Environmental Pollution 172 (2013) 70–75

Contents lists available at SciVerse ScienceDirect

Environmental Pollution

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

Effects of selenium accumulation on phytotoxicity, herbivory, and pollination ecology in radish (*Raphanus sativus* L.)

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ARTICLE INFO

Article history:
Received 11 April 2012
Received in revised form
2 August 2012
Accepted 17 August 2012

Keywords:
Radish
Apis mellifera
Floral traits
Plant reproduction
Pollen limitation

ABSTRACT

Selenium (Se) has contaminated areas in the western USA where pollination is critical to the functioning of both agricultural and natural ecosystems, yet we know little about how Se can impact pollinators. In a two-year semi-field study, the weedy plant *Raphanus sativus* (radish) was exposed to three selenate treatments and two pollination treatments to evaluate the effects on pollinator–plant interactions. Honey bee (*Apis mellifera* L.) pollinators were observed to readily forage on *R. sativus* for both pollen and nectar despite high floral Se concentrations. Se treatment increased both seed abortion (14%) and decreased plant biomass (8–9%). Herbivory by birds and aphids was reduced on Se-treated plants, indicating a potential reproductive advantage for the plant. Our study sheds light on how pollutants such as Se can impact the pollination ecology of a plant that accumulates even moderate amounts of Se.

1. Introduction

Up to 80% of the world's 250,000 flowering plant species (Kremen et al., 2007) and 60% of crop species (Roubik, 1995) are animal pollinated, with insect pollinators such as honey bees being critical components to the crop species in particular. Pollinators such as honey bees and their honey products have been investigated as potential bioindicators pollutants, and varying amounts of elements that are toxic to insects have been found in honey, propolis, and pollen of honey bee hives located in proximity to polluted sites (Bogdanov, 2006). However, few studies have focused on pollutants effects on plant–pollinator interactions or the fitness consequences on bee populations.

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 dissolves Se from these shales, causing accumulation of toxic levels of selenate (SeO_4^{2-}) in water and soil (Brown et al., 1999). Selenate is the most common species of Se found in the root zone (Tokunaga et al., 1991) and can contaminate both water and soil (Cutter, 1982; Frankenberger and Benson, 1994; Dhillon and Dhillon, 2001; Trumble and Sorensen, 2008).

Several studies have reported elevated levels of metals in the flowers and fruits of specialized plant species known as hyperaccumulators that have evolved to use certain elements as a defense against herbivores (Jaffre et al., 1976; Reeves et al., 1981; Freeman et al., 2006; Boyd, 2007). High levels of Se have been found in flowers relative to leaf tissues (up to 9000 mg Se kg^{-1} for *Astragalus bisulcatus* ((Hook.) A. Gray, Galeas et al., 2007)), but this study did not distinguish which specific parts of the flower (pollen, nectar, or petal) contained Se. These hyperaccumulators tend to be found in rather limited areas where elevated concentrations of specific elements naturally occur (Boyd, 2007; Feist and Parker, 2001). However, certain species of Brassicaceae that have not evolved elemental defense can also have moderately high Se levels (Brown and Shrift, 1981) when growing on Se-polluted soils. Foliar herbivores fed plant tissues containing high levels of metals, metalloids, or other accumulated elements have shown reduced developmental rates and survival (Boyd, 2007; Butler and Trumble, 2008). Several reports have indicated some insect species cannot detect detrimental levels of Se (Trumble et al., 1998; Vickerman et al., 2002), but there is no published study to date examining the effects of the pollutant Se on the pollination ecology of a non-hyperaccumulator plant.

Certain crop species can accumulate Se when grown in soils with elevated Se (Carvalho et al., 2003). Members of the Brassicaceae such as *B. juncea* experience reduced growth when grown in soil containing 2 mg Se kg^{-1} (Bañuelos et al., 1997), suggesting

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http://dx.doi.org/10.1016/j.envpol.2012.08.009

there is a cost to accumulating Se. Se may have similar phytotoxic effects on *Raphanus sativus* L. (radish), which is known to accumulate Se mostly as selenate (Pedrero et al., 2006). Selenate can be reduced to selenite (SeO_3^{2-}) and then incorporated into amino acids as selenomethionine or selenocysteine and then into proteins, which can also have toxic effects (Brown and Shrift, 1981). Se volatilizes from foliar tissues as dimethylselenide (DMSe) and other Se-containing volatiles (Meija et al., 2002; Kubachka et al., 2007), and may cause changes in feeding site preferences and deterrence for herbivores as well as pollinators. The potential effects on pollination and subsequent plant reproductive success is largely unknown for non-specialist plants.

Raphanus sativus has been examined as a model for studying plant responses to pollutants (Kostka-Rick and Manning, 1993). This species is a common weed throughout California and is cultivated throughout the world (Snow and Campbell, 2005). It is an annual, self-incompatible plant (thus ideal for pollination studies) that has been examined extensively in herbivore and pollinator studies (Stanton, 1987; Strauss et al., 2004) as well as for its hybridization with *Raphanus raphanistrum* L. (Hedge et al., 2006). Our previous greenhouse studies confirm that radish can accumulate Se into its leaves and roots, as well as into its pollen and nectar (Hladun et al., *unpublished data*) at concentrations well above the LC_{50} for an insect herbivore (*Spodoptera exigua* Hübner, Lepidoptera: Noctuidae, Trumble et al., 1998).

We conducted a manipulative semi-field study to examine how the soil-borne pollutant Se can affect plant performance and reproduction, herbivory, and pollinator visitation. Our main objectives were to test the hypotheses: 1) the pollutant Se will cause a reduction in plant reproduction due to pollinator deterrence or phytotoxicity to the plant, and 2) Se will have a beneficial effect by reducing herbivore damage without a plant losing attractiveness to pollinators and therefore maintaining plant reproductive output.

2. Materials and methods

2.1. Experimental treatments

For year 1, on 27-Jan-2010, *R. sativus* (crop radish, cv. "White Globe", Livingston Seed Co., Columbus, OH USA) was planted in steam sterilized potting mix (50% sand, 25% bark, 25% peat moss) within 18.93 l pots. Pots were placed approximately 0.5 m apart, placed within bins to capture runoff, in a plot of land measuring 35 m × 22 m. Experiments were conducted at the Department of Agricultural Operations at the University of California (Riverside, CA). Two Se treatments (0 and 0.51 mg Se l^{-1}) and 2 pollination treatments (natural and hand) were manipulated in a factorial design for a total of 4 treatment combinations. Plants were assigned to treatments in a randomized block design, with 3 plants per treatment combination and 12 plants per block, for a total of 6 blocks and 72 plants. Block was included as a fixed factor to account for differences in proximity to the honey bee hive.

For year 2, on 2-Feb-2011, crop radish seeds were planted as described above. Two Se treatments (0 and 0.51 mg Se l^{-1}) were applied along with an additional high Se concentration (1.53 mg Se l^{-1}) for a total of three Se treatments. Two pollination treatments (natural and hand) were again included in a factorial design for 6 total treatment combinations. Plants were assigned to treatments in a randomized block design, with 1 replicate plant per treatment combination per block and 6 plants per block, for a total of 12 blocks and 72 plants. During both years, we watered plants with Se-treated tap water three times a week with 500 ml of treatment water. Se treatments were added as sodium selenate (Na_2SeO_4, Sigma-Aldrich, St. Louis, MO), the form commonly found in contaminated waters and soils (Tokunaga et al., 1991) and concentrations are reported in elemental Se. Se treatment levels were ecologically relevant because concentrations were within the range of the high end of reported concentrations for contaminated sites (2 mg l^{-1}) (Seiler et al., 1999) and the highest mean Se concentrations from stream sediments and soils in CA (0.58 mg kg^{-1}) (Grossman et al., 2007), but were below 4 mg l^{-1}, the maximum Se concentrations contaminating the western San Joaquin Valley in CA (Burau, 1985; Presser and Barnes, 1985).

Pollination was also manipulated to determine if Se accumulation in the plant altered pollen limitation. Pollination treatments were applied twice during the peak flowering period (Year 1: April 30 2010 and May 21 2010, Year 2: April 29 2011 and May 20 2011). Two unopened flowers of similar age per plant were arbitrarily chosen and covered with mesh bags the day before pollination treatment to prevent any visitation. The next day, pollen was collected from 5 different greenhouse-grown radish plants used for the sole purpose of pollen donation for the application of hand pollination treatments on the field plants. Pollen viability was evaluated for each paternal line using Alexander's stain (Alexander, 1980) and averaged 92.9 ± 1.4% ($n = 20$). Bags were then removed and saturating amounts of pollen were applied evenly to the stigmas as the hand pollination treatment. Plants assigned the natural pollination treatment were also bagged to control for any bag effects, and were removed to allow pollen deposition from bee visitation. A honey bee hive maintained adjacent to the plots, was the main source of natural pollination at this site.

2.2. Plant performance and reproduction

For floral traits, two flowers per pot were measured during peak flowering period using morphological measurements based on Conner and Via (1993). Floral trait measurements included display width (distance across flower from the tip of one petal to the other), petal area (estimated as length × width), corolla tube length, pistil and stamen length. The total number of flowers produced per day was counted for each plant throughout the experiment, and then summarized within plant to calculate total flower number.

Aboveground biomass and root biomass were harvested at the end of the experiment, dried in an oven at 70 °C and weighed. Fruit on the whole plant were examined at the end of each experiment year and scored as intact, frugivory, or aborted (only the pedicel present). Seed production was measured for 5 randomly chosen fruit per plant. Fruit were broken open and total seed number and weight were quantified using a microbalance (weighing to 0.00001 g, model 1712 MP8, Sartorius Corp., Goettingen, Germany).

2.3. Herbivory

Herbivory was scored once a week for 11 weeks beginning on Feb 12 2010 and on Feb 23 2011. Each week, the total number of leaves were counted and damage to three randomly chosen leaves were estimated and averaged as the percent of leaf tissue removed. Herbivore damage by the imported cabbageworm (*Pieris rapae* L., Lepidoptera: Pieridae) was rare. The predominant herbivore found both years was cabbage aphid (*Brevicoryne brassicae* L., Hemiptera: Aphididae), and their total numbers on leaves and flower buds were quantified. Aphid mummies were also counted during each weekly herbivory census in order to collect data on Se's effects on higher trophic levels. A previous study found Se can impair the development and weight of a parasite in a host that had been feeding on Se-treated plants (Vickerman et al., 2004). The observation of aphid mummies was based on the characteristic swollen, papery brown stature an aphid turns into when parasitized by a wasp. Ladybird beetles (*Coccinella septempuctata* L.) were observed to be feeding on the aphids, and were also collected. Frugivory was observed to be from house finches, *Carpodacus mexicanus*. Fruits were ripped open by the birds, the seeds inside eaten, and therefore the fruit was scored as "frugivory" only if a torn, empty husk remained.

2.4. Pollen limitation and pollinator visitation

Seed production and viability from flowers used in the pollination treatments was quantified as described above. Visitation by the predominant pollinator, the honey bee, was observed during peak flowering period from May 15 2010 to May 20 2010 (Year 1) and from May 13 2011 to May 26 2011 (Year 2) for 5 min observation periods per day at the same time of day (between 1400 and 1600 h). The total number and duration of honey bee visits were recorded for each plant. Seed viability was confirmed in two randomly chosen fruit per plant 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. Final germination percentage (FGP) was calculated as the total number of seeds that germinated after 7 days divided by the total number of seeds produced in each cross.

2.5. Se analyses in plant and insect tissues

Se treatment effects on plant tissue Se content was examined by measuring the concentration of Se in floral and leaf tissues. Two leaves and five flowers of similar age were collected from each plant during the peak flowering period. Honey bees were collected as they foraged during peak flowering period. Pollen loads were removed from corbiculae and analyzed separately. Cabbage aphids and ladybird beetle (*C. septempuctata*) predators were also collected from plants treated with Se. All floral, leaf and insect 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. After freeze drying, leaf and flower tissues were ground to a fine powder using a mortar and pestle to homogenize tissues. All freeze-dried plant tissues were stored in a −60 °C freezer until digestion. All Se concentrations in plant tissues are reported in mg kg^{-1} dry weight.

All plant tissues were weighed using a microbalance prior to digestion. Plant material was microwaved 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). Insect tissues were weighed using a microbalance prior to microwave digestion. Insect material was microwave digested in vessels containing 10 ml concentrated HNO_3, then were heated for 30 min in the microwave. Plant and insect tissues were then analyzed using inductively coupled plasma optical emission spectroscopy (ICP-OES) (PerkinElmer Inc., Shelton CT). Se concentrations in plant and insect tissues are reported in mg kg^{-1}. Samples were run in duplicate and Se spikes were added as internal standards to determine precision and recovery. The NIST Standard Reference Material 8436 (durum wheat flour) was used as a standard for plant tissues, and NIST 1566B (oyster) was used for insect tissues. Duplicate sample concentrations were within 10% of each other, and Se spike recovery and NIST Se recovery were over 90%.

2.6. Statistical analyses

Results were analyzed with general linear models (PROC GLM, SAS 9.2; SAS Institute, Cary NC, USA) with type III sum of squares; independent variable included Se treatment, pollination treatment, year, and their interactions. Block was included as a fixed factor, and the experiment was blocked in space to minimize variation. MANOVAs were conducted on plant performance, herbivory, pollination, and Se in insect and plant tissues. When MANOVAs were significant, subsequent ANOVAs were conducted. Mean separations were conducted between groups ($\alpha = 0.05$) using *post hoc* Tukey's HSD test. Assumptions of normality were examined using the Shapiro–Wilks test. The response variables aboveground biomass, root biomass, leaf damage, total bee visits and bee visit duration per bout were log-transformed to meet assumptions of normality and homogeneity of variance.

3. Results

3.1. Plant performance and reproduction

Se treatment or its interaction with year had no significant effect on floral traits or flower number (MANOVA, Wilks' $\lambda < 1.16$, $P > 0.34$). Block (Wilks' $\lambda = 1.63$, $P < 0.005$) and year (Wilks' $\lambda = 9.19$, $P < 0.001$) significantly affected floral morphology. Year significantly affected display width (ANOVA, $F_{1,32} = 7.52$, $P < 0.01$), corolla tube length ($F_{1,32} = 6.60$, $P < 0.02$), short stamen length ($F_{1,32} = 13.36$, $P < 0.001$), and long stamen length ($F_{1,32} = 4.96$, $P < 0.04$). Block had a significant effect on total flower number ($F_{1,32} = 5.77$, $P < 0.001$).

Se treatment (MANOVA, Wilks' $\lambda = 4.01$, $P < 0.005$) and year (MANOVA, Wilks' $\lambda = 72.68$, $P < 0.001$) had a significant effect on plant performance and reproduction.

The interaction of Se treatment × year and block were not significant (MANOVA, Wilks' $\lambda < 2.14$, $P > 0.09$). The 1.53 mg l^{-1} Se treatment reduced aboveground biomass by 20% compared to controls (Table 1; Fig. 1a). In year 2, Se treatments significantly increased the proportion of aborted fruit up to 15% (Table 1; Fig. 1b), whereas the proportion of frugivory on fruit was reduced by 14% (Fig. 1c). Se treatments reduced the number of seeds per fruit by up to 21% (Table 1; Fig. 1d). Aboveground biomass weighed more in year 1 (mean ± SE: Year 1 187.58 ± 11.27 g; Year 2 43.96 ± 2.63 g), and the proportion aborted (Year 1 0.33 ± 0.02; Year 2 0.29 ± 0.02) and frugivory (Year 1 0.21 ± 0.04; Year 2 0.30 ± 0.02) fruit was higher in year 1. Plants also produced more seeds in year 1 (Year 1 5.7 ± 0.22; Year 2 3.99 ± 0.15). Se did not have a significant effect on dry belowground biomass (mean ± SE: 0 mg l^{-1} Se = 27.46 g ($N = 18$); 0.51 mg l^{-1} Se = 27.57 g ($N = 18$), 1.53 mg l^{-1} Se = 22.68 g ($N = 12$)).

3.2. Herbivory

Se treatment (MANOVA, Wilks' $\lambda = 10.02$, $P < 0.001$) had a significant effect on herbivory. Year and block did not significantly affect the number of aphids per g dry foliar biomass, or the number of mummies per aphid (MANOVA, Wilks' $\lambda < 2.74$, $P > 0.08$). Therefore the herbivory data for both years were pooled. The interaction of Se treatment and year was also not significant (MANOVA, Wilks' $\lambda = 0.29$, $P = 0.19$). Low and high Se treatments significantly reduced aphid numbers compared to control plants (Fig. 2; ANOVA, $F = 14.75$, $P < 0.001$). The number of mummies were also significantly reduced by both Se concentrations (Fig. 2; $F = 12.91$, $P < 0.001$). There was no effect of Se treatment on leaf number or average leaf damage (ANOVA, $F < 0.91$, $P > 0.41$).

3.3. Pollen limitation and pollination

Pollination treatment (Wilks' $\lambda = 0.95$, $P = 0.43$) and the interaction of Se treatment × pollination treatment were not significant (Wilks' $\lambda = 1.01$, $P = 0.43$), indicating plants were not pollen limited due to Se treatment. Overall, plants that received the natural pollination treatment (pollen deposited only by naturally occurring pollinators, mostly honey bees) produced 25% more seed than plants given the hand pollination treatment (mean ± SE: Natural pollination: 2.30 ± 0.52 seeds, hand pollination: 1.71 ± 0.25 seeds), although the difference was not significant (Wilks' $\lambda = 0.95$, $P = 0.43$). There was no significant difference in final germination percentage for plants treated with natural pollination compared to hand pollination (Natural pollination: 50.4%, hand pollination: 54.5%). Block, Se treatment, year, pollination and their interactions also had no significant effect on pollen limitation (Wilks' $\lambda < 2.08$, $P > 0.13$).

Overall, the primary pollinator, the honey bee, visited flowers frequently and was an efficient pollinator, as indicated by the lack of pollen limitation in the pollination treatments listed above. Se treatment, Se treatment × year, and block had no significant effect on pollinator visitation (MANOVA, Wilks' $\lambda < 0.96$, $P > 0.53$). Year had a significant effect on pollinator visitation (Wilks' $\lambda = 33.75$, $P < 0.001$). Year had a significant effect on visit duration per flower ($F = 9.42$, $P < 0.005$) and total honey bee visits ($F = 104.40$, $P < 0.001$). Honey bee visit durations were 51% shorter in year 2 (mean ± SE: Year 1 21.25 ± 1.61; Year 2 10.48 ± 1.39). There were also far fewer total honey bee visits to plants in year 2 compared to year 1 (Year 1 15.85 ± 2.30; Year 2 1.26 ± 0.19).

Table 1
ANOVA showing the effects of selenium treatment, year, their interaction and block on aboveground biomass, root biomass, proportion of aborted fruit, proportion of frugivory, number of seeds per fruit and weight per seed.

Source	*df*	Aboveground biomass		Root biomass		Proportion of aborted fruit		Proportion of frugivory fruit		Number of seeds per fruit	
		F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Selenium treatment	2	25.28	0.01*	2.11	0.14	10.20	0.0004***	5.11	0.01*	3.98	0.03*
Year	1	1291.28	0.0001***	2.91	0.10	8.00	0.008***	8.18	0.008**	28.44	0.0001***
Selenium treatment × year	1	10.02	0.88	0.33	0.57	6.15	0.02*	1.42	0.24	0.73	0.40
Block	11	114.54	0.0004***	0.73	0.70	1.03	0.45	0.85	0.60	1.71	0.12
Error	32										

$^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

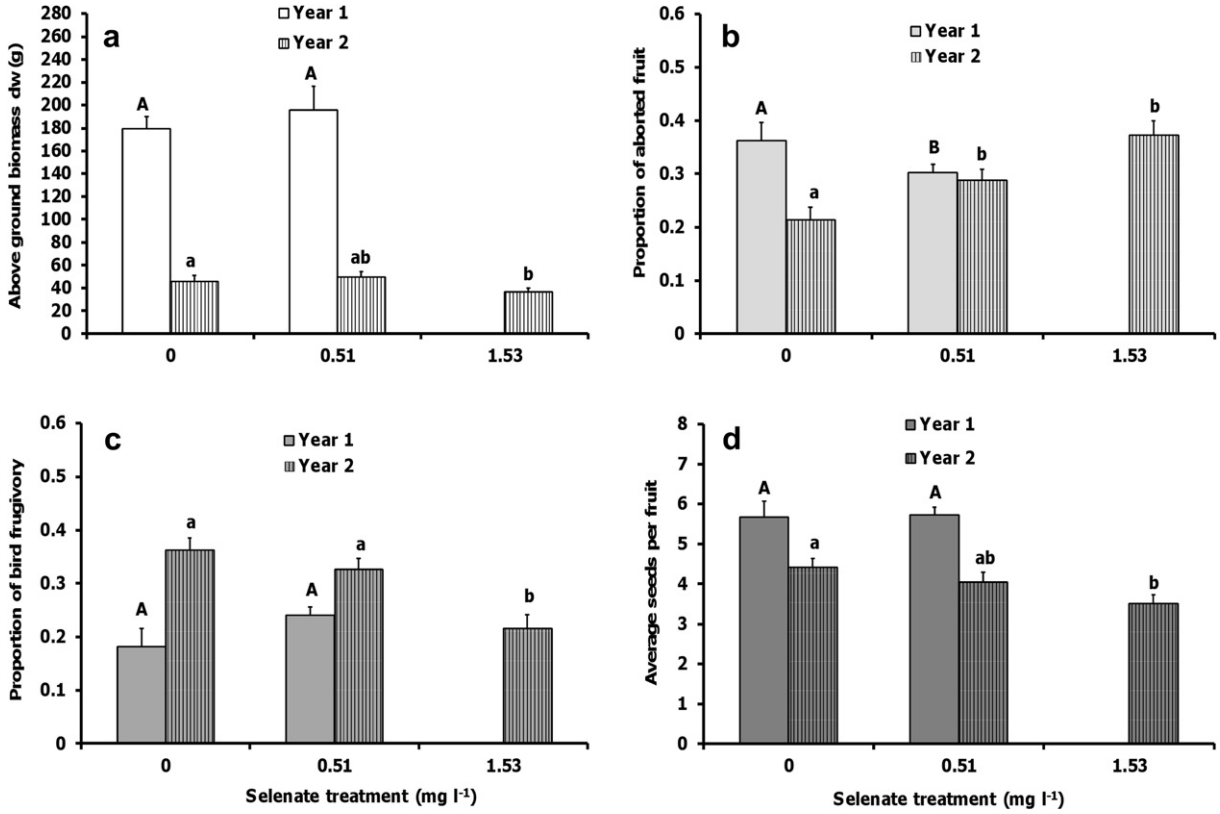

Fig. 1. Se treatment and year effects on aboveground biomass (**a**), proportion of aborted fruit (**b**), proportion of seeds with bird (house finch, *Carpodacus mexicanus*) frugivory (**c**), and the average number of seeds per fruit (**d**) in *Raphanus sativus* (radish). Se treatment levels: 0.0 mg l^{-1} (control), 0.51 mg l^{-1}, and 1.53 mg l^{-1}. Values are means ± standard error (SE). Letters above the means indicate statistically significant differences between groups ($\alpha = 0.05$).

3.4. Se concentrations in plant and insect tissues

Selenate-treated plants significantly accumulated Se into the flowers and leaves (Fig. 3; ANOVA, $F > 16.98$, $P < 0.001$). Pollen loads collected from the corbicula of honey bees observed to visit both control and Se-treated plants contained 6–2830 mg Se kg^{-1} ($n = 7$). Honey bee forager bodies contained 3–27 mg Se kg^{-1} ($n = 11$). Cabbage aphids collected from plants treated with Se contained 20–60 mg Se kg^{-1} ($n = 5$). Ladybird beetles collected near the cabbage aphids contained 141–217 mg Se kg^{-1} ($n = 4$).

4. Discussion

This study reveals the effects of a plant-accumulated pollutant on pollinators, frugivores and insect herbivores. Herbivory by birds and aphids was reduced at the highest Se treatment level, whereas pollinator visitation by honey bees was maintained at rates similar to control plants. Field studies have demonstrated reduced insect and mammalian herbivory (Galeas et al., 2008; Quinn et al., 2008) and fewer flower visitors present on Se-hyperaccumulating plants

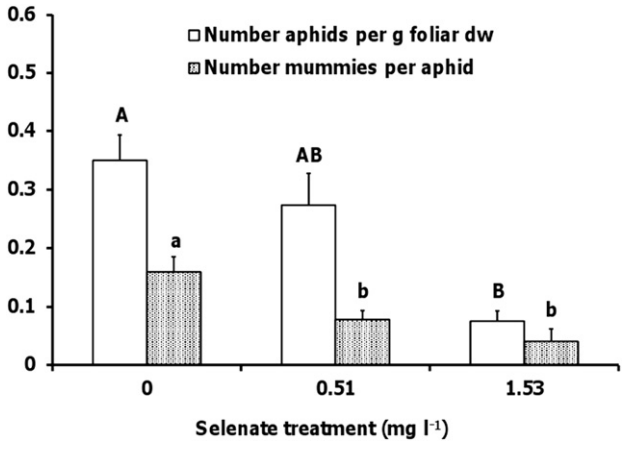

Fig. 2. Se treatment effects on the number of aphids (*Brevicoryne brassicae*) per gram dry weight of foliar biomass and the number of mummies per aphid. Se treatment levels: 0.0 mg l^{-1} (control), 0.51 mg l^{-1}, and 1.53 mg l^{-1}. Values are means ± standard error (SE). Letters above the means indicate statistically significant differences between groups ($\alpha = 0.05$).

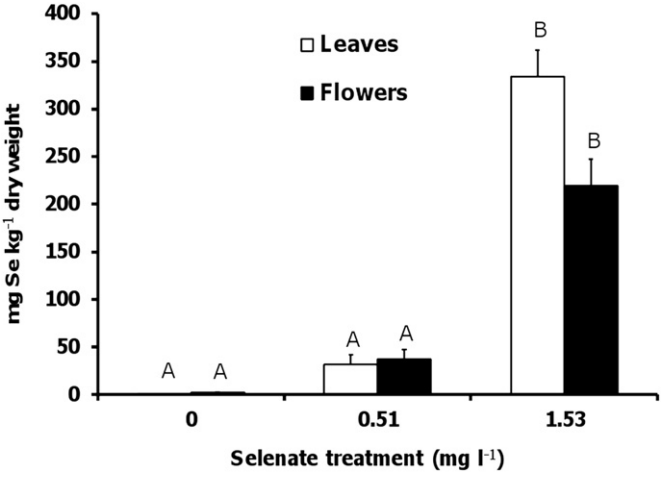

Fig. 3. Se treatment effects on Se accumulation levels in leaves and flowers. Se treatment levels: 0.0 mg l^{-1} (control), 0.51 mg l^{-1}, and 1.53 mg l^{-1}. Values are means ± standard error (SE). Letters above the means indicate statistically significant differences between groups ($\alpha = 0.05$).

(Galeas et al., 2008). Our manipulative semi-field study suggests that while *R. sativus* plants experience some phytotoxicity from Se, these effects are minimized by the preservation of attractive floral traits as well as the reduction in herbivory, thus maintaining pollination and reproductive output in Se-accumulating plants.

The phytotoxic effects of Se in radish included reduced biomass and increased fruit abortion. Greenhouse-grown radish plants irrigated with similar levels of Se showed reduced biomass and seed set in the absence of herbivores (Hladun, unpublished data), suggesting non-hyperaccumulator plants will suffer reductions in plant performance when exposed to Se concentrations of 1.53 mg Se l^{-1}in the field. *Brassica juncea* showed phytotoxic effects of reduced dry matter yield and leaf surface area when grown in soils containing 2 mg Se kg^{-1} (Bañuelos et al., 1997). In greenhouse studies, *B. juncea* suffered toxic effects from Se irrigation in terms of reduced flower size and number (Hladun et al., 2011). However, in the presence of herbivores, Se may protect plants, allowing them to outcompete non-accumulators that may also be present in the polluted landscape.

In several laboratory and field studies, herbivores fed plant tissues containing high levels of metals, metalloids, or other accumulated elements have exhibited toxic effects (Boyd, 2007; Butler and Trumble, 2008). In our study, aphid numbers were significantly reduced on plants watered with both Se treatment levels. Even the low Se treatment acted as a deterrent. Leaf concentrations as low as 10 mg Se kg^{-1} sufficed in deterring green peach aphids (*Myzus persicae* (Sulzer), Hemiptera: Aphididae, Hanson et al., 2004). In other insect species, Se ingestion increases mortality and development time, including the leaf-chewing herbivores *Heliothis virescens* F. (Lepidoptera: Noctuidae, Popham and Shelby, 2007) and *S. exigua* (Trumble et al., 1998; Vickerman and Trumble, 1999; Vickerman et al., 2002) as well as the predator *Podisus maculiventris* Say (Hemiptera: Pentatomidae, Vickerman and Trumble, 2003). Several insect herbivore species and their predators are susceptible to Se toxicity, and the primary herbivore in our study was not tolerant of even low levels of Se.

Pollutants can alter tritrophic interactions (Heliövaara and Väisänen, 1993), particularly if it is a soil-borne contaminant accumulated by a plant, passed onto the herbivore, and then biotransferred to the natural enemy. Parasitoids can be more susceptible to certain pollutants than their herbivore hosts (Fuhrer, 1985), although the pollutant may have a direct toxic effect on the insect, or an indirect effect by reducing the number of prey available to the natural enemy. One study examining the effects of Se on a tritrophic system found detrimental effects on the parasitoid *Cotesia marginiventris* (Vickerman et al., 2004). The braconid wasp weighed less and took longer to develop when parasitizing a herbivore host that was fed Se-containing plant material. In our study, there were fewer aphid mummies (most likely caused by a parasitoid wasp) on Se-treated plants. In addition, a common generalist predator, the ladybird beetle (Coleoptera: Coccinellidae), was collected and analyzed for Se. High Se concentrations in the predators from Se-treated plants indicates biotransfer of the contaminant across several trophic levels. The predator accumulated about three times more Se than the aphid host. At Se-contaminated sites such as Kesterson Reservoir in CA, predatory invertebrates generally had higher Se concentrations than the herbivores (Vickerman and Trumble, 2003). Additional studies are required to determine whether Se can biomagnify from the second to third trophic levels, and whether this can alter natural enemy populations.

A recent study using both hyperaccumulator (*Stanleya pinnata* (Pursh) Britton, Desert Prince's Plume) and non-hyperaccumulator (*B. juncea*) plants found honey bee and bumble bee pollinators visited control and Se-containing plants equally, further confirming that certain pollinators will not discriminate against hyperaccumulating plants despite concentrations as high as 3200 mg Se kg^{-1} in the flowers (Quinn et al., 2011). Our study revealed that honey bee pollinators will visit *R. sativus* that have accumulated selenium into flowers at concentrations well above the LC_{50} for a common insect herbivore, the beet armyworm (*S. exigua*, Trumble et al., 1998). Despite the high levels of Se (up to 219 ± 28 mg Se kg^{-1} dw), pollinators foraged on radish flowers and were observed to collect both pollen and nectar. At naturally seleniferous field sites, hyperaccumulator plants absorb up to 9000 mg Se kg^{-1} dw into the flowers (Galeas et al., 2007). Although there were fewer floral visitors to hyperaccumulators, the insects that did visit flowers contained up to 75 mg Se kg^{-1} dw (Galeas et al., 2008). Pollen collected by honey bees from aster plants growing in fly ash from coal-burning electrical power plants contained 14 mg Se kg^{-1} Se (De Jong et al., 1977), and nectar from radish plants grown in the greenhouse contained up to 100 μg Se ml^{-1} (Hladun, *unpublished data*). Based on these concentrations, honey bees have the potential to bring food resources back to the hive that are contaminated with Se at levels shown to be toxic to other insect species.

Pollutants found at toxic levels in the plant tissues honey bees forage upon and feed to their progeny may cause fitness effects for the colony that are not currently recognized. If a weedy plant such as *R. sativus* grows in a Se-contaminated area, and can maintain its attractiveness to pollinators as our study has demonstrated, there is the potential for biotransfer of Se from the accumulating plant to the colony. Several weedy Brassicaceae species have the ability to accumulate Se (White et al., 2004, 2007), and may concentrate the element in the flowers, allowing Se to biotransfer to pollinators through the portal of an accumulating plant. In addition, certain species of plants are used to accumulate and disperse Se in contaminated soils through phytoremediation, which has developed into an important strategy for land reclamation (Vickerman et al., 2004; Pilon-Smits and Freeman, 2006). Such large-scale Se accumulation by phytoremediating plant species has the potential to alter local ecosystems. This may adversely affect plant mutualists such as pollinators and efforts should be made to minimize pollinator exposure to Se-rich flowers.

5. Conclusions

Our study confirms that Se can accumulate in the flowers of *R. sativus*, and will be foraged upon by pollinators. If pollinators do visit Se-accumulating plants in polluted areas, depending on the widespread nature of the contamination, they may not have many alternate resources and will receive significant doses of the element. However, selenium is also a micronutrient that is essential to many organisms, including mammals, fish, and bacteria (Burau, 1985). Pollinators may dilute the amount of Se they receive by foraging on both non-accumulator and accumulator plants, and low levels of Se may have beneficial impacts on colony health such as reduced disease or predation (Barillas et al., 2011). Studies are currently underway to elucidate the fitness consequences of Se on honey bee adult and larval development and survival. Further studies are needed to determine the impact of soil-borne pollutants such as Se and their impact on plant–pollinator interactions.

Acknowledgments

The authors thank Woody Smith and David Thomason for their helpful discussions and assistance. We thank William Carson, Deborah De La Riva, Greg Kund, Christina Mogren, John Diaz-Montano and Beatriz Vindiola for their review of this manuscript. Thank you to P. Kirk Visscher for providing us with the honey bee

colony used in this study. We also thank Nichole Drew, Kris Gilbert, Sam Gilbert, and Ray Morton for their assistance. This work was supported by the Department of Entomology at University of California – Riverside 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 the authors and the EPA does not endorse any products or commercial services mentioned in this publication. All the work described in the manuscript complies with the current laws of the United States of America.

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