* grown under increasing levels of SeO_4^{-2} in nutrient solutions (*12*, *38*, *53*, *54*). The concentration of Se in the leaf tissue of kale grown under SeO_3^{-2} treatments remained fairly consistent, averaging 209 μg Se/g over all of the treatments (**Table 5**). Selenate can accumulate in plants in concentrations much greater than those present in the surrounding media. In contrast, SeO_3^{-2} does not accumulate to levels surpassing those of the external environment (*5*). When broccoli (*B. oleracea* L. var. *botrytis*), Indian mustard (*B. juncea* L.), or rice (*Oryza sativa* L.) were grown under SeO_4^{-2}, SeO_3^{-2}, or selenomethionine treatments, plants accumulated the greatest amount of shoot Se under SeO_4^{-2}, followed by those given selenomethionine (*55*). Broccoli, Swiss chard (*Beta vulgaris* var. *cicla*), collards (*B. oleracea* L. var. *acephala*), and cabbage (*B. oleracea* L. var. *capitata*) grown in soils treated with 4.5 mg Se/kg as either SeO_3^{-2} or SeO_4^{-2} ranged in tissue Se concentration from 0.013 to 1.382 g/kg DM and absorbed 10 times the amount of Se if treated with SeO_4^{-2} than with SeO_3^{-2} (*8*). Time-dependent kinetic studies showed that Indian mustard absorbed SeO_4^{-2} up to 2-fold faster than SeO_3^{-2} (*56*). The current study demonstrates that kale can accumulate much higher levels of Se from SeO_4^{-2} than from SeO_3^{-2}.

Kale can accumulate high concentrations of Se, as well as lutein and β-carotene. Results from this study show that kale can accumulate high levels of Se (provided as SeO_4^{-2}) without any negative effects on carotenoid concentrations. Selenite fertilization did not have a detrimental effect on the accumulation of lutein and β-carotene; however, kale did not accumulate Se from SeO_3^{-2} to the extent of that from SeO_4^{-2}. With the important health benefits associated with increased consumption of plant-derived lutein, β-carotene, and Se, current results demonstrate that kale may be a good candidate crop to deliver all three to human diets.

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