Environmental and Experimental Botany 74 (2011) 90–97

Contents lists available at ScienceDirect

Environmental and Experimental Botany

journal homepage: www.elsevier.com/locate/envexpbot

# Selenium accumulation in the floral tissues of two Brassicaceae species and its impact on floral traits and plant performance

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ARTICLE INFO

*Article history:*
Received 23 April 2010
Received in revised form 19 April 2011
Accepted 3 May 2011

*Keywords:*
*Brassica juncea*
Floral traits
Hyperaccumulator
Pollinators
Selenium
*Stanleya pinnata*

ABSTRACT

Selenium (Se) is a metalloid that can occur naturally in soils from the Cretaceous shale deposits of a prehistoric inland sea in the western United States. Agricultural irrigation and runoff solubilizes Se from these shales, causing buildups of toxic levels of selenate ($SeO_4^{2-}$) in water and soil. Our main objective was to investigate the accumulation of Se in two Brassicaceae species chosen for their potential as phytoremediators of Se contaminated soils. We tested the hypothesis that Se will accumulate in the pollen and nectar of two plant species and negatively affect floral traits and plant reproduction. Certain species of Brassicaceae can accumulate high concentrations of Se in their leaf tissues. In this study Se accumulation in plant tissues was investigated under greenhouse conditions. Se accumulator (*Brassica juncea*) and Se hyperaccumulator (*Stanleya pinnata*) plants were irrigated in sand culture with 0 μM selenate (control), 8 μM selenate, and 13 μM selenate.

Nectar and pollen in *S. pinnata* contained up to 150 μg Se $mL^{-1}$ wet weight and 12900 μg Se $g^{-1}$ dry weight when irrigated with 8 μM selenate. Se levels in nectar (110 μg Se $mL^{-1}$ wet weight) and pollen (1700 μg Se $g^{-1}$ dry weight) were not as high in *B. juncea*. Floral display width, petal area and seed pod length were significantly reduced in the 13 μM selenate Se treatment in *B. juncea*. *S. pinnata* floral traits and seeds were unaffected by the Se treatments.

This study provides crucial information about where some of the highest concentrations of Se are found in two phytoremediators, and may shed light on the potential risks pollinators may face when foraging upon these accumulating plants. In the field, duration of the plant's exposure, Se soil and water concentrations as well as other environmental factors may also play important roles in determining how much Se is accumulated into the leaf and floral tissues. Our greenhouse study shed light on two species' ability to accumulate Se, as well as determined the specific plant tissues where Se concentrations are highest.

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## 1. Introduction

Plants employ several tactics for defending against herbivory, ranging from physical structures and escape in time or space to chemical defenses that are produced within the plant. Most plants employ innate defenses such as secondary compounds to guard against herbivore attack. However, plant-made defenses can be costly and acquiring defenses from the environment may prove to be a less expensive tactic. Certain species of plants have evolved on naturally metalliferous soils and may accumulate toxic levels of the elements to defend against herbivores, as described by the elemental defense hypothesis (Boyd and Martens, 1992). A growing number of studies support the elemental defense hypothesis by revealing the toxic and deterrent effects of metal and metalloid-containing plant tissues on herbivores (for reviews see Boyd, 2007; Trumble and Sorensen, 2008).

Hyperaccumulator plants can sequester large amounts of metals or metalloids (such as As, Co, Cr, Cu, Mn, Ni, Pb, Zn, or in this case, selenium, Se) in their foliar tissues (Baker and Brooks, 1989). They can absorb 1000 mg $kg^{-1}$ Se dry weight (dw) or higher into shoot tissues (Brown and Shrift, 1981; Reeves and Baker, 2000), and may contain levels of elements several orders of magnitude higher than what is normally found in species at the same site. Se hyperaccumulators include plant species in the genera *Astragalus* (Fabaceae), *Stanleya* (Brassicaceae), *Oonopsis* and *Xylorhiza* (Asteraceae), and these species mainly occur on naturally seleniferous soils such as in the western USA. At least twenty Se hyperaccumulator plant species have been described (Reeves and Baker, 2000). Secondary accumulators, on the other hand, can typically absorb up to 1000 mg $kg^{-1}$ Se when grown on contaminated soils con-

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0098-8472/$ – see front matter. Published by Elsevier B.V.
doi:10.1016/j.envexpbot.2011.05.003

taining moderate levels of the metalloid (Brown and Shrift, 1981). Secondary accumulator plants do not accumulate extremely high concentrations of Se like hyperaccumulators. Certain Brassicaceae species growing in seleniferous soils can accumulate high levels of Se within their tissues (Brown and Shrift, 1981). Non-accumulators such as forage or crop plants accumulate less than 100 mg $kg^{-1}$ of Se and suffer toxic effects when growing in high-element soils. Plants normally accumulate 0.05–1 mg $kg^{-1}$ Se dw, but hyperaccumulators can absorb concentrations hundreds of times greater than the normal range of elements found in non-accumulator plants.

An extensive body of research has examined the role of Se accumulation in plants. Two plant species, *Stanleya pinnata* and *Brassica juncea*, have recently been investigated as potential phytoremediators of polluted soils due to their ability to accumulate and volatilize Se from the soil through their plant tissues (Bañuelos et al., 2002; Parker et al., 2003; Pilon-Smits and Freeman, 2006; Terry et al., 2000). *S. pinnata* is a Se hyperaccumulator species that grows on naturally formed seleniferous soils in the Western USA (Rosenfeld and Beath, 1964), and can absorb up to 10,000 mg $kg^{-1}$ Se dw even when growing on soils containing only 2–10 mg $kg^{-1}$ Se dw (Virupaksha and Shrift, 1965). *S. pinnata* will preferentially take up Se even when S is present as a competitive inhibitor (Bañuelos et al., 1997; Bell et al., 1992; Feist and Parker, 2001; Terry et al., 2000; White et al., 2007). *B. juncea* is a Se secondary accumulator that typically contains up to 350 mg Se $kg^{-1}$ dw when grown in soils contaminated with moderate levels of Se (Terry et al., 2000), and it preferentially accumulates sulfur (S) over Se (Feist and Parker, 2001; Parker et al., 2003). *B. juncea* accumulates Se mostly as selenate ($SeO_4^{2-}$, Parker et al., 2003; Terry et al., 2000), and experiences reduced growth when grown in soil containing 2 mg Se $kg^{-1}$ (Bañuelos et al., 1997), suggesting there may be toxic effects of accumulating Se in secondary accumulator plants. In secondary accumulator plants, selenate can be reduced to selenite ($SeO_3^{2-}$) and then incorporated into amino acids and proteins as selenomethionine or selenocysteine, which can also have toxic effects (Brown and Shrift, 1981).

Two recent studies by Freeman et al. (2006) and Galeas et al. (2007) found high levels of Se in the flowers of *S. pinnata* relative to its leaf tissues, suggesting the defense of fitness-linked reproductive organs (McKey, 1979). However, these studies did not distinguish which specific parts of the flower (pollen, nectar, or petal) contained Se. Selenium concentrations in specific *B. juncea* and *S. pinnata* floral tissues such as pollen and nectar have not been examined to date.

The first objective of this study was to determine whether plants that accumulate Se in their leaves will also accumulate Se in their pollen, nectar, and other floral tissues. The second objective was to determine the toxic effects of Se uptake in terms of floral traits and plant performance in a hyperaccumulator and accumulator plant species.

## 2. Materials and methods

### 2.1. Plant growth conditions

Seeds from the Se hyperaccumulator plant species *S. pinnata* (Pursh) Britton (Desert Prince's Plume) were obtained from a commercial seed company (Western Native Seed, Coaldale, CO, USA). Seeds from the secondary Se accumulator plant species *B. juncea* (L.) Czern (Indian mustard, cv. "Southern Giant Curled") were also obtained from a commercial seed company (Seedway Vegetable Seeds, Hall, NY).

Seeds of both species for Experiment 1 were germinated in the greenhouse (Environmental Sciences Greenhouses, University of California, Riverside, CA) in University of California Standard Soil Mix III and transplanted in 2007. Se treatments were then begun 20 days after transplanting. For Experiment 2, seedlings were transplanted to the greenhouse in 2008 and Se treatments were begun 24 days after transplanting. Seedlings were removed from germination flats and roots were rinsed with tap water to remove as much soil as possible, and were then transplanted to the irrigation sand culture after nutrients had already been added and passed through the sand so that carbonates in the sand would buffer the pH. Seedlings were transplanted to 7.5 l pots filled with silica sand (Weist Rentals and Sales, Riverside, CA). Five plants were transplanted per pot, and any plants that had died were replaced during the following week. Four pots were irrigated from a 120 L tank filled with water and nutrient solution. The basal nutrient solution and Se treatments were added according to Parker et al. (1991). The basal nutrient solution contained 1 mM $NH_4NO_3$, 1 mM $CaCl_2$, 0.25 mM KCl, 0.1 mM $MgSO_4$, 10 μM $NaH_2PO_4$, 1 μM $MnCl_2$, 1 μM $ZnCl_2$, 0.1 μM $CuCl_2$, 3 μM $H_3BO_3$, 0.1 μM $Na_2MoO_4$, and 10 μM Fe–EDTA. Nutrient solution irrigation was activated on a daily timer, pumping solution into each pot five times a day for 5 min. Nutrient solution then drained out of the pots and back into the 120 L tanks. Water levels were maintained at 120 L in the tank by replacing evaporated water with deionized water. Solution N and P levels were checked throughout the experiments and replenished as necessary. However, solution Se levels were not replenished, and were added only once at the start of the experiments (using protocols from Feist and Parker, 2001). *B. juncea* showed reduced growth when irrigated with 2 mg Se $kg^{-1}$ that was maintained at this concentration throughout the experiment (Bañuelos et al., 1997), thus only an initial exposure to the high Se concentration was used to minimize the toxic effects of Se and allow for greater flower production. In addition, a multi-year field study using Se-contaminated soils from the Kesterson Reservoir of California found *B. juncea* depleted the total soil Se inventory by almost 50% (Bañuelos et al., 1995), thus phytoremediators planted in Se-contaminated soils can deplete the Se in the soils around them from an initially higher concentration to a lower concentration over time. Tank pH was monitored in both experiments and averaged 7.78 ± 0.05 (Experiment 1) and 7.50 ± 0.08 (Experiment 2). Greenhouse temperatures were monitored throughout the experiments using a Hobo temperature sensor (Onset Computer Corp., Bourne, MA) and averaged 26.1 °C.

### 2.2. Experimental design and Se treatments

Selenium treatments were started after 20–24 days of seedling establishment in the sand culture. Selenium was added as sodium selenate ($Na_2SeO_4$, Sigma–Aldrich, St. Louis, MO) and is reported as concentrations of elemental Se. Treatment water concentrations were chosen based on Se treatment concentrations used in Feist and Parker (2001), as well as concentrations below 4 mg $L^{-1}$, the maximum Se concentrations contaminating the western San Joaquin Valley in CA (Burau, 1985; Mikkelsen et al., 1986; Presser and Barnes, 1985). The three treatment levels of elemental Se added to the tanks were 0 μM selenate (0.0 mg Se $L^{-1}$) (control, nutrient solution only), 8 μM selenate (0.63 mg Se $L^{-1}$), and 13 μM selenate (1.0 mg Se $L^{-1}$). Pots from each experiment were arranged in a randomized block design in order to minimize the variation in temperature and light in the greenhouse. Each pot was used as a unit of replication for all responses measured except Se content in nectar for *B. juncea* because it produced such low quantities of nectar (<0.02 μL per flower per pot). Nectar from the four pots irrigated by individual tanks were pooled together, thus irrigation tank became the unit of replication for this response.

In Experiment 1, *B. juncea* and *S. pinnata* plants were subjected to the three levels of treatments (0 μM selenate, 8 μM selenate, and 13 μM selenate). In Experiment 2, *B. juncea* plants were subjected

to the 0 μM selenate and 8 μM selenate levels of Se treatments and *S. pinnata* was subjected to the 0 μM selenate, 8 μM selenate, and 13 μM selenate treatments. Each treatment was replicated with up to 58 pots. The datasets of both Experiment 1 and Experiment 2 were compared for each response variable using a *t* test. Datasets of Se content in floral and leaf tissues were combined for both experiments due to no significant differences between the two (*t* test, $P > 0.23$). Experiments 1 and 2 datasets for display width ($P < 0.03$), anther length ($P < 0.004$) and petal area ($P < 0.0001$) were analyzed separately for *B. juncea*. Experiments 1 and 2 plant performance responses that showed no significant differences between the two experiments (total flower number, nectar per flower, seed pod length and total seed weight and proportion of developed seeds, $P > 0.05$ for all) were combined into one dataset. *S. pinnata* plants did not flower in Experiment 1, thus Se content and plant performance data from Experiment 2 only are reported. The photosynthetic photon fluence rate (PPFR, 400–700 nm) was 621–895 $\mu mol\ m^{-2}\ s^{-1}$. Additional high intensity lighting was provided in the greenhouse and programmed on a 16:8 day:night cycle.

### 2.3. Collection of plant tissues for Se uptake

We examined the effects of Se irrigation on plant tissue Se content by measuring the concentration of Se in floral and leaf tissues. Irrigation solution samples were collected 0, 41, 60, and 95 days after the selenate treatments were started. Irrigation solution was analyzed for S and Se.

Floral tissues were collected throughout the experiments, and included: pollen, nectar, anthers/stigmas, and petals. Petals and anther/stigmas were dissected away from other floral tissues and placed in microcentrifuge tubes. Eighty percent ethanol was added to tubes containing anthers/stigmas then sonicated for 3 min (Bransonic Ultrasonics Corp., Danbury, CT) to remove pollen. The anther/stigma portion of the flowers was then removed from the tubes with forceps and placed into separate microcentrifuge tubes. Tubes with ethanol and pollen were then centrifuged at 10,000 rpm for 3 min to pellet the pollen (Fisher Scientific accuSpin Micro 17R microcentrifuge, Fisher Scientific, Pittsburg, PA), and tubes were then placed in a fume hood to evaporate the ethanol. Leaf tissues were also collected at the end of the experiments to compare leaf Se concentrations to floral tissue concentrations. Two leaves of similar age were collected from each plant, rinsed with tap water, and then dried with clean paper towels. All floral and leaf tissues were frozen in a −60 °C freezer (Fisher Scientific, Pittsburg, PA) and then freeze-dried (Labconco Corp., Kansas City, MO) at −40 °C and −25 psi for at least 3 days. Nectar was not freeze-dried and is reported as wet weight in $\mu g\ Se\ mL^{-1}$. After freeze drying, leaf tissues were ground to a fine powder using a mortar and pestle. Floral tissues and seeds were not ground due to their small weights. All freeze-dried plant tissues and nectar were stored in a −60 °C freezer until digestion.

### 2.4. Plant performance measurements

We examined the effects of Se irrigation on plant performance by measuring both floral traits and seed production in both *B. juncea* and *S. pinnata*. For floral traits, we measured two flowers per pot. Floral trait measurements included display width (distance across flower from the tip of one petal to the other), petal area (estimated as length × width), anther length (length of one anther from two flowers per pot), total flower number, and nectar produced per flower (collected from two flowers per pot). The total number of flowers produced per day were counted for each pot replicate throughout the experiment, and then summarized within pot to calculate total flower number. Nectar production was measured using microcapillary tubes (20 μL size for *B. juncea* and 50 μL size for *S. pinnata*) (Drummond "Microcaps", Drummond Scientific Co., Broomall, PA). Nectar volume was collected by first measuring the length of the microcapillary tube using digital calipers (Fisher Scientific, Pittsburg, PA). The microcapillary tube was positioned at the bottom of the nectary, collecting the entire nectar volume in the flower, and the length of the nectar in the tube was then measured using digital calipers. The total volume of nectar was calculated as the tube size (20 μL or 50 μL) divided by the length of nectar in the tube (mm) which was also divided by the length of entire tube (mm). The total sum of nectar collected during the entire experiment (for each pot replicate) was then summed and divided by the total number of flowers collected for nectar to calculate the nectar produced per flower.

Seed production was measured from up to two seed pods per pot as the seed pod length, proportion of developed seeds, and total seed weight. Seeds were categorized as developed or undeveloped; undeveloped seeds were small and wrinkled, indicating an undeveloped embryo. Seed viability was confirmed for developed and undeveloped seeds by germinating them on filter paper moistened with tap water in a growth chamber kept at a constant temperature of 21 °C and a 16:8 day:night cycle.

### 2.5. Atomic absorption and inductively coupled plasma optical emission spectroscopy measurements

Plant tissues were weighed using a microbalance (weighing to 0.00000 g, model 1712 MP8, Sartorius Corp., Goettingen, Germany) prior to microwave digestion. Plant material was microwave digested in 110 mL teflon-lined vessels containing a mixture of 1 mL $H_2O$, 2 mL 30% (v/v) $H_2O_2$, and 2 mL concentrated $HNO_3$ (Sah and Miller, 1992). The vessels were heated for 20 min using a 570 W microwave oven (CEM Corp., Matthews, NC). Plant tissue filtrates and irrigation solution samples were then diluted with 6 M HCl, heated in a 90 °C water bath for 20 min and analyzed using hydride vapor-generated atomic absorption spectroscopy (HVG-AAS). Sulfur was analyzed using inductively coupled plasma optical emission spectroscopy (ICP-OES). Se and sulfur concentrations in irrigation water are reported in μM. Selenium concentrations in plant tissues are reported in ppm ($\mu g\ g^{-1}$ for plant tissues or $\mu g\ mL^{-1}$ for nectar). Samples were run in duplicate and Se spikes were added as internal standards to determine precision and recovery. Duplicate sample concentrations were within 10% of each other, and Se spike recovery was over 90%.

### 2.6. Statistical analyses

We examined the effects of Se irrigation on Se concentration in plant tissues and plant performance in *B. juncea* and *S. pinnata*. All data were averaged within pot using pot as the unit of replication for all responses except *B. juncea* nectar, which was averaged within tank due to the small volumes. Data were analyzed with SAS version 9.2 (SAS Institute, 2008, Cary, NC) using the General Linear Models (GLM) procedure with type III sums of squares. The basic model analyzed the effects of Se irrigation treatment and block (a fixed factor) on several response variables. The Se concentration response variables were analyzed in the following plant tissues: pollen, nectar, anther/stigmas, petals and leaves. Plant performance was analyzed as several responses, including: floral traits (display width, petal area, anther length, total flower number and nectar per flower) as well as seed traits (seed pod length, proportion of developed seeds and total seed weight). A standard Bonferroni correction was applied to the Se in plant tissue and plant performance analyses due to the large number of ANOVAs conducted. Sulfur and Se concentrations in irrigation tank water were analyzed using regression in the REG procedure (SAS, 2008). Assumptions of normality were examined using normal probability plots and the Shapiro–Wilks

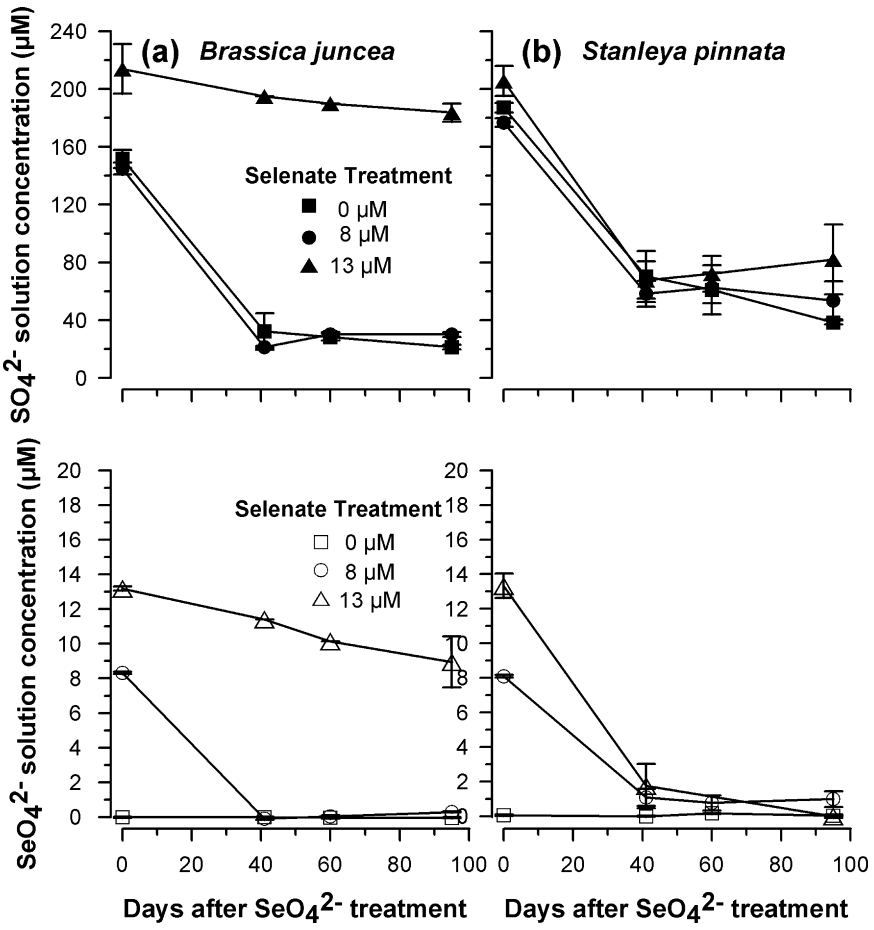


**Fig. 1.** Mean concentrations of S (closed symbols, top panels) and Se (open symbols, bottom panels) in irrigation tank water over time in 0, 8, and 13 µM selenate treatments for (a) *B. juncea* (first flower = 35 days) and (b) *S. pinnata* (first flower = 45 days). Shown are means ± SE.

test in the UNIVARIATE procedure (SAS, 2008). Se concentrations in plant tissues were log transformed to meet assumptions of normality for both *B. juncea* and *S. pinnata*. Floral traits and seed data were normally distributed without transformation for *S. pinnata*. *B. juncea* display width, petal area, nectar per flower, and total seed weights were log transformed to meet assumptions of normality.

## 3. Results

### 3.1. S and Se concentrations in irrigation tanks

Sulfur and Se concentrations were monitored at four timepoints during the experiments. For all irrigation tanks, the 0 µM selenate treatment contained less than 0.006 ± 0.02 µM Se during the entire duration of the experiment. *B. juncea* initial irrigation tank water concentrations averaged 8.32 µM Se (8 µM selenate treatment) and 13.01 µM Se (13 µM selenate treatment) (Fig. 1A). *S. pinnata* initial tank concentrations averaged 8.10 µM Se (8 µM selenate treatment) and 13.20 µM Se (13 µM selenate treatment) (Fig. 1B). After the experiments concluded 95 days later, the final Se concentrations for *B. juncea* averaged 0.30 µM Se (8 µM selenate treatment) and 8.94 µM Se (13 µM selenate treatment). *S. pinnata* final Se concentrations averaged 0.98 µM Se (8 µM selenate treatment) and 0.01 µM Se (13 µM selenate treatment). Sulfur and Se levels in irrigation tanks were correlated in both *B. juncea* ($r = 0.98$, $P < 0.0001$) and *S. pinnata* ($r = 0.96$, $P < 0.0001$) in the 8 µM selenate treatment. Both elements decreased in the irrigation solution over time. Sulfur and Se levels were not correlated in the 0 or 13 µM selenate treatments for *B. juncea* ($r < 0.02$, $P > 0.46$ for both) or *S. pinnata* ($r < 0.23$, $P > 0.08$ for both).

### 3.2. Leaf and floral tissue weights in B. juncea and S. pinnata

Pollen tissue weights averaged 0.008 ± 0.001 g for *B. juncea* ($n = 42$) and 0.01 ± 0.002 g for *S. pinnata* ($n = 32$). Anther/stigma tissue weights averaged 0.02 ± 0.002 g for *B. juncea* ($n = 20$) and 0.08 ± 0.008 g for *S. pinnata* ($n = 34$). Petal weights averaged 0.04 ± 0.008 g for *B. juncea* ($n = 33$) and 0.06 ± 0.008 g for *S. pinnata* ($n = 22$). Leaf tissues averaged 0.10 ± 0.002 g for *B. juncea* ($n = 29$) and 0.10 ± 0.0003 g for *S. pinnata* ($n = 31$). Nectar volumes analyzed ranged from 0.004 ± 0.0004 mL for *B. juncea* ($n = 19$) and 0.04 ± 0.006 mL for *S. pinnata* ($n = 33$).

### 3.3. Se accumulation in B. juncea and S. pinnata plant tissues

*B. juncea* plants irrigated with 8 and 13 µM selenate treatments significantly accumulated Se into pollen, anthers/stigmas, petals, and leaves (ANOVA, $P < 0.0001$ for all) (Fig. 2A). Petal and anther/stigma tissue contained the highest Se concentrations (2800 µg Se $g^{-1}$ dw and 2700 µg Se $g^{-1}$ dw in the 13 µM selenate treatment). Pollen concentrations were also high (1700 µg Se $g^{-1}$ dw in the 13 µM selenate treatment). *B. juncea* nectar irrigated with 8 and 13 µM selenate treatments significantly accumulated Se into nectar (up to 110 µg Se $mL^{-1}$ wet weight (ww), $P < 0.01$) (Fig. 2A). Leaf and nectar concentrations were low relative to the other plant tissues. Block had no significant effect on Se accumulation in any *B. juncea* plant tissues ($P > 0.02$ for all, insignif-

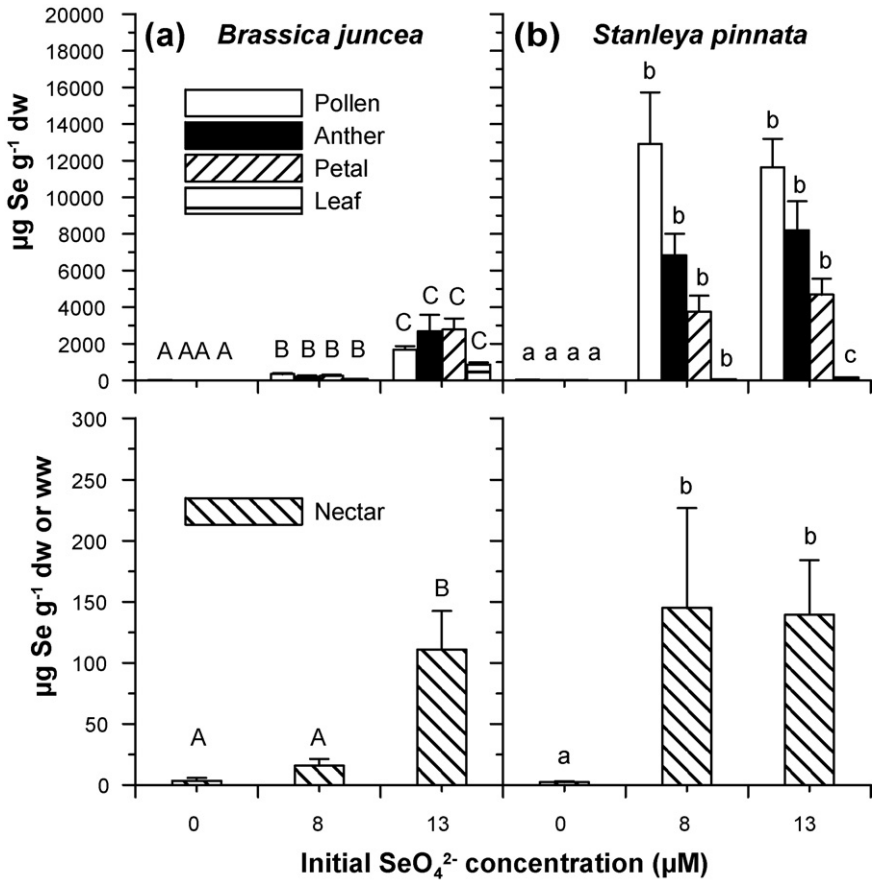


**Fig. 2.** Se concentrations in (a) *B. juncea* and (b) *S. pinnata* after treatment with 0, 8, and 13 μM initial selenate treatments in floral tissues (top panels) (pollen (*B. juncea,* $n = 13$, 18, and 11 respectively, *S. pinnata,* $n = 14$, 11, 7), anther/stigmas (*B. juncea,* $n = 8$, 8, 4, *S. pinnata,* $n = 13$, 14, 7) and petals (*B. juncea,* $n = 14$, 10, 9, *S. pinnata,* $n = 7$, 9, 6)), leaf tissues (*B. juncea,* $n = 9$, 10, 10, *S. pinnata,* $n = 13$, 10, 8) and nectar (bottom panels) (*B. juncea,* $n = 5$, 9, 5, *S. pinnata,* $n = 15$, 11, 7). *Brassica juncea* and *S. pinnata* plants irrigated with selenate treatments significantly accumulated Se into floral and leaf tissues (ANOVA, $P < 0.0001$ for all except *B. juncea* nectar, $P < 0.04$). Shown are means ± SE. Letters next to the means indicate statistically significant differences between groups (alpha = 0.05) using Tukey's HSD test. All plant tissue concentrations are reported in μg of elemental Se.

icant with a Bonferroni correction). Seeds from *B. juncea* treated with 8 and 13 μM selenate contained 220 and 940 μg Se $g^{-1}$ dw respectively.

*S. pinnata* plants irrigated with 8 and 13 μM selenate treatments also significantly accumulated Se into pollen, nectar, anthers/stigmas, petals and leaves ($P < 0.0001$ for all) (Fig. 2B). Pollen contained the highest concentrations of Se compared to all other tissues (12900 μg Se $g^{-1}$ dry weight in the 8 μM selenate treatment), followed by anther/stigma tissues (8200 μg Se $g^{-1}$ dw in the 13 μM selenate treatment) and petal tissues (4700 μg Se $g^{-1}$ dw in the 13 μM selenate treatment). Nectar contained up to 150 μg Se $mL^{-1}$ wet weight in the 8 μM selenate treatment. Leaf tissues had the lowest Se concentrations (130 μg Se $g^{-1}$ dw in the 13 μM selenate treatment). Seeds from *S. pinnata* plants irrigated with 8 and 13 μM selenate contained 3300 and 6000 μg Se $g^{-1}$ dw respectively. Block had no significant effect on Se accumulation in *S. pinnata* plant tissues ($P > 0.44$ for all).

### 3.4. Effects of Se on plant performance in Brassica juncea and Stanleya pinnata

For Experiment 1, the 13 μM selenate treatment reduced *B. juncea* floral display width by 31% (ANOVA, $P < 0.0001$) and petal area by 44% ($P < 0.0001$, Table 1). However, in both Experiments 1 and 2, the 8 μM selenate treatments had no effect on these floral traits ($P > 0.08$ for all), only the highest Se treatment reduced display width and petal area. In Experiment 1, block had a significant effect on display width ($P < 0.006$). Se treatment had no significant effect on anther length ($P > 0.05$).

For Experiments 1 and 2 combined, both the 8 and 13 μM selenate treatments reduced total flower number, but it was not significant with a Bonferroni correction. For Experiments 1 and 2 combined, the 13 μM selenate treatment reduced seed pod length by almost 50% ($P < 0.0001$, Table 1), but the 8 μM selenate treatment actually produced slightly larger seed pods. Se treatments had no effect on nectar per flower, proportion of developed seeds, or total seed weight ($P > 0.20$ for all). For both experiments combined, block had no significant effect on *B. juncea* flower number, nectar per flower, seed pod length, proportion of developed seeds, or total seed weight ($P > 0.10$ for all).

Se treatments had no significant impact on any aspect of *S. pinnata* floral or seed traits ($P > 0.20$ for all, Table 1). *S. pinnata* could tolerate these treatment levels and maintain its floral traits and seed production. Block had no significant effect on all *S. pinnata* plant performance responses ($P > 0.10$).

## 4. Discussion

The objectives of this study were to investigate (1) whether plants that accumulate Se in their leaves will also accumulate Se in their pollen, nectar, and other floral tissues, and (2) to determine the toxic effects of Se uptake in terms of floral and seed traits in a secondary accumulator and hyperaccumulator plant species. Our predictions were that Se would minimally accumulate in the pollen

**Table 1**
Effects of 0, 8, and 13 μM initial selenate treatments on *S. pinnata* and *B. juncea* floral traits (display width, anther length and petal area) and plant performance (flower number and seed pod length). There was no significant difference between Experiments 1 and 2 for *B. juncea* flower number and seed pod length (*t* test, $P > 0.05$), and data shown are for the two experiments combined. Shown are means ± SE. Letters next to the means indicate statistically significant differences between groups (alpha = 0.05) using Tukey's HSD test.

| | Floral display width (mm) | | Anther length (mm) | | Petal area ($mm^2$) | | Flower number | | Seed pod length (mm) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *N* | Mean ± SE | *N* | Mean ± SE | *N* | Mean ± SE | *N* | Mean ± SE | *N* | Mean ± SE |
| Experiment 2 | | | | | | | | | | |
| *Stanleya pinnata* | | | | | | | | | | |
| 0 μM selenate | 16 | 24.14 ± 1.85a | 16 | 3.21 ± 0.33a | 16 | 41.96 ± 4.45a | 14 | 43 ± 7a | 15 | 33.50 ± 3.85a |
| 8 μM selenate | 15 | 23.30 ± 1.14a | 15 | 3.60 ± 0.21a | 15 | 46.84 ± 3.86a | 7 | 57 ± 11a | 8 | 31.27 ± 4.86a |
| 13 μM selenate | 6 | 26.88 ± 1.92a | 16 | 3.38 ± 0.25a | 6 | 43.61 ± 4.69a | 5 | 23 ± 3a | 7 | 38.28 ± 4.58a |
| Experiment 1 | | | | | | | Experiments 1 and 2 | | | |
| *Brassica juncea* | | | | | | | | | | |
| 0 μM selenate | 34 | 12.84 ± 0.34a | 34 | 1.86 ± 0.03a | 34 | 58.66 ± 2.39a | 58 | 108 ± 27a | 36 | 22.23 ± 1.15b |
| 8 μM selenate | 10 | 12.76 ± 0.63a | 11 | 1.88 ± 0.07a | 10 | 57.66 ± 5.70a | 39 | 53 ± 11a | 22 | 27.67 ± 1.03a |
| 13 μM selenate | 11 | 8.92 ± 0.59b | 11 | 1.62 ± 0.06a | 11 | 32.68 ± 2.77b | 11 | 22 ± 6b | 4 | 11.41 ± 3.82c |
| Experiment 2 | | | | | | | | | | |
| 0 μM selenate | 24 | 11.35 ± 0.31a | 24 | 1.67 ± 0.06a | 24 | 43.14 ± 2.05a | | | | |
| 8 μM selenate | 25 | 10.75 ± 0.32a | 25 | 1.68 ± 0.05a | 25 | 37.13 ± 2.20a | | | | |

and nectar of both species and that Se would have a stronger negative effect on plant performance and floral traits in the secondary accumulator *B. juncea* compared to the Se hyperaccumulator *S. pinnata.*

### 4.1. Effects of Se treatments on uptake into leaves and floral tissues

*B. juncea* accumulated up to 22% and 85% less Se in the nectar and pollen, respectively, compared to the hyperaccumulator plant, *S. pinnata*. *B. juncea* plants showed no significant difference in nectar Se concentration between the 0 and 8 μM selenate treatments. However, *S. pinnata* accumulated similar concentrations of Se in nectar at both treatment levels (about 140 μg Se $mL^{-1}$, Fig. 2B). Se accumulation in *S. pinnata* may have peaked at the 8 μM selenate treatment level, since there were no significant differences between Se concentrations at the 8 and 13 μM treatment levels in any of the floral tissues. Se follows the same sulfate assimilation pathway in both plant types, but *B. juncea* preferentially accumulates S instead of Se (Feist and Parker, 2001; Terry et al., 2000). $MgSO_4$ was added to the irrigation tanks once at the beginning of the experiments at a concentration of 0.1 mM, and this was the only significant source of S available to the plants. Sulfur was not completely depleted from the *B. juncea* irrigation tanks at the end of the experiments. However, *B. juncea* contained almost seven times as much Se in its leaf tissues compared with *S. pinnata.* At the end of the experiments, tanks irrigating *S. pinnata* contained less Se than *B. juncea* tanks in the 13 μM selenate treatment (0.01 μM vs. 8.94 μM), suggesting *S. pinnata* removed more Se from the solution. The hyperaccumulator *S. pinnata* may have mobilized much of its leaf Se into the reproductive tissues or volatilized the Se out of its leaves into the atmosphere. Field studies using *S. pinnata* have demonstrated an increase in Se in reproductive tissues (flowers and seeds) corresponding with a reduction in leaf Se (Galeas et al., 2007). Selenium may be utilized as an elemental defense by protecting fitness linked organs such as flowers and sequestering high concentrations of Se in the floral parts instead of the leaves at later developmental stages.

### 4.2. Secondary accumulators vs. hyperaccumulators: effects of Se uptake on plant performance

We hypothesized that Se would have a stronger negative effect on plant performance and floral traits in the accumulator *B. juncea* compared to the hyperaccumulator *S. pinnata*. Hyperaccumulators can take up over 4000 mg Se $kg^{-1}$ without showing reduced growth (Shrift, 1969), whereas in our study, *B. juncea* suffered toxic effects on plant performance in terms of reduced flower size, flower number, and seed pod length. In addition, plants appeared smaller at the highest Se treatment (personal observation). Several *Brassica* land races showed signs of Se toxicity in terms of reduced dry matter yield and leaf surface area (Bañuelos et al., 1997). Selenium's toxicity is attributed to its similarity to sulfur (S). Se replaces S in amino acids and can change protein folding, causing reduced growth and deformities (Daniels, 1996 and Lemly, 1997). However, Se hyperaccumulators can circumvent these toxic effects by methylating the selenocysteine for storage or volatilization (Terry et al., 2000). Se accumulators such as the crop plant *B. juncea* take up low to moderate levels of Se into their plant tissues when growing on soils with moderate levels of Se, whereas Se hyperaccumulators such as *S. pinnata* can take up high levels of Se into their plant tissues even when growing on soils with low levels of Se (Terry et al., 2000). Hyperaccumulators such as *S. pinnata* are thought to have evolved on seleniferous soils, and can metabolize and biotransform selenate into non-protein selenoamino acids (such as Se-methylselenocysteine), which secondary accumulators cannot (Brown and Shrift, 1981; Brown and Shrift, 1982; Terry et al., 2000). Methylation of the selenoamino acids may protect the hyperaccumulators such as *S. pinnata*, but not secondary accumulators such as *B. juncea*, from the toxic effects of these compounds.

A large portion of the Se was depleted from the tanks at the beginning of the flowering period for both species (Fig. 1A and B). In particular, *S. pinnata* began flowering 45 days after the Se treatments were started. Within 41 days after treatments were added, Se concentrations in the irrigation tank water dropped to 1.08 μM Se (8 μM initial selenate treatment), and 1.74 μM Se (13 μM initial selenate treatment). A recent study by Galeas et al. (2007) found that Se mobilizes to different plant tissues in Se hyperaccumulator plants. In the early part of the growing season, hyperaccumulators transport Se to the leaf tissues, whereas later in the season, Se is moved from leaf tissues into reproductive tissues such as flowers and seeds. In our study, Se may have been mobilized within the plant from leaf tissues into the flowers, although leaves were collected for Se testing only at the end of the experiment. In hyperaccumulators, Se mobilization to the fitness-linked floral tissues such as flowers and seeds may provide support for optimal defense theory (McKey, 1979) and the elemental defense hypothesis (Boyd, 1998; Boyd, 2007). However, in order to link the adaptive significance of Se accumulation in terms of increased fitness and as a defense of reproductive tissues, additional studies will be required.

Also, leaf and floral tissues would have to be collected at several timepoints throughout the experiment to determine whether Se was being mobilized within the plant.

## 5. Conclusions

Although Se levels were high in the floral tissues of our greenhouse study, Se concentrations in the leaves of *B. juncea* and *S. pinnata* have varied across field studies. Galeas et al. (2007) found *S. pinnata* leaf concentrations of 500–2000 mg Se $kg^{-1}$ dw and flower concentrations of 1800 mg $kg^{-1}$ dw in the field throughout a 7-month growing season. In addition, a study by Bañuelos et al. (2007) found the leaves of transgenic *B. juncea* grown for phytoremediation of soil contaminated with 4 mg Se $kg^{-1}$ contained only about 30–50 μg Se $g^{-1}$ dw in the field. In our greenhouse study, *B. juncea* accumulated Se concentrations in the pollen and nectar that could be potentially toxic to pollinators, but Se concentrations of leaves in field studies (such as Bañuelos et al., 2007) suggest flower concentrations may be lower. The duration and soil concentration of Se exposure as well as other environmental factors may play important roles in determining how much Se is accumulated into the leaf and floral tissues. Although the leaves in our study had higher *B. juncea* concentrations and lower *S. pinnata* concentrations compared to the studies mentioned above, our experiments are relevant because they focused on a 3 month period which captured the peak flowering period of both species when irrigated with ecologically relevant Se concentrations (up to 1.4 mg Se $L^{-1}$). Our study provides a snapshot of the Se concentrations during the flowering period that could be available to pollinators visiting flowers on Se-accumulating plants.

Several studies have found evidence for plant-produced (secondary chemical) defenses in floral tissues such as petals, nectar (Adler, 2000; Detzel and Wink, 1993; Gegear et al., 2007; Kessler and Baldwin, 2007; McCall and Karban, 2006) and even pollen (Praz et al., 2008). Some hyperaccumulator plant species also accumulate elevated levels of metals and metalloids in their flowers and fruits (Freeman et al., 2006; Jaffre et al., 1976; Reeves et al., 1981), possibly as an elemental defense. Certain insect species cannot detect and avoid Se (Trumble et al., 1998; Vickerman et al., 2002), but there are no studies to date examining the effects of Se-containing plant tissues on insect pollinator visitation in terms of deterrence. If insect pollinators cannot detect and avoid toxic compounds in the floral tissues they are foraging upon and collecting for their progeny, they may suffer similar adverse effects such as mortality and reduced development as has been seen in other insect guilds (Trumble et al., 1998; Vickerman et al., 2002; Hanson et al., 2003; Hanson et al., 2004; Freeman et al., 2007; Sorensen et al., 2009). Alternatively, Se is a micronutrient that is essential to many animals when ingested in low quantities (Burau, 1985) and may be a beneficial antioxidant to pollinators that feed upon Se-containing floral tissues.

*B. juncea* and *S. pinnata* have gained interest as phytoremediators of Se-contaminated soils (Parker et al., 2003, Pilon-Smits and Freeman, 2006). In particular, *B. juncea* has been genetically modified to increase its ability to accumulate and volatilize Se (Bañuelos et al., 2007, Pilon-Smits and LeDuc, 2009). In our study, *S. pinnata* had low concentrations of Se in the leaves, suggesting this species may volatilize Se as well. Phytoremediation using these species may expose pollinators to Se-containing tissues, unless plants are harvested before flowering. Transgenic plants are harvested when 25% of the plants flower (as mandated by the USDA-Animal and Plant Health Inspection Service) and a similar approach to managing non-transgenic phytoremediators may protect beneficial pollinators from exposure to potentially toxic floral tissues. This study provides crucial information about where some of the highest concentrations of Se are found in two phytoremediators, and may shed light on the potential risks pollinators may face when foraging upon these accumulating plants.

## Acknowledgements

The authors thank Woody Smith, David Thomason and Kelly Thrippleton-Hunter for their helpful discussions and assistance. We thank Casey Butler, William Carson, Greg Kund and Christina Mogren for their review of this manuscript. We also thank Lianne Pilon-Smits and Colin Quinn for their helpful comments, as well as Ray Morton and Amber Coffman for their help in the greenhouse. This work was supported by the Department of Entomology at University of California – Riverside, a University of California Toxic Substances Research and Teaching Program fellowship (UC TSR and TP) to KRH, and an Environmental Protection Agency Science to Achieve Results fellowship (EPA STAR) to KRH. This publication was developed under a STAR Research Assistance Agreement No. F08F20896 awarded by the U.S. Environmental Protection Agency. It has not been formally reviewed by the EPA. The views expressed in this document are solely those of Kristen Hladun and the EPA does not endorse any products or commercial services mentioned in this publication.

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