Selenium hyperaccumulation reduces plant arthropod loads in the field

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Summary

• The elemental defense hypothesis proposes that some plants hyperaccumulate toxic elements as a defense mechanism. In this study the effectiveness of selenium (Se) as an arthropod deterrent was investigated under field conditions.

• Arthropod loads were measured over two growing seasons in Se hyperaccumulator habitats in Colorado, USA, comparing Se hyperaccumulator species (*Astragalus bisulcatus* and *Stanleya pinnata*) with nonhyperaccumulators (*Camelina microcarpa*, *Astragalus americanus*, *Descurainia pinnata*, *Medicago sativa*, and *Helianthus pumilus*).

• The Se hyperaccumulating plant species, which contained 1000–14 000 μg Se g^{-1} DW, harbored significantly fewer arthropods (*c.* twofold) and fewer arthropod species (*c.* 1.5-fold) compared with nonhyperaccumulator species that contained < 30 μg Se g^{-1} DW. Arthropods collected on Se-hyperaccumulating plants contained three- to 10-fold higher Se concentrations than those found on nonhyperaccumulating species, but > 10-fold lower Se concentrations than their hyperaccumulator hosts. Several arthropod species contained > 100 μg Se g^{-1} DW, indicating Se tolerance and perhaps feeding specialization.

• These results support the elemental defense hypothesis and suggest that invertebrate herbivory may have contributed to the evolution of Se hyperaccumulation.

Key words: *Astragalus bisulcatus*, *Astragalus americana*, *Camelina microcarpa*, *Descurainia pinnata*, elemental defense, *Helianthus pumilus*, *Medicago sativa*, *Stanleya pinnata*.

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Introduction

A wide variety of defense mechanisms exists within the plant kingdom. Being nonmotile, plants must have the ability to protect themselves effectively from a range of biotic stresses, including pathogen and herbivore attacks. Plant pathogen and herbivore defenses range from structures (e.g. trichomes, thorns) to chemical pathways (e.g. alkaloids). Chemical defenses include pathways that provide whole-plant protection from pathogens or herbivores, which can be a general response to several threats or specific to a certain type of pathogen. These chemical defenses are typically produced as a by-product of an already existing plant metabolic pathway. However, recent studies have shown that plant accumulation of elements, which are not by-products of metabolism, may also serve as an 'elemental defense' (Martens & Boyd, 1994; Coleman *et al.*, 2005).

The elemental defense concept was proposed as one hypothesis for why certain plants hyperaccumulate elements (Boyd & Martens, 1992). Hyperaccumulating plant species typically contain tissue concentrations of metals or trace elements 100 times higher than those found in other species from the same site (Baker & Brooks, 1989). This degree of accumulation occurs even when the element is present at a low external concentration, suggesting that accumulation of the

element gives the plant some advantage, rather than being the result of inadvertent uptake (Sors *et al.*, 2005). Recent studies have investigated some of the proposed advantages (drought resistance, metal tolerance, defense) to determine which may have served as a selection pressure for the evolution of plant hyperaccumulation. Most studies currently support the elemental defense hypothesis (Pollard & Baker, 1997; Jhee *et al.*, 1999; Vickerman & Trumble, 1999, 2003; Ghaderian *et al.*, 2000; Bañuelos *et al.*, 2002; Hanson *et al.*, 2003, 2004; Vickerman *et al.*, 2004; Freeman *et al.*, 2006, 2007). These laboratory and glasshouse experiments have been performed on a variety of hyperaccumulated elements, including arsenic, cadmium, nickel, zinc and selenium (for a review, see Boyd, 2007).

Hyperaccumulation of selenium (Se) has been observed in the plant families *Asteraceae*, *Brassicaceae*, *Chenopodiaceae*, *Lecythidaceae*, *Fabaceae*, *Rubiaceae*, and *Scrophulariaceae* (Reeves & Baker, 2000). The Se concentration of these plants in the field is typically 1000–5000 mg kg^{-1} DW, or 0.1–0.5% of plant DW. Selenium hyperaccumulating plants are found in soils derived from Se-rich Cretaceous shale, such as in the western United States, where soil Se concentrations are often > 10 mg kg^{-1} (Reeves & Baker, 2000). Selenium is an essential element for the health of humans and arthropods, with a very narrow margin between deficiency and toxicity (Diwadkar-Navsariwala *et al.*, 2006). In areas with seleniferous soils, Se hyperaccumulating plants pose a problem for livestock, as consumption of these plants, or 'locoweeds', can cause disease and/or death (Draize & Beath, 1935).

The overall goal of our research is to elucidate the ecological and environmental pressures that contributed to the evolution of Se hyperaccumulation. Studies to date investigating the elemental defense hypothesis for the hyperaccumulation of Se have typically been done in glasshouse and laboratory settings (Pollard & Baker, 1997; Jhee *et al.*, 1999; Vickerman & Trumble, 1999; Ghaderian *et al.*, 2000; Hanson *et al.*, 2003, 2004). In the study described here, the potential role of Se in hyperaccumulator defense was investigated through field studies to determine whether previous glasshouse study results are relevant in nature. To test the elemental defense hypothesis in the field, arthropod loads were surveyed on two hyperaccumulating species (*Astragalus bisulcatus*, *Stanleya pinnata*) and five nonhyperaccumulating species (*Astragalus americana*, *Camelina microcarpa*, *Descurainia pinnata*, *Medicago sativa*, and *Helianthus pumilus*) in two experiments conducted in 2004 and 2005. If Se is a deterrent, it would be expected that fewer arthropods would be collected on Se-hyperaccumulating plants relative to nonhyperaccumulating plant species.

Materials and Methods

Field sampling

Plants were sampled for arthropod loads at Pine Ridge Natural Area (PRNA) in Fort Collins (CO, USA). Pine Ridge is a 247 ha area chosen for the presence of Se-hyperaccumulating plant populations. Three sites were designated for sampling at PRNA. These were approx. 30 × 30 m, had similar topography and soil type and at least 30 individuals of each of the two hyperaccumulator species on each site. Comparison nonhyperaccumulator species were then chosen at those sites. During 2004, over 300 plants were sampled over 48 d from April to September. Two Se-hyperaccumulating plant species, *Stanleya pinnata* (Pursh) Britt. (prince's plume, family Brassicaceae) and *Astragalus bisulcatus* (Hook.) A. Gray (two-grooved milkvetch, family Fabaceae), were sampled for arthropod loads. For comparison, three nonhyperaccumulator species from the same families were sampled in parallel: *Astragalus americanus* (Hook.) (American milkvetch, family Fabaceae), *Descurainia pinnata* (Walt.) Britt. (Western tansymustard, family Brassicaceae), and *Camelina microcarpa* Andrz. ex DC. (littlepod false flax, family Brassicaceae). All plant species were naturally present on the sites and all except *C. microcarpa* are native to the United States. Besides being from the same families, and thus as similar as possible in terms of other plant defense compounds, these comparison species were chosen because they were very similar to the hyperaccumulators with respect to flower color, flowering time (all plant species flowered during the sampling time for arthropods), leaf hairiness, and general appearance, that is factors that might affect the number and type of arthropods. However, these comparison species were substantially smaller than the hyperaccumulators, and of course not all ecologically relevant factors could be controlled for, such as other plant defense compounds. This study utilized the only other Brassicaceae available at the sites. For statistical analysis the data from the three sites were combined, as no significant differences were found between sites.

A second field survey was conducted during the following year (May to July, 2005). Owing to the size discrepancy between the hyperaccumulators and nonhyperaccumulators used during season 1, the hyperaccumulators *S. pinnata* and *A. bisulcatus* were sampled again, with the more similarly sized nonhyperaccumulators *Medicago sativa* L. (alfalfa, family Fabaceae) and *Helianthus pumilus* Nutt. (little sunflower, family Asteraceae) used for comparison. During 2005, more than 200 plants were sampled over 30 d from May to July. All plant species sampled in 2005 are native to the United States with the exception of *M. sativa*. Again, factors that might affect the number and type of arthropods were taken into account as much as possible, including plant flower color, plant flowering time, leaf hairiness, and general appearance. No Brassicaceae similar in size to *S. pinnata* was available at the study site, explaining the use of *H. pumilus* as a comparison species with *S. pinnata.* For both years, arthropod sampling occurred in the morning hours and plants were selected at each site arbitrarily. Specimens from each plant were collected by shaking the arthropods into a collection container, followed by collection into vials with an aspirator. The arthropods were

then stored at –20°C until identification (Triplehorn & Johnson, 2004). Specimens were grouped into taxa (class, family, subfamily), and also classified according to feeding mode or trophic position (Triplehorn & Johnson, 2004). For statistical analysis, the data from the three sites were combined, as no significant differences were found between sites. Additionally, the statistical analyses were performed on the individual morphotypes collected. We use the term morphotype in order to group specimens with separate distinctive phenotypes, without true knowledge of individual genetic relationships. The full lists of morphotypes collected in 2004 and 2005 are shown in Supplementary material, Tables S1 and S2, respectively.

Analyses

In order to determine the relationship between plant Se concentration and arthropod loads, a young leaf was taken for Se analysis from every plant sampled for arthropods. These leaves were dried for 2 d at 50°C and approx. 100 mg of plant material was then acid-digested as described by Zarcinas *et al*. (1987) in order to extract the Se. The Se concentration in the digests was quantified by inductively coupled plasma atomic emission spectrometry (ICP-AES, Fassel, 1978). The detection limit of this method is 0.05 mg l^{-1} in the acid digest. In 2004, leaf surface area was measured by harvesting three whole plants for each species from three size classes (small, medium and large), pressing the leaves for each sample, and analyzing the surface area using Image J, an image and processing tool available for download from the National Institutes of Health's website (http://rsb.info.nih.gov/ij/). In 2005, biomass data were collected by harvesting three whole plants for each species from three size classes (small, medium and large). Plants were dried for 2 d at 50°C and then weighed. Arthropods gathered from the field were analyzed for Se concentration if at least 5 mg of biomass was available, and where possible three replicates of 10 mg each were used. Arthropod material was acid-digested as described by Zarcinas *et al*. (1987) and the Se concentrations of these digests were quantified by ICP-AES. While in 2004 all arthropods of a morphotype were pooled for Se analysis, in 2005 they were separated based on whether they were collected from hyperaccumulators or nonhyperaccumulators.

For statistical analyses the software package JMP-IN version 3.2.6 was used (SAS Institute, Cary, NC, USA). In this study we collected data for several plant species on a variety of factors (e.g. plant selenium concentrations, and plant arthropod loads). We then determined if there was a significant difference in the means of these factors between hyperaccumulators and nonhyperaccumulators. In 2004 the differences between *A. bisulcatus* and *A. americanus* in plant Se concentration, leaf surface area, arthropods per plant (corrected and uncorrected) and arthropod species per plant were determined using *t*-tests (Fig. 1). Analysis of variance (ANOVA) was used (for the same factors) for the comparisons between *S. pinnata*, *D. pinnata*, and *C. microcarpa*. When a one-way ANOVA was used, post-hoc analyses (Tukey-Kramer HSD) were performed to determine which means differed significantly (Fig. 1). In 2005, when each hyperaccumulator was compared with only one nonhyperaccumulating species, *t*-tests were performed to analyze the differences in means of the different factors (plant Se concentration, plant biomass, arthropods per plant, and arthropod species per plant) between hyperaccumulators and their comparative nonhyperaccumulator. To determine the relationship between overall plant Se concentration and arthropod load across all plant species, linear regression and second-order fits were performed (Fig. 2). Differences in Se concentration of arthropods (where available) collected on hyperaccumulators vs comparative nonhyperaccumulators were analyzed using a *t*-test. Statistically significant differences ($P < 0.05$) are reported in the text and shown in the figures, as are mean, standard error of the mean, and number of replicates.

Results

To determine whether Se hyperaccumulators harbored fewer arthropods than comparable nonhyperaccumulators, the number of arthropods harbored per plant was determined, as well as plant Se concentration. In 2004 it was found that both hyperaccumulators (*A. bisulcatus* and *S. pinnata*) had leaf Se concentrations two orders of magnitude higher than nonhyperaccumulating species (*A. americanus*, *C. microcarpa*, and *D. pinnata*) (Fig. 1a, $P < 0.001$). Measurements of leaf surface area revealed that hyperaccumulating plant species were at least 20-fold larger in size than their related nonhyperaccumulators (Fig. 1b). To account for this difference, the data are presented as nonnormalized values (Fig. 1c,e) as well as normalized based on plant surface area (Fig. 1d,f). The number of arthropods per plant, without correction for size, was not significantly different for the hyperaccumulator *A. bisulcatus* and its nonhyperaccumulating relative *A. americanus* (Fig. 1c). The number of arthropods per plant was also not significantly different for the hyperaccumulator *S. pinnata* and its nonhyperaccumulating relative *C. microcarpa* (Fig. 1c). However, there were on average five times more arthropods on the nonhyperaccumulator *D. pinnata* than on *S. pinnata* (Fig. 1c). When corrected for size, there were at least 10 times greater loads of arthropods on all nonhyperaccumulating plant species (*A. americanus*, *C. microcarpa*, and *D. pinnata*) compared with their related hyperaccumulating species (*A. bisulcatus*, and *S. pinnata*; Fig. 1d, $P < 0.001$). Similar results were found for the number of arthropod species per plant. The nonhyperaccumulator *D. pinnata* harbored *c.* 2.5-fold more arthropod species than its hyperaccumulating relative *S. pinnata*, despite its 20-fold smaller size. Besides this, there was no difference between hyperaccumulating species and their comparison nonhyperaccumulators with respect to the raw data (Fig. 1e). When the data were corrected for plant size,

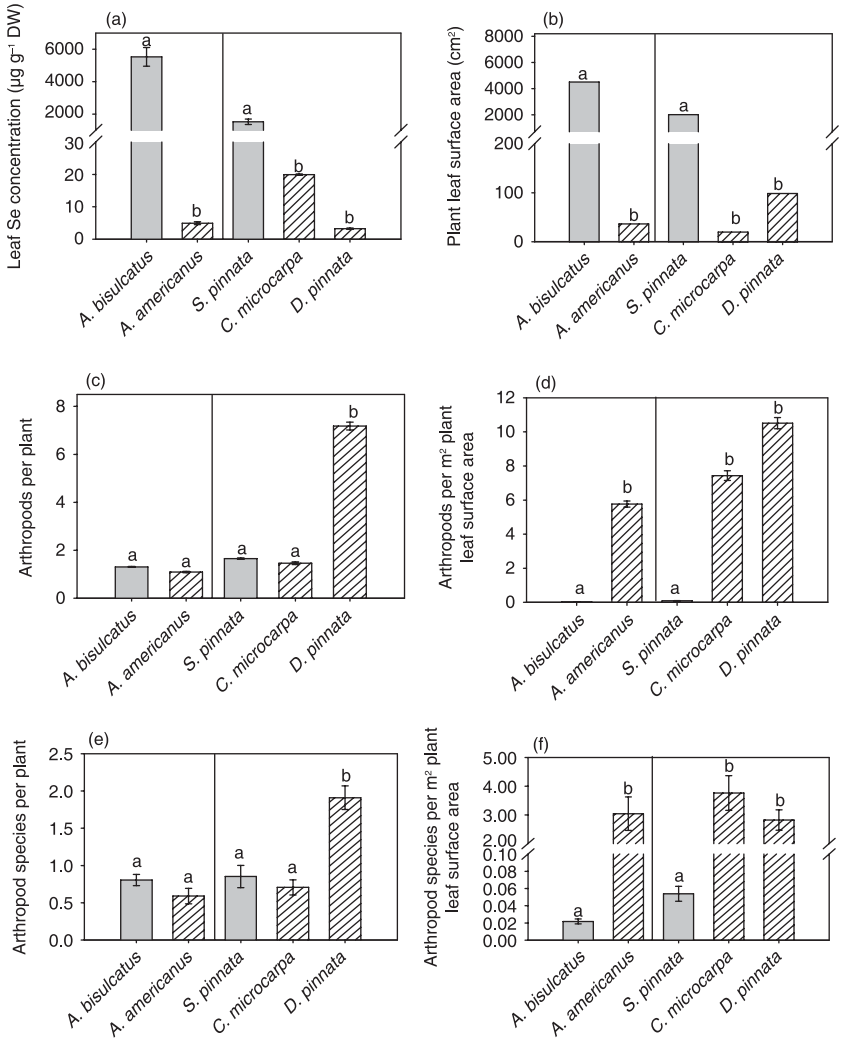

Fig. 1 Field results for 2004 for the hyperaccumulators *Astragalus bisulcatus* ($n = 111$) and *Stanleya pinnata* ($n = 78$) and the nonhyperaccumulators *A. americanus* ($n = 65$), *Camelina microcarpa* ($n = 37$), and *Descurainia pinnata* ($n = 33$). (a) Leaf selenium (Se) concentration in the hyperaccumulators (gray bars) and nonhyperaccumulators (hatched bars) ($P < 0.001$; *t*-test, ANOVA: $F = 31.46$; d.f. = 2, 145) (b) plant leaf surface area by plant species ($n = 3$; $P < 0.001$; *t*-test, ANOVA: $F = 109.66$; d.f. = 2, 6); (c) arthropods per plant by plant species nonnormalized for plant leaf surface area ($P < 0.001$; *t*-test, ANOVA: $F = 39.42$; d.f. = 2, 145); (d) arthropods per plant by plant species normalized for plant leaf surface area ($P < 0.001$; *t*-test, ANOVA: $F = 52.33$; d.f. = 2, 145); (e) number of arthropod species per plant nonnormalized for plant leaf surface area ($P < 0.001$; *t*-test, ANOVA: $F = 20.62$; d.f. = 2, 145); (f) number of arthropod species per plant normalized for plant leaf surface area ($P < 0.001$; *t*-test, ANOVA: $F = 47.51$; d.f. = 2, 145). Analysis: *t*-tests were used for the comparison between *A. bisulcatus* and *A. americanus*. One-way ANOVAs were performed to compared *S. pinnata*, *C. microcarpa*, *and D. pinnata*, followed by a post-hoc analysis (Tukey-Kramer HSD) to determine which means differed significantly. Data are means ± SE. The letters above the bars indicate statistically significant differences between groups ($\alpha = 0.05$). Note: In some cases the standard error was too small to be recognized by the graphing programs; in those cases no error bar is shown.

there were significantly higher numbers of arthropod species on all nonhyperaccumulator species compared with their respective hyperaccumulating relatives (Fig. 1f, $P < 0.001$).

To determine the relationship between plant Se and the number of arthropods per plant across all plant species, the data from all plant species were pooled and the number of arthropods collected plotted against plant Se concentration. As plant Se increased, there was a sharp decline in the number of arthropods collected (Fig. 2a, linear regression: $y = 0.00158x + 10.9029$, $R = 0.03$, $P = 0.0125$). Most arthropods were found on plants containing < 600 μg Se g^{-1} (Fig. 2, inset). To determine if arthropods found on Se hyperaccumulators were actually ingesting the plants, the Se concentration of arthropods found on hyperaccumulating plants was compared with arthropods collected from related nonhyperaccumulators. It was found that arthropods collected from Se hyperaccumulator plant species had significantly (*c.* 10 times) higher Se concentration than arthropods collected from nonhyperaccumulator plants: arthropods collected from hyperaccumulators contained 55.5 ± 18.6 μg Se g^{-1} (mean ± SE), while arthropods collected from nonhyperaccumulators contained 4.9 ± 2.2 μg Se g^{-1} (d.f. = 20, $t = 2.48$, $P = 0.02$).

After collection, specimens were identified to class, family, or subfamily, and then classified according to feeding mode or trophic position. This information was used to determine the arthropod feeding mode composition (as a fraction of the total number of arthropods) for each plant species (Fig. 3). In season 1 it was found that both hyperaccumulating species contained a relatively larger proportion of herbivores compared with nonhyperaccumulating plant species (58–68% of arthropods collected on hyperaccumulators were herbivores, compared with 42–45% for nonhyperaccumulators). In addition, hyperaccumulators harbored fewer floral visitors (e.g. nectar robbers or pollinators) than their comparison nonhyperaccumulators (8–22% on hyperaccumulators were floral visitors compared with 25–40% for nonhyperaccumulators) with the exception of *D. pinnata* (no floral visitors were collected). Hyperaccumulators also had relatively fewer predators than nonhyperaccumulating species (22–33% arthropods collected on hyperaccumulators were predators

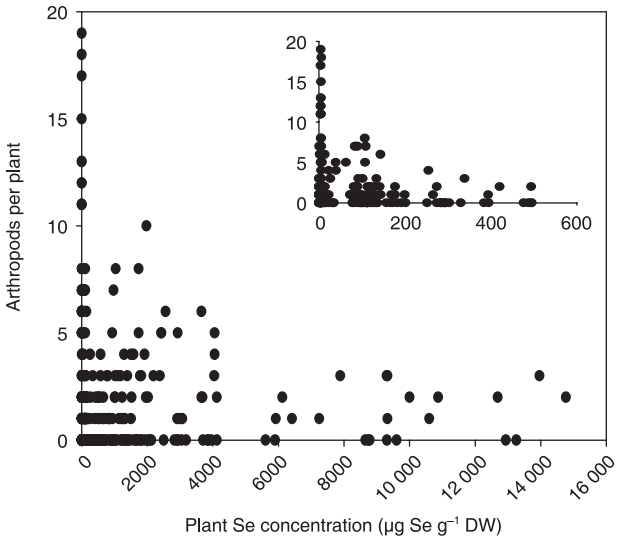

Fig. 2 Arthropods per plant, all plant species combined, as a function of plant tissue selenium (Se) concentration for 2004. Inset shows magnification of origin area.

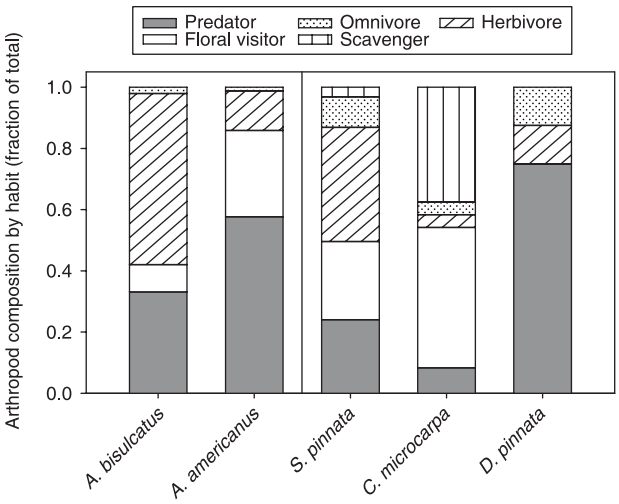

Fig. 3 Arthropod composition in 2004 by feeding mode (predator, floral visitor, herbivore, omnivore, scavenger) for the hyperaccumulators *Astragalus bisulcatus* and *Stanleya pinnata*, and the nonhyperaccumulators *A. americanus*, *Camelina microcarpa* and *Descurainia pinnata*.

compared with 53–77% for nonhyperaccumulators), with the exception of *C. microcarpa* (8% predators). Table S1 lists the arthropods collected in 2004, with their accession number, taxonomic classification, feeding mode, and plant species from which they were collected. For close to 30 of these arthropod accessions, there was sufficient material to determine Se concentration; these are shown in Table 1. Of the 27 arthropod accessions tested for Se in 2004, 10 contained ≥ 100 μg Se g^{-1} DW, two contained intermediate Se concentrations (39 and 50 μg Se g^{-1} DW), 11 contained ≤ 13 μg Se g^{-1} DW, and the remaining four contained nondetectable concentrations of Se. For arthropods collected across all plant species, floral visitors contained a range of 2–136 μg Se g^{-1} DW, herbivores contained a range of 3–188 μg Se g^{-1} DW, and predators contained a range of 10–29 μg Se g^{-1} DW. Sufficient biomass was not available to determine omnivore or scavenger Se concentration. The average Se concentrations for herbivores, floral visitors and predators were 55, 46 and 18 μg Se g^{-1} DW, respectively.

A second replicate of the field experiment was conducted in 2005 to investigate whether the results from 2004 were repeatable, and to avoid a large size discrepancy between the hyperaccumulators and nonhyperaccumulators. This time the hyperaccumulators *S. pinnata* and *A. bisulcatus* were compared with the similarly sized nonhyperaccumulators *M. sativa* and *H. pumilus*. Though different nonhyperaccumulating species were used as comparison plant species, the results obtained in 2005 were very similar to those from 2004. The hyperaccumulators *A. bisulcatus* and *S. pinnata* had leaf Se concentrations around two orders of magnitude higher than nonhyperaccumulating species (*M. sativa* and *H. pumilus*) (Fig. 4a, $P < 0.001$). Measurements of plant biomass showed that hyperaccumulating plant species were not significantly different in size from their comparison nonhyperaccumulators, allowing us to compare plant arthropod loads directly (Fig. 4b). Hyperaccumulating plants (*A. bisulcatus* and *S. pinnata*) had approx. twofold lower arthropod loads compared with their respective nonhyperaccumulating plant species (*M. sativa* and *H. pumilus*; Fig. 4c, $P < 0.001$). Similar results were found for the number of arthropod species per plant, with a significantly (approx. 1.5-fold) lower number of arthropod species found on hyperaccumulators compared with their respective nonhyperaccumulators (Fig. 4d, $P < 0.001$).

The relationship between plant Se and the number of arthropods per plant was compared across all plant species. As in season 1, the number of arthropods declined sharply as Se increased (Fig. 5, inverse second order: $y = 25.3x - 7.5x^2 + 8$, $R = 0.1882$, $P = 0.0167$). Most arthropods were again found on plants containing below 600 μg Se g^{-1} DW (Fig. 5 inset). The Se concentration of arthropods found on hyperaccumulating plants compared with their related nonhyperaccumulators was also determined. Arthropods found on Se-hyperaccumulating plants had significantly (over twofold) higher Se concentration than arthropods collected from nonhyperaccumulating species: 40.4 ± 0.62 and 15.2 ± 0.20 μg Se g^{-1} DW, respectively (d.f. = 171, $t = 4.58$ $P < 0.001$).

Table S2 shows the list of the arthropods collected in 2005, including accession number, taxonomic classification, feeding mode, and plant species from which it was collected. Of the 96 accessions collected in 2004, 43 were found again in 2005. Additionally, 68 new morphotypes were found for a total of 160 arthropod morphotypes collected over the two seasons. All accessions collected in 2004 may not have been collected again in 2005, since collection in 2005 occurred during a shorter time period (May–July). For a subset of these arthropods

Table 1 Feeding mode, classification, accession number and selenium (Se) concentration for select arthropods in 2004 (pooled from all plant species) and 2005 (arthropods collected from hyperaccumulators and nonhyperaccumulators analyzed separately)

		Se concentration (μg g^{-1} DW)		
		2004	2005	
Classification	Accession number	Pooled	Hyperaccumulator	Nonaccumulator
Herbivore				
Anthicidae	84	–	81	33
	93	49	–	–
Aphididae	67	> 200*	–	–
	70	> 200*	–	–
	87	ND	–	–
Chrysomelidae	47	9	–	–
	60	119	–	–
	69	> 200*	–	–
	75	> 200*	12	–
	79	–	159	–
	80	188	–	–
	83	> 200*	–	–
	86	13	–	–
	1034	–	50	10
Cicadellidae	41	–	11	10
	66	–	12	10
	1038	–	12	7
	1103	–	32	11
	1161	–	3	5
Curculionidae	15	–	7	20
	73	–	43	–
	1102	–	–	18
Membracidae	20	7	–	–
	1022	–	7	10
Nitidulidae	94	3	–	–
Pentatomidae	29	–	59	2
	49	ND	–	–
	1099	–	65	37
Phalacridae	1047	–	21	16
Rhopalidae	50	3	–	–
Tingidae	34	102	103	43
	59	ND	–	–
Formicidae	3	136	–	–
	5	39	26	5
	37	2	30	1
	76	7	–	
	1188	–	75	24
Predator				
(Class) Arachnida	1	10	–	–
	9	29	–	–
	10	20	–	–
Anthocoridae	82	> 200*	–	24
Coccinellidae	1018	–	14	5
Dictynidae	46	–	16	10
Melyridae	32	ND	113	76
Nabidae	38	–	8	2
Reduviidae	27	14	87	11
	57	–	62	12
Thomisidae	54	–	17	8
	1065	–	–	8

ND, nondetectable concentrations of Se.
*Values calculated from low sample weights (< 5 mg).
Note: The detection limit of ICP-AES is approximately 2 μg g^{-1} DW.

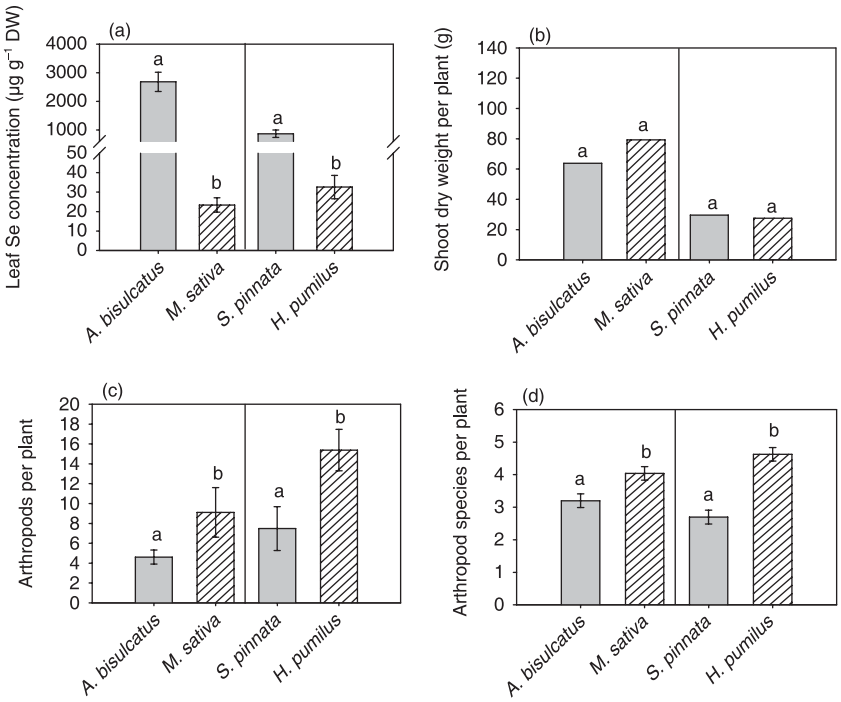

Fig. 4 Field results for 2005 for the hyperaccumulators (gray bars) *Astragalus bisulcatus* ($n = 111$) and *Stanleya pinnata* ($n = 112$) and the nonhyperaccumulators (hatched bars) *Medicago sativa* ($n = 107$) and *Helianthus pumilus* ($n = 113$). (a) Leaf selenium (Se) concentration ($P < 0.001$; *t*-test); (b) shoot dry weight per plant ($n = 3$; note, in this case the standard error was too small to be recognized by the graphing program); (c) arthropods per plant ($P < 0.001$; *t*-test); (d) number of arthropod species per plant ($P < 0.001$; *t*-test). Data are means ± SE. The letters above the bars indicate statistically significant differences between groups ($\alpha = 0.05$).

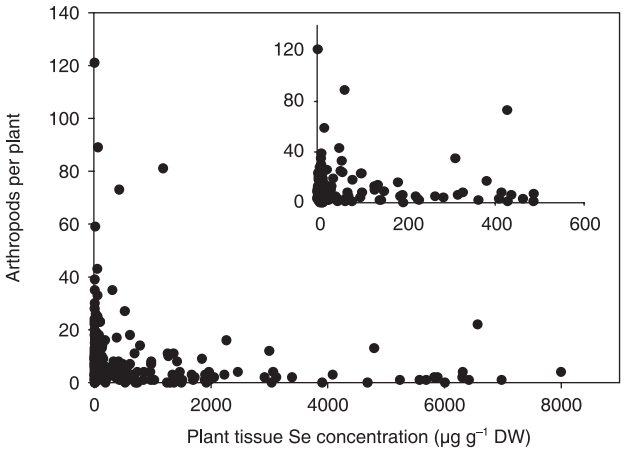

Fig. 5 Arthropods per plant, all plant species combined, as a function of plant tissue selenium (Se) concentration for 2005. Inset shows magnification of origin area.

there was sufficient material to determine Se concentration; these are listed in Table 1. Seven of the 16 accessions collected from both hyperaccumulator and nonhyperaccumulator plants showed a significantly (1.5- to 30-fold) higher Se concentration when collected from hyperaccumulator plants than from nonhyperaccumulators (Fig. 6). These include two ant species (accession nos 5 and 37) that are floral visitors (nectar robbers or pollinators), a leaf hopper (accession no. 1103) that is a fluid-feeding herbivore, an ant-like flower beetle herbivore (accession no. 84), a damsel bug predator (accession no. 38), a soft-winged flower beetle predator (accession no. 32), and an assassin bug predator (accession no. 27). Ten of the 29 arthropod species tested for Se in 2005 contained tissue Se concentrations ≥ 50 μg Se g^{-1} DW and three species had concentrations ≥ 100 μg Se g^{-1} DW. For arthropods collected across all plant species in 2005, floral visitors contained a range of 1–75 μg Se g^{-1} DW, herbivores contained 2–159 μg Se g^{-1} DW, and predators contained 2–113 μg Se g^{-1} DW (Table 1). There was not sufficient biomass to determine omnivore or scavenger Se concentration. The average Se concentrations for herbivores collected from hyperaccumulators and nonhyperaccumulators, respectively, were 42 and 17 μg Se g^{-1} DW. For floral visitors and predators, the Se concentration differences were similar between hyperaccumulators and nonhyperaccumulators (44 and 10 μg Se g^{-1} DW for floral visitors and 45 and 17 μg Se g^{-1} DW for predators), respectively, although a large range of Se values for each feeding mode was present.

In 2005, the collected arthropods were identified to family and classified according to feeding mode. The arthropod feeding mode composition (as a fraction of the total number of arthropods) for each plant species was determined, with results similar to the previous year (Fig. 7). The two hyperaccumulating species (*A. bisulcatus*, *S. pinnata*) harbored relatively more herbivores compared with nonhyperaccumulators (58–65% of arthropods collected on hyperaccumulators were herbivores compared with 45–49% for nonhyperaccumulators). Also, the hyperaccumulators harbored relatively fewer floral visitors (6–11% on hyperaccumulators were floral visitors compared with 26–30% for nonhyperaccumulators). The number of predators per plant species did not display an

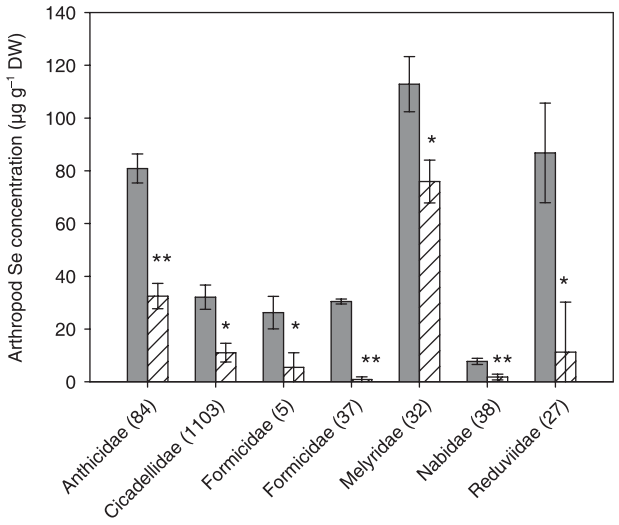

Fig. 6 Comparison of arthropod selenium (Se) concentration in seven morphotypes collected from both hyperaccumulator plant species (gray bars, *Astragalus bisulcatus* and *Stanleya pinnata*) and nonhyperaccumulator plant species (hatched bars, *Medicago sativa* and *Helianthus pumilus*). Data are means ± SE. Asterisks above bars indicate the degree of statistical significance between arthropods collected from hyperaccumulators vs nonhyperaccumulators within each accession (*, $P < 0.05$; **, $P < 0.01$; *t*-test, $n = 3$–5 samples each containing multiple arthropods). Additional accessions that did not show significant differences when collected from hyperaccumulators or nonhyperaccumulators are shown in Table 1.

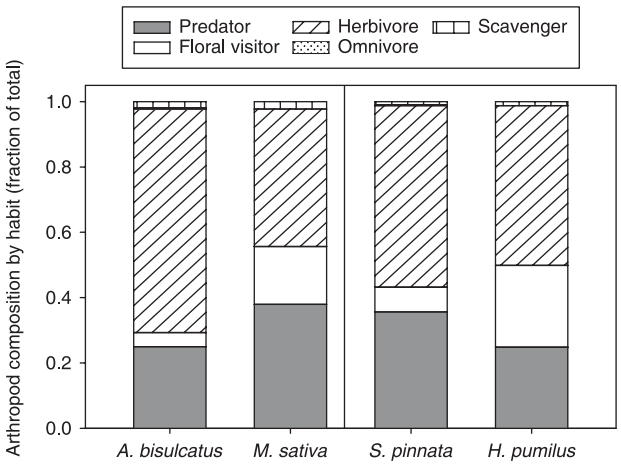

Fig. 7 Arthropod composition (fraction of total) in 2005 by feeding mode (predator, floral visitor, herbivore, omnivore, scavenger) for the hyperaccumulators *Astragalus bisulcatus* and *Stanleya pinnata* and the nonhyperaccumulators *Medicago sativa* and *Helianthus pumilus*.

obvious difference between hyperaccumulators and nonhyperaccumulators. Scavengers and omnivores were a negligible percentage of the arthropods collected across all plant species.

Discussion

The main finding of this study is that Se hyperaccumulators harbored significantly fewer arthropods and arthropod species when compared with nonhyperaccumulators, supporting the hypothesis that Se serves as an elemental defense. Our findings are particularly interesting because this study is one of the first to address and support the elemental defense hypothesis in the field (Martens & Boyd, 2002; Freeman *et al.*, 2007). Our study was robust in that it was conducted over two consecutive seasons, using two Se hyperaccumulator species and five nonhyperaccumulator species. The finding that Se hyperaccumulators harbor fewer arthropods in the field is in agreement with a recent manipulative field study (Freeman *et al.*, 2007) where elevated Se was shown to protect *S. pinnata* from grasshopper herbivory in a seleniferous habitat. Together, these studies suggest that protection from herbivory has contributed to the evolution of plant Se hyperaccumulation.

The Se concentrations in the two hyperaccumulating plant species (*A. bisulcatus* and *S. pinnata*) were around two orders of magnitude higher than in the five nonhyperaccumulating plant species, confirming that this was a suitable group of plants to study the effect of plant Se accumulation on arthropod load. In earlier laboratory studies, Se in leaves was shown to be detected by herbivores and to act as a deterrent (Hanson *et al.*, 2003, 2004; Freeman *et al.*, 2006, 2007). Although the factors that attract or repel an arthropod to a particular plant vary greatly between species and environmental conditions, it can be assumed that arthropods in the field can also detect the high Se concentrations found in hyperaccumulator species, part of which is volatilized as dimethyl selenide, and may act as a deterrent (Terry *et al.*, 2000).

When pooling all species, most arthropods were collected on plants with a Se concentration of 600 μg Se g^{-1} DW or less (Figs 2, 5). Arthropods collected from hyperaccumulators contained at least three times the Se concentration of arthropods collected from nonhyperaccumulating species, even within single morphotypes (Fig. 6). This suggests that the presence of these arthropods on Se hyperaccumulators was not by chance, and that the insects were feeding on the plants they were collected from. Additionally, the Se concentrations found in several of the arthropods collected from hyperaccumulator plants were > 50 μg Se g^{-1} DW (Table 1), which is several-fold higher than tissue Se concentrations found to be lethal to other arthropods (Hanson *et al.*, 2003, 2004; Freeman *et al.*, 2006, 2007). This suggests that several of the arthropods collected from hyperaccumulator plants are unusually Se-tolerant, which may indicate specialization. Indeed, in 2005, 11 herbivore morphotypes, four predator morphotypes, three omnivore morphotypes, and one parasite morphotype were collected only from hyperaccumulating plants, indicating potential Se-tolerant or specialist species at multiple trophic levels. Two of these potential specialists, both leaf beetles (accession nos 80 and 83; see Table S2),

contained close to 200 μg Se g^{-1} DW, strongly indicating Se tolerance. Conversely, 16 herbivore morphotypes, five floral visitor morphotypes, and one predator morphotype were collected only from nonhyperaccumulating plants, suggesting deterrence by Se and/or Se sensitivity. In this context it is interesting to note that a recently discovered diamondback moth variety collected from *S. pinnata* in this same area was found to tolerate and accumulate Se significantly better than a variety from the eastern USA, suggesting local adaptation (Freeman *et al.*, 2006).

It was surprising that the hyperaccumulators harbored relatively more herbivores than nonhyperaccumulators, and fewer floral visitors. The opposite would be expected, since herbivores would be expected to consume the largest amount of toxic hyperaccumulator plant material. However, we have recently found that the reproductive tissues of hyperaccumulators contain the highest Se concentrations (Galeas *et al.*, 2007) of all plant tissues except young leaves. Mature and old leaves contained 2- to 10-fold lower Se concentrations than young leaves or flowers. Thus it may be possible that the hyperaccumulator herbivores targeted older leaves. Additionally, these studies were conducted in areas with Se-rich soils and high densities of Se hyperaccumulators. Therefore, some degree of coevolution may have already occurred, as supported by the diamondback moth study (Freeman *et al.*, 2006).

Among the different arthropod feeding modes, herbivores, floral visitors and predators all showed higher average tissue Se concentration when collected from hyperaccumulator plants than nonhyperaccumulators. This suggests that arthropods interacted with the plants and ingested the plant Se either directly or indirectly, although this is not the only way the arthropods could have obtained elevated Se concentrations. As mentioned earlier, hyperaccumulator flowers contain extremely high Se concentrations (Galeas *et al.*, 2007), which apparently can be transferred to their floral visitors. Since arthropod Se was analyzed using whole arthropods, it is unknown whether the Se on the floral visitors was located internally (ingested nectar or pollen) or externally (pollen). Owing to their high Se concentrations, hyperaccumulators are a likely portal for entry of Se into the food chain via Se-tolerant herbivores. From a trophic interaction perspective, it is interesting that herbivores, floral visitors and predators collected from hyperaccumulator plants contained similar Se concentrations, in the range of 2–188 μg Se g^{-1}, although of course the range was very large within each group. The Se concentrations of herbivores, floral visitors and predators collected are an order of magnitude lower than those found in their host plants. The lack of biomagnification of tissue Se concentrations from plant to herbivores to predators suggests that the risk of bioaccumulation of Se is low. This is in agreement with a study that found biotransfer, but not biomagnification, of Se in the generalist predator *Podisus maculiventris* (Hemiptera) when fed *Spodoptera exigua* (Lepidoptera) larvae with varying Se concentrations (Vickerman & Trumble, 2003). The lack of bioaccumulation of Se in our study has implications in phytoremediation, specifically phytoextraction. It has been noted that the risks associated with a field application of phytoremediation technologies should be carefully considered (Angle & Linacre, 2005). While further studies should be conducted, knowledge (as we present here) about the movement of plant Se into the food chain has implications for the use of Se-enriched plants for phytoremediation or as fortified foods: if the bioaccumulation of Se from the plant to the second and third trophic levels is low, plants grown for Se phytoremediation or Se-enhanced foods likely pose little risk for bioaccumulation of Se in the surrounding environment.

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Supplementary Material

The following supplementary material is available for this article online:

Table S1 Data on arthropods collected in 2004, including accession number, taxonomic classification, feeding mode, and plant species from which the specimens were collected

Table S2 Data on arthropods collected in 2005, including accession number, taxonomic classification, feeding mode, and plant species collected from which the specimens were collected

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