

# The defense hypothesis of elemental hyperaccumulation: status, challenges and new directions

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**Abstract** Elemental hyperaccumulation may have several functions, including plant defense against natural enemies. A total of 34 studies, including 72 experimental tests, have been conducted to date. At least some tests have demonstrated defense by hyperaccumulated As, Cd, Ni, Se and Zn, but relatively few plant taxa and natural enemies have been investigated. Defense by hyperaccumulated Ni has been shown for most leaf/root chewing herbivores and pathogens tested (20 of 26 tests) but not for herbivores of other feeding modes (1 of 8 tests). Most tests (5 of 6) using Ni concentrations below accumulator levels found no defensive effect, and the single test using plants in the accumulator range also found no effect. For Zn, mixed results have been reported for both hyperaccumulator (3 of 6 tests showed defense) and accumulator levels (3 of 4 tests showed defense). These tests have focused exclusively on leaf chewing/scraping herbivores: no herbivores of other feeding modes, or pathogens, have been tested. Both hyperaccumulator and accumulator concentrations of Se generally have shown defensive effects (12 of 14 tests). Most (75%) of these positive results used plants with

accumulator Se concentrations. The three tests of Cd showed defensive effects in two cases, one for hyperaccumulator and one for sub-accumulator Cd concentrations. Arsenic has been tested only once, and was found effective against a leaf-chewing herbivore at a concentration much less than the hyperaccumulator level. Defense studies have used a variety of experimental approaches, including choice and no-choice experiments as well as experiments that use artificial diet or growth media. Investigations of hyperaccumulation as a defense against natural enemies have led to two emerging questions. First, what is the minimum concentration of an element sufficient for defense? Evidence suggests that plants other than hyperaccumulators (such as accumulators) may be defended by elements against some natural enemies. Second, do the effects of an element combine with the effects of organic defensive compounds in plants to produce enhanced joint defensive effects? Recent investigation of this “joint effects hypothesis,” using Ni and secondary plant compounds in artificial insect diet, has demonstrated joint effects. Initial answers to both these questions suggest that defensive effects of elements in plants are more widespread than previously believed. These results also suggest an evolutionary pathway by which elemental hyperaccumulation may have evolved from accumulation. In this “defensive enhancement” scenario, defensive benefits of elevated levels of elements

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may have led to stepwise increases in element concentrations that further magnified these benefits. This series of steps could have led to increased accumulation, and ultimately hyperaccumulation, of elements by plants.

**Keywords** Elemental plant defense · Heavy metal · Herbivory · Hyperaccumulation · Plant pathogen

### Element accumulation and plant defense

Plants are the major primary producers in terrestrial ecosystems. This fundamental role makes them an important direct source of energy for primary consumers (herbivores, parasitic plants, pathogens). These primary consumers constitute plant “natural enemies,” a term that captures the antagonistic nature of their relationship with plants. Plants have traits that reduce their susceptibility to attack by natural enemies. These traits, intuitively termed plant defenses, include physical defenses such as thorns and spines (e.g., Gomez and Zamora 2002), associational defenses such as ants (e.g., Oliveira et al. 1999) and a tremendous variety of chemical defenses (alkaloids, terpenes, etc., Harborne 1988). To date, most studies of plant chemical defenses have focused upon organic plant constituents (secondary compounds). A great many secondary compounds have been discovered and the roles of some of these in plant defense have been well studied (Harborne 1988).

Some plants, termed hyperaccumulators by Brooks et al. (1977a), are chemically unusual in that they contain large amounts of elements (often metals) not normally found in abundance in plants. As discussed below, these elements may play a role in plant defense. Other plants have less elevated levels than hyperaccumulators yet greater concentrations than normal (Reeves 1992). The term accumulator is sometimes used (e.g., Berzain Iturralde 2004) for plants with elevated but not extraordinary element levels. The boundaries between accumulator and hyperaccumulator categories are somewhat arbitrary (Macnair 2003; Pollard et al. 2002; Reeves and Baker 2000) but are useful because they subdivide the wide range of element concentrations found in plants. Table 1

summarizes the element concentrations of plants into accumulator and hyperaccumulator categories for those elements often discussed in this context (mostly metals). Counts of hyperaccumulator species are available (Baker et al. 2000); the element most commonly hyperaccumulated is Ni (Table 1). New reports of hyperaccumulator species (e.g., Robinson et al. 2006; Srivastava et al. 2006; Sun et al. 2006) accrue each year, so that the counts in Table 1 are minimum numbers. Besides those listed in Table 1, the hyperaccumulation concept has been applied less frequently to some other elements, including Al (Jansen et al. 2002), B (Babaoğlu et al. 2004) and Fe (Rodríguez et al. 2005).

Most research into plant element concentrations has targeted identification of hyperaccumulators (e.g., Reeves 2003), with the result that much less information is available regarding accumulator plants. For example, because of the research emphasis on hyperaccumulators and the lack of discrete definitions of accumulation, the numbers of accumulator species are poorly known. It also should be noted that some elements are co-accumulated by plants. Co-accumulation is the simultaneous accumulation of more than one element (usually multiple metals). Reeves and Baker (2000) list Co and Cu, Zn and Pb, and Zn and Ni as pairs of metals that are sometimes reported as co-accumulated.

Several explanations have been offered for the unusual elemental composition of metal hyperaccumulators. As originally summarized by Boyd and Martens (1992) for metal hyperaccumulators, these include: (1) metal tolerance/disposal; (2) drought resistance; (3) interference with neighboring plants; (4) inadvertent uptake and (5) defense against natural enemies. This last explanation, termed the “defense hypothesis” by Boyd and Martens (1992), has garnered the most supporting evidence (Boyd 2004; Boyd and Martens 1998a), although at least in part because tests of the other hypotheses are rare (Boyd 2004). Martens and Boyd (1994) suggested that elevated concentration of an element in plant tissues be considered as a new category of plant chemical defense: “elemental defense.” Boyd (1998) pointed out several differences between elemental and organic (secondary chemical) plant defenses. First, elemental defenses are acquired

**Table 1** Normal range, minimum value for status as accumulator and minimum value for status as hyperaccumulator for ten elements reported to be hyperaccumulated by at

least some plants. Values for normal range and both accumulator and hyperaccumulator thresholds are expressed in mg/kg tissue dry mass

Element	Normal range <sup>a</sup>	Accumulator threshold <sup>b</sup>	Hyperaccumulator threshold <sup>c</sup>	Number of hyperaccumulator taxa <sup>d</sup>
As	–	–	1,000	Not reported
Cd	0.1–3	20	100	1
Co	0.03–2	20	100	28
Cr	0.2–5	50	100	Unknown
Cu	5–25	100	1,000	37
Mn	20–400	2,000	10,000	9
Ni	1–10	100	1,000	317
Pb	0.1–5	100	1,000	14
Se	0.05–1	10	1,000	20
Zn	20–400	2,000	10,000	11

<sup>a</sup> Reported by Reeves and Baker (2000). Discovery of hyperaccumulation of As (Ma et al. 2001) was too recent to include that element in the Reeves and Baker (2000) review

<sup>b</sup> Accumulator threshold values are the “high” level in plant leaves reported by Reeves and Baker (2000). Discovery of hyperaccumulation of As was too recent for Reeves and Baker (2000) to include that element in their review

<sup>c</sup> The threshold value of hyperaccumulation for As according to Ma et al. (2001)

<sup>d</sup> Numbers of hyperaccumulator taxa for each element are from Baker et al. (2000), except value for Se is from Reeves and Baker (2000). The number of taxa for Cr is “Unknown” because Reeves and Baker (2000) are uncertain whether reports of Cr hyperaccumulation are due to sample contamination or represent genuine plant uptake and sequestration, whereas for As it is listed as “Not reported” due the relatively recent discovery of As hyperaccumulation

from the soil and are not synthesized by a plant. Second, elements cannot be chemically degraded so that this herbivore counterdefense mechanism is not an option. Finally, elemental defenses may be less expensive metabolically than organic plant defenses. These elements are obtained from the soil solution and often are complexed with relatively small organic molecules (Callahan et al. 2006). The cost implication is that plants with elemental defenses may be able to decrease levels of putatively more expensive organic defenses (the “trade-off hypothesis” of Boyd 1998). Little evidence regarding this hypothesis is available, but Tolrà et al. (2001) showed that Zn hyperaccumulating plants of *Thlaspi caerulescens* contained lower glucosinolate concentrations than non-hyperaccumulating plants. A similar investigation of the Ni hyperaccumulator *Streptanthus polygaloides* (Jhee et al. 2006a) showed no difference in total glucosinolate concentrations, but some differences in concentrations of specific glucosinolates, between Ni hyperaccumulating and non-hyperaccumulating plants.

The term “plant defense” is an intuitive term yet is difficult to define. Ecologists often discuss plant “resistance” to natural enemies in this con-

text (e.g., Rausher 2001), referring to a plant’s ability to reduce damage in the face of an attack, but I will use the term “defense” for its intuitive appeal. Using as a model Karban and Agrawal’s (2002) approach to defining “herbivore offense,” I will define “plant defense” as a trait that: (1) decreases natural enemy performance (fitness) while (2) simultaneously increasing the fitness of the plant possessing it. Thus, in this definition, the effect of the trait from the perspective of both the natural enemy and the plant are included. While ideally both of these perspectives should be included in a study testing whether or not a trait (such as element hyperaccumulation) is a plant defense, evidence that a trait either decreases natural enemy performance or increases plant fitness in the face of enemy attack constitutes what I call here a “defensive effect:” evidence that is consistent with the defensive function that trait.

Again following Karban and Agrawal (2002), I use “adapted plant defense” to denote a trait that has evolved because it reduces natural enemy damage to a plant. The crucial difference is that an “adapted plant defense” requires that defense be the factor that selected for evolution of the trait, whereas “plant defense” simply denotes that the

trait reduces damage whether or not reduction in damage by a natural enemy was the selective benefit that underlay its evolution. In the sense used here, “plant defense” includes what Gould and Vrba (1982) called “exaptations,” features that evolved for one reason but have utility in another selective context. Here I will use “elemental defense” in the less strict sense, as an elevated concentration of an element that simultaneously results in decreased natural enemy performance and increased plant fitness. In this sense, an adapted plant elemental defense is a special (more restrictive) case of plant elemental defense.

My purpose here in focusing on the elemental defense hypothesis is threefold:

- (1) To provide a summary of the results of studies testing this hypothesis to date, examining evidence for defensive effects of elements at hyperaccumulator and lesser concentrations;
- (2) To discuss the experimental approaches that have been used to address this hypothesis, including the rationale behind each approach and the challenges facing them. Pointing out the challenges and pitfalls regarding research in this area will be useful to investigators as they continue to frame and test research questions. Explicit discussion of these topics may help investigations of plant elemental defenses to avoid some of the problems that have occurred for studies of plant defense in general (Stamp 2003a); and
- (3) To highlight new hypotheses and research directions involving the defense hypothesis that merit experimental attention. I do this to stimulate research into these areas and to point out that the defense hypothesis may provide, in at least some cases, an ecological explanation for the evolution of hyperaccumulation and, perhaps, accumulation.

### **Hyperaccumulation as elemental plant defense: status of the defense hypothesis**

A total of 34 studies published during the past 13 years have tested the elemental defense hypothesis by using plants raised on high and low element substrates (Table 2). These are summa-

rized in Table 2 into 72 individual tests. Most of these tests used a single natural enemy and a single plant species, but some tests were more complex by involving suites of natural enemies or plants (e.g., Freeman et al. 2007; Noret et al. 2006; Vickerman et al. 2002a; Martens and Boyd 2002) and are simplified as shown for convenience. Because the studies included in Table 2 are restricted to those that have used plants raised on high and low element substrates, reports of natural enemies that attack high element plants in the field (e.g., Boyd et al. 2006a, b; Pilon-Smits and Freeman 2006; Szwedo 2005; Mesjasz-Przybylowicz and Przybylowicz 2001) are not included in the table. An exception was made for studies that compared natural enemy performance on congeneric pairs of high and low element plant species (Augustyniak et al. 2002; Balkwill and Burt 1997), as this was considered a modification of the high vs. low element experimental approach within a species.

Tests of the elemental defense hypothesis for many elements have yet to be attempted. Elements tested to date (As, Ni, Zn, Se and Cd; Table 2) include only half of the ten listed in Table 1 as hyperaccumulated by plants. In addition, no experiments have targeted the elements that are not included in Table 1 but are considered to be hyperaccumulated by some plants (e.g., Al, B, Fe).

Tested elements are listed in Table 2 from that with the most published studies (Ni) to that with the fewest (As). Table 2 also summarizes the defensive results reported for each natural enemy tested, listing the results from both the natural enemy and plant perspectives. For a natural enemy, a defensive effect (signified by “+” in Table 2) was defined as significantly decreased performance (lesser survival, slower growth, smaller population size, preference for low element tissues, reduced consumption of high element tissues, less extensive area of infection, etc.) of a natural enemy when performance was compared between individuals or populations reared on high vs. low element plants. From the plant perspective, a defensive effect (“+” in Table 2) was evidence of better plant performance (greater plant survival, less leaf damage, larger plant size) of high element plants in the face of

**Table 2** Summary of studies testing the elemental defense hypothesis

Study <sup>a</sup>	Maximum concentration <sup>b</sup> (mg/kg dry wt.)	Plant species <sup>c</sup>	Natural enemy species <sup>d</sup> (type, feeding mode)	Experimental approach used <sup>e</sup>	Defense effect Enemy; Plant (+, 0, -) <sup>f</sup>
<b>Nickel</b>					
Wall and Boyd (2006)	N.R.	<i>Streptanthus polygaloides</i> Gray	<i>Melanotrichus boydi</i> Schwartz and Wall* (plant bug, cell disruptor)	3	0; N.D.
	N.R.	<i>Streptanthus polygaloides</i>	<i>Lygus hesperus</i> Knight* (plant bug, cell disruptor)	3 (modified)	0; N.D.
Jhee et al. (2006a)	6,800	<i>Streptanthus polygaloides</i>	<i>Plutella xylostella</i> L. (moth, leaf chewer)	2,3	++; +
Freeman et al. (2005)	N.R.	<i>Thlaspi goesingense</i> Hálačsy	<i>Erysiphe cruciferarum</i> Opiz ex Junell (fungus, pathogen)	1	++; N.D.
Jhee et al. (2005)	7,300	<i>Streptanthus polygaloides</i>	<i>Melanoplus femurrubrum</i> DeGeer (grasshopper, leaf chewer)	1,3	++; +
	6,800	<i>Streptanthus polygaloides</i>	<i>Evergestis rimosalis</i> Guenée (moth, leaf chewer)	1,3	++; +
	N.R.	<i>Streptanthus polygaloides</i>	<i>Delia radicum</i> L. (fly, root chewer)	1,3	++; +
	7,400	<i>Streptanthus polygaloides</i>	<i>Philaenus spumarius</i> L. (spittlebug, xylem feeder)	1	0; N.D.
	6,800	<i>Streptanthus polygaloides</i>	<i>Lipaphis erysimi</i> Kaltenbach (aphid, phloem feeder)	1	0; N.D.
	8,000	<i>Streptanthus polygaloides</i>	<i>Tetranychus urticae</i> Koch (whitefly, phloem feeder)	1	0; N.D.
	5,400	<i>Streptanthus polygaloides</i>	<i>Trialeurodes vaporariorum</i> Westwood (mite, cell disruptor)	1,3	++; +
	5,300	<i>Streptanthus polygaloides</i>	<i>Lygus lineolaris</i> Palisot de Beauvois (plant bug, cell disruptor)	1	0; N.D.
Boyd and Jhee (2005)	3,800	<i>Streptanthus polygaloides</i>	<i>Limax maximus</i> L. (slug, leaf chewer)	1	++; N.D.
	3,800	<i>Streptanthus polygaloides</i>	<i>Lehmannia valentiana</i> Férussac (slug, leaf chewer)	3	N.D.; +
	48	<i>Streptanthus breweri</i> A. Gray	<i>Limax maximus</i> (slug)	1	0; N.D.
	53	<i>Streptanthus albidus</i> E. Greene	<i>Limax maximus</i> (slug)	1	0; N.D.
	64	<i>Streptanthus insignis</i> Jepson	<i>Limax maximus</i> (slug)	1	0; N.D.
	41	<i>Streptanthus tortuosus</i> Kellogg	<i>Limax maximus</i> (slug)	1	0; N.D.
Augustyniak et al. (2002)	37,000	<i>Berkheya coddii</i> Roessl.	<i>Chrysolina pardalina</i> Fabricius* (beetle, leaf chewer)	3 (modified)	0; 0
Martens and Boyd (2002)	9,100	<i>Streptanthus polygaloides</i>	Unknown invertebrates*	4	N.D.; +
	9,100	<i>Streptanthus polygaloides</i>	Unknown vertebrates*	4	N.D.; 0
Boyd et al. (2002)	12,000	<i>Senecio coronatus</i> (Thunberg) Harvey	<i>Helix aspersa</i> Müller (snail, leaf chewer)	1,2,3	++; +

Table 2 continued

Study <sup>a</sup>	Maximum concentration <sup>b</sup> (mg/kg dry wt.)	Plant species <sup>c</sup>	Natural enemy species <sup>d</sup> (type, feeding mode)	Experimental approach used <sup>e</sup>	Defense effect Enemy; Plant (+, 0, –) <sup>f</sup>
Davis et al. (2001)	N.R.	<i>Streptanthus polygaloides</i>	<i>Turnip mosaic virus</i> (TuMV) (virus, pathogen)	1	–; 0
Ghaderian et al. (2000)	N.R.	<i>Streptanthus insignis</i>	<i>Turnip mosaic virus</i>	1	0; 0
	18,000	<i>Alyssum serpyllifolium</i> Desf. subsp. <i>lusitanicum</i> Dudley & Pinto da Silva	<i>Pythium mamillatum</i> Meurs* (fungus, pathogen)	1,2	+; +
Boyd et al. (1999)	18,000	<i>Alyssum serpyllifolium</i> Desf. subsp. <i>lusitanicum</i> Dudley & Pinto da Silva	<i>Pythium ultimum</i> (fungus, pathogen)	1,2	+; +
	30,000	<i>Alyssum murale</i> Waldst. & Kit.	<i>Pythium mamillatum</i> *	1,2	+; +
Boyd and Moar (1999)	30,000	<i>Alyssum murale</i> Waldst. & Kit.	<i>Pythium ultimum</i>	1,2	+; +
	2,300	<i>Aurinia saxatilis</i> (L.) Desv.*	<i>Pythium mamillatum</i> *	1,2	0; 0
Boyd et al. (1999)	2,300	<i>Aurinia saxatilis</i> (L.) Desv.*	<i>Pythium ultimum</i>	1,2	0; 0
	470	<i>Cuscuta californica</i> Hook. & Arn. var. <i>breviflora</i> Engelm.*	<i>Spodoptera exigua</i> Hübner (moth, leaf chewer)	1	0; N.D.
Boyd and Moar (1999)	1,500	<i>Streptanthus polygaloides</i>	<i>Spodoptera exigua</i> (moth, leaf chewer)	1,2	+; N.D.
	40	<i>Streptanthus breweri</i> *	<i>Spodoptera exigua</i> (moth, leaf chewer)	1	0; N.D.
Boyd and Martens (1999)	93	<i>Streptanthus tortuosus</i> *	<i>Spodoptera exigua</i> (moth, leaf chewer)	1	+; N.D.
	6,000	<i>Streptanthus polygaloides</i>	<i>Acyrtosiphon pisum</i> Harris (aphid, phloem feeder)	1,2,3	0; –
Balkwill and Burt (1997)	1,100	<i>Senecio coronatus</i>	Unidentified slug (slug, leaf chewer)	3 (modified)	N.D.; 0
	4,000	<i>Berkheya coddii</i>	Unidentified slug (slug, leaf chewer)	3 (modified)	N.D.; 0
Boyd and Martens (1994)	3,000	<i>Thlaspi montanum</i> L. var. <i>montanum</i>	<i>Pieris rapae</i> L. (butterfly, leaf chewer)	1,2	+; N.D.
	5,800	<i>Streptanthus polygaloides</i>	<i>Erysiphe polygoni</i> DC (fungus, pathogen)	1	+; N.D.
Boyd et al. (1994)	N.R.	<i>Streptanthus polygaloides</i>	<i>Xanthomonas campestris</i> pathovar <i>campestris</i> [Pam.] Dow (bacterium, pathogen)	1,2	+; N.D.
Martens and Boyd (1994)	N.R.	<i>Streptanthus polygaloides</i>	<i>Alternaria brassicicola</i> (Schw.) Wilsh. (fungus, necrotrophic pathogen)	1	+; N.D.
	7,400	<i>Streptanthus polygaloides</i>	<i>Pieris rapae</i> (butterfly, leaf chewer)	1,2,3	+; +
Martens and Boyd (1994)	7,400	<i>Streptanthus polygaloides</i>	<i>Euchloe hyantis</i> subsp. <i>hyantis</i>	1	+; N.D.
	7,400	<i>Streptanthus polygaloides</i>	W.H. Edwards* (butterfly, leaf chewer)	1	+; N.D.
Zinc	7,400	<i>Streptanthus polygaloides</i>	Grasshopper nymphs* (grasshopper, leaf chewer)	1	+; N.D.
	3,800	<i>Viola calaminaria</i> (Gingins) Lej.	<i>Issoria lathonia</i> L.* (butterfly, leaf chewer)	1	+; –
Noret et al. (2007)	15,000	<i>Thlaspi caerulescens</i> J. & C. Presl.	Gastropods*? (snails and slugs, leaf chewers)	4	N.D.; 0
Jiang et al. (2005)	2,700	<i>Thlaspi caerulescens</i>	<i>Frankliniella occidentalis</i> Pergande (thrips, leaf scraper)	3	N.D.; 0

Table 2 continued

Study <sup>a</sup>	Maximum concentration <sup>b</sup> (mg/kg dry wt.)	Plant species <sup>c</sup>	Natural enemy species <sup>d</sup> (type, feeding mode)	Experimental approach used <sup>e</sup>	Defense effect Enemy; Plant (+, 0, -) <sup>f</sup>
Behmer et al. (2005)	5,770	<i>Thlaspi caerulescens</i>	<i>Schistocerca gregaria</i> Forskal (grasshopper, leaf chewer)	1,2,3	+, +
Noret et al. (2005)	13,500	<i>Thlaspi caerulescens</i>	<i>Helix aspersa</i> * (snail, leaf chewer)	1	N.D.; 0
Huitson and Macnair (2003)	10,000	<i>Arabidopsis halleri</i> (L.) O Kane & Al-Shehbaz	<i>Helix aspersa</i> * (snail, leaf chewer)	3	N.D.; 0
Jhee et al. (1999)	34,000	<i>Thlaspi caerulescens</i>	<i>Pieris napi oleracea</i> Harris (butterfly, leaf chewer)	3	N.D.; +
Pollard and Baker (1997)	14,000	<i>Thlaspi caerulescens</i>	<i>Schistocerca gregaria</i> (grasshopper, leaf chewer)	3	N.D.; +
	14,000	<i>Thlaspi caerulescens</i>	<i>Deroceras caruanae</i> Pollonera (slug, leaf chewer)	3	N.D.; +
	7,400	<i>Thlaspi caerulescens</i>	<i>Pieris brassicae</i> * (butterfly, leaf chewer)	3	N.D.; +
<b>Selenium</b>					
Freeman et al. (2007)	550	<i>Brassica juncea</i> (L.) Czern.	<i>Acheta domestica</i> (L.) (cricket, leaf chewer)	1,3	+, +
	230	<i>Stanleya pinnata</i> (Pursh.) Britt.	Grasshoppers (mix of 11 genera) (grasshopper, leaf chewer)	1,3	+, +
	<250	<i>Stanleya pinnata</i>	Natural field herbivore fauna*	4	N.D.; +
Freeman et al. (2006)	2,000	<i>Stanleya pinnata</i>	<i>Plutella xylostella</i> "Stanleyi" (moth, leaf chewer)	1,3	0; 0
		<i>Stanleya pinnata</i>	<i>Plutella xylostella</i> "G88" (moth, leaf chewer)	1,3	+, +
		<i>Stanleya pinnata</i>	<i>Pieris rapae</i> (butterfly, leaf chewer)	1,3	+, +
Hanson et al. (2004)	750	<i>Brassica juncea</i>	<i>Myzus persicae</i> Sulzer (aphid, phloem feeder)	1,3	+, +
Hanson et al. (2003)	1,600	<i>Brassica juncea</i>	<i>Pieris rapae</i> (butterfly, leaf chewer)	1,3	+, +
	1,500	<i>Brassica juncea</i>	<i>Mesodon ferrisi</i> Pilsbry (snail, leaf chewer)	1,3	0; -
	800	<i>Brassica juncea</i>	<i>Fusarium</i> sp.* (fungus, pathogen)	1,2	+, N.D.
	300	<i>Brassica juncea</i>	<i>Alternaria brassicicola</i> (fungus, pathogen)	1,2	N.D.; +
Bañuelos et al. (2002)	465	<i>Brassica juncea</i>	<i>Trichoplusia ni</i> Hübner* (moth, leaf chewer)	3	+, N.D.
Vickerman et al. (2002a)	83	<i>Atriplex</i> (17 species)*	<i>Spodoptera exigua</i> (moth, leaf chewer)	1	+, N.D.
Vickerman et al. (2002b)	310	<i>Medicago sativa</i> L.*	<i>Spodoptera exigua</i> (moth, leaf chewer)	1,3	+, -
<b>Cadmium</b>					
Scheirs et al. (2006)	4.4	<i>Holeus lanatus</i> L.*	<i>Chromatomyia militi</i> Kaltenbach* (fly, leaf miner)	1,3	+, +
Jiang et al. (2005)	1,900	<i>Thlaspi caerulescens</i>	<i>Frankliniella occidentalis</i> (thrips, leaf scraper)	3	N.D.; +
Noret et al. (2005)	1,700	<i>Thlaspi caerulescens</i>	<i>Helix aspersa</i> * (snail, leaf chewer)	1	N.D.; 0



Table 2 continued

Study <sup>a</sup>	Maximum concentration <sup>b</sup> (mg/kg dry wt.)	Plant species <sup>c</sup>	Natural enemy species <sup>d</sup> (type, feeding mode)	Experimental approach used <sup>e</sup>	Defense effect Enemy; Plant (+,0,-) <sup>f</sup>
<b>Arsenic</b>					
Rathinasabapathi et al. (2007)	46	<i>Pteris vittata</i> L.	<i>Schistocerca americana</i> Drury (grasshopper, leaf chewer)	1,3	+; +

<sup>a</sup> Studies are listed by element and then in reverse chronological order within an element

<sup>b</sup> Maximum concentration is the highest element level in plants used for any experiment using the listed natural enemy. N.R. means that specific values were Not Reported for plant material used, but hyperaccumulator levels were likely in all cases except that of *Streptanthus insignis* (Davis et al. 2001), for which species Boyd and Jhee (2005) reported values <100 mg Ni/kg under similar culture conditions

<sup>c</sup> Plant species denoted by an asterisk are not reported to be hyperaccumulator species (Reeves and Baker 2000). Note that the study of Vickerman et al. (2002a) used 17 *Atriplex* species and in some cases multiple lines of species: for simplicity and to be conservative, that study is listed here as one test but in fact contains multiple cases of positive defensive effects

<sup>d</sup> The type of natural enemy tested is described parenthetically below its scientific name, along with the general feeding mode of animal natural enemies. An asterisk for a natural enemy denotes an enemy native to sites on which the plant species tested naturally grows (a question mark is provided if it was unclear whether the natural enemy is native or not)

<sup>e</sup> Experimental approaches are: (1) no choice *in planta* lab experiment, (2) artificial growth medium lab experiment, (3) choice *in planta* lab experiment, (4) choice *in planta* field experiment

<sup>f</sup> Defense effect denotes whether a defensive effect was detected, listing results from both the natural enemy and the plant perspectives. From the natural enemy perspective: + indicates reduced feeding/survival for a high element concentration plant treatment, 0 denotes no significant effect and – indicates increased feeding or survival for a high concentration plant treatment (see text for exceptions). From the plant perspective: + indicates greater biomass/survival for a high element concentration plant treatment, 0 denotes no significant treatment effect and – indicates reduced biomass/survival for a high concentration plant treatment



natural enemy attack. For studies of herbivores, this usually meant lesser damage to high element plants during choice experiments. For pathogen studies, positive evidence required that plant performance (survival, growth, etc.) be measured rather than a simple comparison of infection levels. Most tests (42 of 72) did not include measures of defensive effects from both the natural enemy and the plant perspectives: in such cases the defensive effect for the missing component is indicated as “Not Determined” (N.D.) in Table 2.

Half of the studies (17 of 34) have targeted Ni: this emphasis on Ni may be justified because Ni is the element hyperaccumulated by most (about 75%) hyperaccumulator taxa (Baker et al. 2000). Of the 44 tests of Ni reported in Table 2, 17 examined elemental defense from both the natural enemy and plant perspectives. Studies using Ni examined a wider range of natural enemies than studies of other elements (Table 2), in terms of both taxonomic range (including insects, gastropods, fungi, bacteria and viruses) and range of animal feeding mode (leaf chewer, root chewer, cell disruptor, phloem feeder, xylem feeder).

Defensive effects were reported for hyperaccumulator levels of Ni in 22 of the 36 tests of Ni in that concentration range. Leaf chewing insects were well represented and defense was reported for 12 of the 14 leaf chewing species included in Table 2. Failing to show defensive effects were the studies of Balkwill and Burt (1997), which used an unidentified gastropod (slug) herbivore, and Augustyniak et al. (2002), which used the native beetle *Chrysolina pardalina*. This latter insect is reported to be a specialist on the Ni hyperaccumulator *Berkheya coddii* (Mesjasz-Przybyłowicz and Przybyłowicz 2001). Defense by hyperaccumulated Ni has rarely been shown against herbivores with other feeding modes: three of four cell disruptors, both phloem feeders and the one xylem feeder tested have shown no evidence of defensive effects when feeding on hyperaccumulating plants (Table 2). These unaffected insects may avoid Ni by their selective feeding, but this question remains unexplored. In one case (Boyd and Martens 1999), high Ni plants supported greater aphid population sizes than low Ni plants, leading to the negative defensive effect (increased susceptibility to attack) from the plant perspective

shown in Table 2. This increase in susceptibility may have been due to more vigorous growth of the plant species when grown on high Ni soil (Boyd and Martens 1999).

Nickel is also the element best tested by studies of pathogens on hyperaccumulating plants, including four fungi, a bacterium and a virus (Table 2). In all cases but the virus, a defensive effect of Ni was reported. However, Ghaderian et al. (2000) reported a lack of defense against two fungi at the hyperaccumulator Ni concentration of 2,300 mg/kg; in their study a defensive effect occurred only for Ni hyperaccumulated at the relatively high concentration of 18,000 mg/kg or greater. The case of the virus (Davis et al. 2001) was unusual in that viral particle counts were greater for hyperaccumulating plants, indicating greater susceptibility of those plants to viral attack. Davis et al. (2001) postulated that more vigorous growth of the hyperaccumulating plants may have supported increased virus reproduction.

Relatively few studies have examined defense by Ni at less than hyperaccumulator concentrations. Only one study (Boyd et al. 1999) used Ni in the accumulator range: that study showed no effect of 470 mg Ni/kg on a leaf chewing insect. Three of the studies in Table 2 (Boyd and Jhee 2005; Davis et al. 2001; Boyd and Moar 1999) included tests using plant species that accumulate Ni to concentrations <100 mg/kg. In only one of the six tests reported was a defensive effect observed, and that was the relatively subtle effect of decreased larval growth on high Ni (93 mg/kg) *Streptanthus tortuosus*.

Defense by Zn has been the topic of eight studies and ten experimental tests. Only two of the ten tests examined defense from both natural enemy and plant perspectives (Table 2), with the remaining tests focusing solely on defense from the plant perspective. Leaf chewing (seven species) and leaf scraping (one species) invertebrates were included, but not herbivores of other feeding modes (Table 2). No studies of pathogens have yet been attempted. Plant Zn concentrations at or greater than the threshold for Zn hyperaccumulation (10,000 mg/kg) either defended (three tests) or failed to defend (three tests) plants against folivores.

Accumulator levels of Zn (2,000–10,000 mg/kg) were used in four tests, and three reported defensive effects. One of these defensive effects, however, is equivocal. Noret et al. (2007) reported that leaves containing 3,800 mg Zn/kg decreased larval growth of *Issoria lathonia* but also documented increased leaf consumption during a larva's lifetime. Thus, a positive defensive effect was found from the natural enemy perspective (decreased performance) yet Noret et al. (2007) point out that the likely impact of Zn accumulation on plant fitness would be negative because high Zn plants would lose more biomass to each larva that completed its development. The defensive effect from the plant perspective is therefore listed as negative in Table 2. Two of the three positive tests used *Thlaspi caerulescens*: Behmer et al. (2005) and Pollard and Baker (1997) reported defensive effects at 5,770 and 7,400 mg Zn/kg, respectively.

Considering studies of Zn at both hyperaccumulator and accumulator levels, defensive effects have been shown for six of the ten tests. These results may be due to differences in the herbivores used, but also may be influenced by organic defensive compounds. All but two of the tests regarding Zn reported in Table 2 used a single plant species: *Thlaspi caerulescens*. Noret et al. (2005, 2006) suggested that the concentration of glucosinolates in *Thlaspi caerulescens*, rather than Zn, determined defense against leaf chewing gastropod herbivores. Their suggestion might also explain the result of Huitson and Macnair (2003), who reported no effect of plant Zn concentration on the leaf chewing gastropod *Helix aspersa*.

Selenium is the topic of seven studies and 14 tests of defense. Nine tests considered defense from both natural enemy and plant perspectives. The range of natural enemies used in studies of Se is relatively broad (second only to studies of Ni) and includes at least seven species of leaf chewers, a phloem feeder and two fungi (Table 2). The exact number of leaf chewing species is inexact because Freeman et al. (2007) used a mix of grasshopper species that included members of eleven genera. Because the responses of these grasshopper taxa were not followed individually, they are listed as a single grouping of natural enemies in Table 2.

Defensive effects of elevated Se were found in 12 of 14 tests (Table 2). Only two of the studies used plants that hyperaccumulated Se (>1000 mg Se/kg). Most (nine) cases of the 12 positive examples of defensive effects were found for plant material that contained less than that level. Selenium is thus the element for which defensive effects most often have been shown to extend to concentrations below the hyperaccumulation level. In some cases, remarkably little Se produced a defensive effect. For example, high Se plants negatively impacted the phloem-feeding insect (aphid) used by Hanson et al. (2004). Relatively low Se plants, containing as little as 10 mg Se/kg, were avoided in choice experiments and plants containing only 1.5 mg Se/kg reduced aphid population growth 50%.

One species, the moth *Plutella xylostella*, is included in Table 2 as both defended against and not defended against by Se. Freeman et al. (2006) reported that a strain of this introduced pest moth, collected from the Se hyperaccumulator *Stanleya pinnata*, has evolved tolerance for a high Se diet. Unadapted moths fed the hyperaccumulator died rapidly, but the adapted strain was unaffected by dietary Se. Another unusual result for Se in Table 2 is the increased consumption of Se hyperaccumulating plants of *Brassica juncea* by the snail *Mesodon ferrissi* (Hanson et al. 2003), resulting in a negative defensive effect from the plant perspective. This high Se diet did not noticeably affect the snails over the 4-day experiment, yielding a neutral ("0") defensive effect from the natural enemy perspective, but their response over a longer timeframe is unknown. Similar to the study by Hanson et al. (2003), Vickerman et al. (2002b) showed a preference of fourth instar *Spodoptera exigua* caterpillars for high Se (300 mg/kg) *Medicago sativa* plants. This result is summarized by a negative defensive effect from the plant perspective in Table 2. In this case, however, the conclusion noted in Table 2 from the natural enemy perspective is a positive defensive effect because a diet of that Se concentration usually was lethal to the insects.

Three relatively recent studies have examined defensive effects of Cd (Table 2). In two cases (Jiang et al. 2005; Noret et al. 2005), the plant species used (*Thlaspi caerulescens*) co-accumulates

Cd and Zn and thus the results of these studies are reported in Table 2 under both of those elements. One study reported no effect of hyperaccumulated Cd (at 1,700 mg/kg) against a leaf chewing gastropod. Two studies of Cd reported defensive effects against folivores: one showed defense at a hyperaccumulator level (1,900 mg/kg) and the other at a sub-accumulator concentration. In the latter case (Scheirs et al. 2006), the Cd concentrations used (maximum was 4.4 mg/kg) were less than the accumulation threshold for Cd (20 mg/kg: Table 1). Scheirs et al. (2006) showed that only 2.5 mg Cd/kg was sufficient to significantly decrease herbivore attack.

Arsenic is the least studied element in Table 2, the subject of a single recent study. Using plant material (maximum of 46 mg As/kg) with much less As than the hyperaccumulation level (1,000 mg/kg: Table 1), Rathinasabapathi et al. (2007) showed a leaf chewing insect preferred low As *Pteris vittata* (Table 2).

A mix of defensive effects and lack of defensive effects has been reported for all elements tested (Table 2) except for As (which only has been tested once). Several factors contribute to these results. First, element concentrations used vary widely and include hyperaccumulator, accumulator and sub-accumulator concentrations. Because defensive effects are likely dose dependent, some differences in defensive outcomes may be due to differences in the element concentrations used. Most (13) of the 21 plant taxa used (counting the *Atriplex* species used by Vickerman et al. (2002a) as one taxon) are hyperaccumulators, but non-accumulator taxa (denoted by asterisks in Table 2) have been used by some studies (e.g., Boyd and Jhee 2005; Vickerman et al. 2002a, b). In 25 of the 72 cases (35%) in Table 2, element concentrations used were below the threshold used to define hyperaccumulation for an element, in some cases even when a hyperaccumulator species was used (e.g., Freeman et al. 2007; Rathinasabapathi et al. 2007; Behmer et al. 2005). It also should be kept in mind that element concentrations reported in Table 2 are maximum values reported by a study. In some tests (e.g., Huitson and Macnair 2003), element concentrations in plants generally may have been below the hyperaccumulator level for an element, yet because

some treatments used plants that equaled or exceeded the hyperaccumulation threshold the study is counted here as involving hyperaccumulator levels. I will discuss the issue of defensive effects at concentrations below hyperaccumulator levels in greater detail below.

Second, natural enemy species will vary in susceptibility to elements. For example, some native natural enemies may possess traits that allow them to tolerate high element diets (Boyd 1998). For example, Wall and Boyd (2006) showed that the plant bug *Melanotrachus boydi*, which is a specialist on the Ni hyperaccumulator *Streptanthus polygaloides*, prefers to attack *S. polygaloides*. Another plant bug, the widely distributed and polyphagous *Lygus hesperus*, also attacks *S. polygaloides* in the wild but this plant is not a preferred host (Wall and Boyd 2006). Experiments on the beetle *Chrysolina pardalina* (Augustyniak et al. 2002) have shown it prefers one Ni hyperaccumulator host (*Berkheya coddii*) over another congeneric Ni hyperaccumulator (*B. rehmannii* var. *rehmannii*). As mentioned previously, recent work with the Se hyperaccumulator *Stanleya pinnata* has identified a race of the introduced pest moth *Plutella xylostella* that has evolved tolerance of a high Se diet (Freeman et al. 2006). This example is particularly interesting, as this race must have evolved since *P. xylostella* was first introduced. Other studies that have documented use of hyperaccumulator hosts by native natural enemies include Mesjasz-Przybyłowicz and Przybyłowicz (2001), Wall and Boyd (2002), Boyd et al. (2004), Boyd et al. (2006a, b), and Pilon-Smits and Freeman (2006). Natural enemies native to sites that host the plant species tested were used in less than one-third of the cases (28%: 20 of the 72 cases) reported in Table 2. Using native natural enemies collected from field sites that host the test plant species is more rare, but has been done in some cases (e.g., Freeman et al. 2007; Noret et al. 2007; Wall and Boyd 2006; Hanson et al. 2003; Augustyniak et al. 2002; Ghaderian et al. 2000; Martens and Boyd 1994).

A third factor contributing to lack of defensive effects is that the effectiveness of a plant defense may be influenced by how a natural enemy exploits its host (including what tissues are

attacked). For example, Jhee et al. (2005) tested the defensive effectiveness of hyperaccumulated Ni in *Streptanthus polygaloides* against eight herbivore species, including species with leaf chewer, root chewer, phloem feeder, xylem feeder or cell disruptor feeding modes (Table 2). Leaf and root chewers were negatively impacted by hyperaccumulated Ni but xylem and phloem feeders were not, with cell disruptors presenting mixed results depending on the species used. Jhee et al. (2005) suggested that these results could be explained by: (1) variation of Ni concentration among tissues of *S. polygaloides*, allowing natural enemies of some feeding modes to avoid the elemental defense and/or (2) differences among herbivores in their sensitivity to Ni. Studies that test these possibilities would help us understand these cases of mixed defensive results.

Another point is evident from the studies summarized in Table 2: relatively few plant taxa have been tested. The studies in Table 2 contain a total of 38 taxa, but this is inflated by the large number (17) of *Atriplex* species used by Vickerman et al. (2002a). Most (86%) of the 72 tests reported use members of the Brassicaceae (species of *Alyssum*, *Arabidopsis*, *Brassica*, *Stanleya*, *Streptanthus* and *Thlaspi*; Table 2). The only other families represented are the Asteraceae (*Berkheya*, *Senecio*), Chenopodiaceae (*Atriplex*), Convolvulaceae (*Cuscuta*), Fabaceae (*Medicago*) and Violaceae (*Viola*). None of the many families and genera of tropical hyperaccumulators (Reeves 2003) have been included. A consequence of this focus on Brassicaceae is that no plant growth forms other than annual (*Arabidopsis*, *Brassica*, *Cuscuta*, most *Streptanthus*) or herbaceous/semi-woody perennial (*Alyssum*, some *Atriplex*, *Berkheya*, *Medicago*, *Senecio*, *Stanleya*, *Thlaspi*, *Viola*) have been included. This is not surprising: time and space constraints favor experimental use of small rapidly growing species, but this limits results to a small subset of potential taxa. The many hyperaccumulator shrub and tree species (Reeves 2003) have not been studied.

The natural enemies listed in Table 2 include a small fraction of the potentially important plant natural enemies present in sites hosting accumulator and hyperaccumulator plants. Most tests (58% of the 72 in Table 2) have focused on leaf

chewing folivores: natural enemies attacking other plant parts (such as roots, flowers, seeds) are almost uninvestigated. The herbivore species identified in Table 2 are exclusively invertebrates: to date no vertebrate herbivore species have been studied, with one exception. The field study of Martens and Boyd (2002) included treatments that allowed or restricted vertebrate herbivore access to some plants. They found no defensive effect of plant Ni concentration against those herbivores and a large impact of their activity on plant damage. Vertebrate herbivores should be included in future research because of their general ecological importance (e.g., Sessions and Kelly 2001) and because some accumulated/hyperaccumulated elements, e.g., Se (Franke and Potter 1936), are reported to influence vertebrate feeding.

Defensive effects reported in Table 2 from the natural enemy perspective can be attributed to two main mechanisms. One is through toxicity of the element-containing plant tissue, in which attack on plants results in enemy mortality (e.g., Jhee et al. 2006a; Freeman et al. 2006) or decreased growth (e.g., Boyd and Moar 1999). Another is by deterrence, in which high element plant tissue is attacked to a lesser extent than low element tissue when a choice is experimentally provided. Deterrence of herbivores has been demonstrated for all elements studied: As (Rathinasabapathi et al. 2007), Cd (Scheirs et al. 2006), Ni (Boyd et al. 2002; Boyd and Martens 1994), Se (Hanson et al. 2004), and Zn (Pollard and Baker 1997). There is overlap between toxicity and deterrence, as natural enemies such as herbivores may form feeding preferences based upon the toxic or growth-reducing effects of a defense. In an example for elemental defenses, Behmer et al. (2005) showed that grasshoppers could associate negative physiological effects of high Zn foods with the secondary chemicals contained in those foods.

### Studying the elemental defense hypothesis: experimental approaches and challenges

The challenges facing studies of the defense hypothesis are reminiscent of those facing studies

Step	Purpose	Experiment
1	Compare enemy performance on high vs. low element plants in controlled setting	No-choice <i>in planta</i> lab experiment
2	Isolate element as factor that reduces enemy performance <i>in planta</i> by using artificial medium	Artificial growth medium lab experiment
3	Demonstrate fitness benefit to high element vs. low element plants when exposed to natural enemy in controlled setting	Choice <i>in planta</i> lab experiment
4	Demonstrate fitness benefit to high element vs. low element plants when exposed to natural enemies in field setting	Choice <i>in planta</i> field experiment

**Fig. 1** Steps taken by experimental approaches to the defense hypothesis, their purposes and the experimental approach used in each case

of plant defense in general (Stamp 2003a, b). Using the framework of Karban and Agrawal (2002), I suggest that a thorough exploration of the defense hypothesis involves four steps (Fig. 1), where each step is associated with a particular experimental approach. Although these approaches are numbered sequentially in Fig. 1, they need not be performed in order, although that in Fig. 1 is a logical arrangement. Below I will briefly discuss the rationale behind, and challenges underlying, each step. Although I will focus on hyperaccumulator plants, studies investigating the defense hypothesis using plants that accumulate lower concentrations of elements (to accumulator and sub-accumulator levels) could use similar approaches and would face similar challenges.

**Step 1: Determine if natural enemy performance is reduced on high element plants compared to low element plants**

**Rationale**—This step documents one of the two criteria used to define a plant defense: reduced enemy performance. Enemy “performance” includes many possible metrics. Reproductive fitness is the best metric but survival is often viewed as a reasonable indicator of reproductive fitness. Sublethal effects, meaning reductions in parameters such as growth rate, reproductive effort or feeding rate, or increases in parameters such as development time, are also measured in some cases. Studies of elemental defenses have an

advantage over studies of many organic chemical defenses (Boyd 1998; Pollard 2000) because plant elemental concentration can be manipulated by manipulating soil elemental concentration. In contrast, studies of most organic plant defenses must use natural variation in defense levels or variation created by generations of artificial selection, induce production of a defense (and thus introduce another variable into the study) or otherwise manipulate plants to produce or not produce a putative defense chemical. Step 1 has been used by most tests of the elemental defense hypothesis (79% of 72: Table 2) because it is a logical first investigation of the defensive potential of elevated element levels in plants. Jhee et al. (2005) began investigations of eight herbivore natural enemies using this step, and only followed up with choice tests (Step 3) if a significant effect was determined for the no-choice (Step 1) experiments.

**Challenges**—A critical feature of Step 1 is the choice of the natural enemy to be used. Often, this choice is constrained by a lack of information on the natural enemies that occur in the habitats of hyperaccumulator plants, as well as a lack of specific information regarding the natural enemies of hyperaccumulator species. In the case of metal hyperaccumulators, which often grow on serpentine soils (Brooks 1987), there is little information on the herbivore communities of serpentine sites (Alexander et al. 2007; Kruckeberg 1984) and few surveys of arthropod herbivores of hyperaccumulator plants. There are a few reports of native pathogens (Pilon-Smits and Freeman 2006; Ghaderian et al. 2000), and one of a parasitic flowering plant attacking a Ni hyperaccumulator species in the wild (Boyd et al. 1999). Partly as a result of this information void, most Step 1 studies have used “bioassay” organisms (sensu Jhee et al. 2006a): those readily obtained and that will accept low element plants as a host, but are not native to areas hosting hyperaccumulators. It was noted above that few tests (28%) in Table 2 have used a native natural enemy (signified with an asterisk next to the name) with any experimental approach. A drawback to bioassay organisms is that results from experiments using them have unknown relevance to plant defense under natural conditions.



Identifying pertinent natural enemies in the field might involve two steps. First, an investigator could survey the hyperaccumulator under field conditions (i.e., when it is hyperaccumulating) to determine its major natural enemies. Second, the investigator could raise hyperaccumulator plants on low element soil, place them into the field, and document which natural enemies attack them. Comparing these two sets of natural enemies would reveal “potential” natural enemies: those that would attack the hyperaccumulator if it lacked its elemental defense but which do not when it is hyperaccumulating. These potential natural enemies are those against which the elemental defense is currently most effective. To my knowledge this experimental approach has yet to be attempted.

Identifying natural enemies suitable for experimentation also could use a comparative approach. For example, suites of natural enemies on hyperaccumulator and closely related non-hyperaccumulator species could be compared. The use of phylogenetic techniques to trace the evolution of hyperaccumulation is still in initial stages (e.g., Broadley et al. 2001; Mengoni et al. 2003), but applying them to both plants and their natural enemies can provide important insights on their joint evolutionary histories (e.g., Agrawal and Fishbein 2006).

A second constraint to Step 1 experiments is obtaining and culturing a hyperaccumulator species. As pointed out above, relatively few plant taxa have been used to date. This is because Step 1 experiments require that plants be raised on high and low element substrates to generate high and low element plant tissues. The plant species in Table 2 are generally small, grow rapidly from seed, are amenable to culture in growth chambers or greenhouses, and are temperate zone species. But many metal hyperaccumulators are woody tropical species (Reeves 2003): the difficulties of obtaining plant material and growing plants in culture are obstacles to their use in tests of the defense hypothesis. Yet a comprehensive evaluation of the defense hypothesis requires inclusion of plants with other growth forms and from tropical habitats.

Step 2: Using artificial media, show that the elevated concentration of an element *in planta* is sufficient to cause the defensive effect observed in Step 1

*Rationale*—This step helps to isolate an element’s concentration as the trait causing a defensive effect against an herbivore *in planta*. Plant-based experiments (Step 1) can show that high element plant tissues are less suitable substrates for a plant enemy than low element tissues, but tissue element concentration probably is not the only characteristic that varies when plants are grown on high or low element soils. Other features of plant tissues, such as water concentration, concentrations of other elements, and organic chemical composition, might all vary in plants grown on element-amended media. For example, Huitson and Macnair (2003) discovered a significant influence of growth medium rather than plant Zn concentration in their investigation of effects of Zn on snail herbivory. A study of the Ni hyperaccumulator *Streptanthus polygaloides* (Martens and Boyd 1994) showed that moisture content, along with Ca, K, Mg, P and Zn concentrations, varied significantly between plants grown on high and low Ni greenhouse soil (although Ni concentration varied to the greatest extent). As noted above, organic defense chemical composition (glucosinolates: Tolrà et al. 2001) may vary between plants raised on high or low element soils: this variable could influence natural enemy response during *in planta* experiments. Besides plant composition, other plant features (such as growth rate) may vary due to soil element concentration. These other plant features should be documented as much as possible during Step 1 experiments and tested to determine if they vary significantly between high and low element plants, but these differences provide a compelling argument for including Step 2 experiments in explorations of the defense hypothesis. Artificial medium experiments allow an investigator to control these other factors so that element effects on a natural enemy can be isolated and revealed. Table 2 shows that relatively few tests of elemental defense (16 of 72) have incorporated this step.

Another advantage of the artificial medium approach is that it allows investigation of a

number of elements simultaneously so that their defensive thresholds can be determined and compared (e.g., Coleman et al. 2005; Boyd and Shaw 2004). These defensive thresholds can also be compared to literature reports of element concentrations in plants, but only if they are expressed in the same units (mass per unit dry mass) as most studies of plant element concentration. Artificial medium experiments are an advantage over *in planta* experiments because most hyperaccumulator species accumulate only one or, at most, a few elements. Thus an investigator desiring to compare the defensive effects of many elements would need to include multiple hyperaccumulator species, complicating the research and confounding the experimental design. Some plants co-accumulate elements, however, such as *Thlaspi caerulescens* (Assunção et al. 2003). Thus two studies in Table 2 (Jiang et al. 2005; Noret et al. 2005) have results listed under more than one element (Zn and Cd) because of this co-accumulation feature.

Note also that artificial medium studies may be a useful first step in exploring the defensive capability of elements in general. By selecting an appropriate natural enemy, response of that enemy to element levels in an artificial medium can show if element levels documented in plants might be sufficient to produce mortality or sublethal effects *in planta*. A handful of recent studies (Gonçalves et al. 2007; Coleman et al. 2005; Boyd and Shaw 2004; Sagner et al. 1998; Vickerman and Trumble 1999; Trumble et al. 1998) have investigated elemental defense using artificial diet (Step 2) alone. All but one of these studies targeted an herbivore: the exception (Boyd and Shaw 2004) investigated a bacterial pathogen. Some of these studies (Vickerman and Trumble 1999; Trumble et al. 1998) complement separate studies investigating natural enemy responses *in planta* (Vickerman et al. 2002a, b: Table 2), thus together covering both Steps 1 and 2.

Huitson and Macnair (2003) suggested using genetically determined variation in elemental accumulation ability as a way to avoid confounding growth medium and elemental effects. However, even this approach is not without potential confounding factors. Many traits are genetically

linked and thus cosegregate, resulting in dependence between traits that one would rather be independent. Perhaps the most powerful approach would include both an artificial diet approach and an approach based on genetically determined variation of element accumulation ability: to my knowledge that has not yet been attempted.

*Challenges*—There are several challenges to this step. One is the difficulty of obtaining and culturing on artificial media those enemies identified from field surveys as being ecologically relevant. Artificial culture conditions have been developed for relatively few organisms and often development of such systems is a major research challenge in its own right (Cohen 2004). On the other hand, the science of raising organisms on artificial diet has greatly progressed (e.g., insects: Cohen 2004) so that our ability to conduct such experiments has improved.

A challenge to the experimental design of artificial medium experiments is the need to control changes other than metal concentration in the artificial medium due to addition of the test element. As examples, pH may be affected by addition of element salts, the salts themselves may change the solute potential of the medium, and anions must necessarily be added when adding cations and vice versa. These issues can be partially addressed by monitoring a particular feature of the medium and trying to maintain consistency among treatments (e.g., adjusting pH to constant value: Boyd and Shaw 2004) or by testing for effects of another added ion by using additional experimental treatments. As an example of the latter approach, Boyd and Martens (1999) evaluated the effects of adding  $\text{CaCl}_2$  as well as  $\text{NiCl}_2$  to artificial diet fed to aphids. Finding that  $\text{NiCl}_2$ -amended diet was more toxic than  $\text{CaCl}_2$ -amended diet at the same concentration of  $\text{Cl}^-$ , they concluded that it was  $\text{Ni}^{2+}$  and not  $\text{Cl}^-$  that likely was toxic in the  $\text{NiCl}_2$ -amended treatment.

Another challenge is that results of artificial medium studies and *in planta* studies of elemental toxicity may only generally correspond to one another. For example, Vickerman et al. (2002b) found that greater doses of Se were needed for tissues of *Medicago sativa* to produce toxicity to



herbivorous *Spodoptera exigua* larvae than were needed to produce toxicity in artificial diet. Toxicity experiments may be affected by the difference in nutritional quality between plant tissues and an artificial medium, in part because an artificial medium is designed to optimize natural enemy growth (Cohen 2004). Results of *in planta* experiments also can be modified by joint effects: these effects can occur between an element and the organic defense chemicals that often are present in plant tissues (discussed below). Finally, it is likely that, in plant tissues, an element may occur in multiple chemical forms or be bound to multiple ligands (Callahan et al. 2006). Thus, to be most realistic, the form of an elemental defense in an artificial medium should match its form *in planta*. The importance of elemental form has been well illustrated in the case of Se, which can be found in several chemical forms in plants and these can vary significantly in toxicity to natural enemies (Vickerman and Trumble 1999; Trumble et al. 1998). Lack of information regarding the *in planta* condition of hyperaccumulated elements in most hyperaccumulator species (Callahan et al. 2006) precludes considering this feature when designing artificial medium experiments for these plants.

**Step 3: Document fitness difference between high and low element plants attacked by a natural enemy**

**Rationale**—From the plant perspective, the most important feature of a defensive trait is its ability to provide a fitness benefit to an individual possessing it (relative to an individual lacking that trait). The relative nature of fitness means that choice experiments are the preferred method to use for experiments taking this step (Fig. 1). Although the no-choice experiments of Step 1 can be viewed as documenting this feature, the relative nature of a defense is best demonstrated when natural enemies are allowed to choose between plants possessing or lacking the trait. This essentially mimics (in a laboratory setting) how a trait can provide a fitness benefit under natural conditions and thus provides an important piece of evidence for investigations of potential defensive traits.

Criteria for denoting a study in Table 2 as having taken Step 3 (by documenting a fitness benefit) were very broad: I assumed that a decrease in damage done by a natural enemy to high element plants will increase the fitness of those plants. In Table 2, a study that compared amount of damage done to high and low element plant tissues (plants, leaves/roots, or leaf/root pieces) was considered to have attempted this step. In the case of Noret et al. (2007), fecal production was used as a measure of feeding rate and thus was included as having taken Step 3. In fact, in the strict sense of plant fitness as reproductive success, to my knowledge fitness has not been measured in any study of elemental defense. None of the studies included in Table 2 continued to the point at which plants became reproductively mature, with the exception of Jhee et al. (2006a). That study, using the annual Ni hyperaccumulator *Streptanthus polygaloides*, exposed plants to a “pulse” of herbivory and then allowed them to continue to grow until they began to senesce. Unfortunately, *S. polygaloides* does not set seed under greenhouse conditions and so flower production was documented as a surrogate for female fitness (seed production).

**Challenges**—As intimated above, one challenge is that defining and measuring fitness is not easy. For plants, fitness can be subdivided into female (seeds) and male (pollen) fitness: the latter component is especially difficult to quantify. Also note that, in choice experiments, the amount of damage that a researcher allows an enemy to inflict on plants must be considered. The damage inflicted must be measurable but not so great as to overwhelm a potential defense.

An issue underlying Step 3 is the challenge of determining the cost of element hyperaccumulation, or at least allowing that cost to be included in the experimental design. For a trait to result in greater fitness, its costs must be less than its benefits. Traits such as plant defenses are presumed to have costs (metabolic or otherwise), although these are difficult to demonstrate experimentally (Stamp 2003a). To my knowledge, we have very little knowledge of the cost of hyperaccumulation in plants. Fortunately, documenting cost is not necessary as long as both the cost and benefit are expressed during an experiment so that the net

result of the hyperaccumulation trait on fitness can be measured for plants under attack.

Whether a choice experiment documenting natural enemy impacts includes both costs and benefits of hyperaccumulation will depend on the experiment's design. For example, a choice experiment that documents damage levels to high versus low element plants (or plant parts) over a short period of time will not include costs of hyperaccumulation. Instead, a better strategy would be to grow plants under high and low element conditions in the presence of a natural enemy for an extensive period, so that costs associated with hyperaccumulation could be expressed simultaneously with the effects of hyperaccumulation on that natural enemy. This would more realistically reflect evolutionary fitness, but would be a much more challenging experiment to conduct. Some investigators (e.g., Jhee et al. 2006a; Huitson and Macnair 2003) have attempted laboratory experiments that at least partially address this issue.

Growing plants under high or low element conditions also has a potential weakness: unwitting inclusion of other natural enemies in the experimental design. Some investigators (e.g., Callahan et al. 2006; Freeman et al. 2005; Jiang et al. 2005; Boyd et al. 1994) have reported that plants grown under low element culture conditions were fortuitously attacked by natural enemies. If such an attack were not noticed by an investigator (e.g., a fungal pathogen attacking plant roots would be difficult to detect), then data from plants growing on high versus low element media and exposed to natural enemy attack might include: (1) effects of any physiological response of the plants to variation in medium element concentration, (2) differential impacts of the experimental natural enemy on high versus low element plants and (3) differential impacts of the undetected natural enemy.

A final difficulty of these experiments deals with rearing plants. In order to create high and low element plants, most experiments have manipulated the element concentration of the growth medium. As mentioned above, this can create “medium effects” that might confound experimental design. A partial solution to this challenge is that used by Jhee et al. (1999) and Huitson and Macnair (2003): use of genotypes

that vary in metal uptake ability so that all can be cultured on the same medium.

**Step 4: Document fitness difference between high and low element plants grown under field conditions and exposed to natural enemy attack**

*Rationale*—Field experiments are generally viewed as the most convincing (realistic) way to demonstrate the importance of ecological factors. For this step, an experimental design might simultaneously measure effects of element concentration on both natural enemy and plant, and thus document defensive effects from both the natural enemy and plant perspectives, but a plant fitness difference generally would be viewed as the best evidence supporting the defense hypothesis.

*Challenges*—Field experiments can face daunting challenges. Some of the challenges mentioned here are more specific to experiments regarding hyperaccumulation whereas others are general problems that arise when attempting to examine evolutionary forces under natural conditions. Due to these challenges, only three studies have tested the defense hypothesis under field conditions (Step 4 in Table 2): Freeman et al. (2007) for Se, Noret et al. (2006) for Zn and Martens and Boyd (2002) for Ni.

A major challenge for field studies of elemental defense is how to create defended and undefended plants under field conditions. Creation of defended and undefended plants in the field is more complicated than in a laboratory setting because it is difficult to grow plants on low element soil in a field setting of high element soil. Transplanting plants directly into field soil (Freeman et al. 2007; Noret et al. 2006), or placing pots into field soil (Martens and Boyd 2002), may result in plants accessing elements in field soil either directly (through root growth into the field soil) or indirectly (by transfer of elements due to movements of soil water). Monitoring plant element status to confirm that low element plants retain their low element status (Freeman et al. 2007; Noret et al. 2006) is one solution, but another may be to use “socket pots” (Fidler 1999), the technique of placing an experimental pot into another (larger) sealed pot to isolate the experimental pot from the field soil. As with

laboratory experiments, however, a better solution would use genotypes that differ in metal accumulation ability so that both high and low element plants could be grown directly in the same (field) soil.

Broader and more general problems faced by ecological studies in the field include the issues of scale in space and time. Factors that affect individual survival can vary greatly between populations and through time. As a result, an experiment may yield different results depending on when and where it is conducted. Little can be done to solve these challenges: perhaps the only option is to replicate studies in space and across time so that the generality of results may be measured. To date the only field study to be spatially replicated (by using more than one field site) is that of Noret et al. (2006).

#### **New directions: exploring the lower limits of elemental defenses**

Most tests of the defense hypothesis have focused on hyperaccumulator species (Table 2). As the most extreme examples of element accumulation, these species are the best starting point to explore hypotheses about the function of accumulated elements. However, we know little about the quantitative limits of the defensive function of elements. In an initial test, Coleman et al. (2005) used artificial diet amended with metal salts and fed to the bioassay herbivore *Plutella xylostella*. They found toxicity at relatively low concentrations for eight metals: 7.5 mg Cd/kg, 40 mg Co/kg, 110 mg Cr/kg, 200 mg Cu/kg, 1,400 mg Mn/kg, 20 mg Ni/kg, 15 mg Pb/kg, and 280 mg Zn/kg. These concentrations are much less than minimum levels for hyperaccumulation for each metal, well into the range of accumulator plants in most cases, and even into the normal range in the case of Zn (Table 1). If these results are confirmed *in planta*, the concept of elemental defense can be broadened to plants with lesser metal levels than hyperaccumulators: to accumulators and, for some elements, even sub-accumulator plants.

Such results have the potential to extend the concept of elemental defenses geographically (beyond metalliferous soils) because other soils

can harbor plants with unusually high elemental concentrations. As examples, high values of Co (some exceeding 400 mg Co/kg) were reported for the widespread forest tree *Nyssa sylvatica* from the southeastern U.S. (Brooks et al. 1977b; Thomas 1975) and 470 mg Ni/kg was reported in *Thlaspi montanum* var. *montanum* from a non-serpentine California soil (Boyd and Martens 1998b). There may be other cases of unusually high elemental concentrations of plants growing on non-metalliferous soils. These may not receive much attention because their potential ecological significance depends upon recognition of the existence of elemental defenses.

#### **New directions: how did hyperaccumulation evolve? Defensive enhancement and the “joint effects” hypothesis**

The premise that even relatively low levels of elements provide a defensive benefit for plants suggests a mechanism for the evolution of hyperaccumulation, and possibly accumulation, of elements by plants. This mechanism, termed here “defensive enhancement,” occurs if defense provides the selective benefit that favors evolution of still greater element levels in a plant population. In this scenario, individuals that take up a defensively effective level of an element have greater fitness than other plants in the population because they are damaged less by natural enemies. This can lead to spread of the traits that allow element uptake and sequestration in the population. Further increases in element concentration, if they provide still greater fitness, can result in stepwise increases in element levels through evolutionary time (Boyd 2004). This scenario would result from defense being the selective benefit stimulating evolution of hyperaccumulation in plants, making hyperaccumulation an adapted plant defense.

Evolution of hyperaccumulation via defensive enhancement requires that concentrations of elements at lesser levels (accumulator or below) have defensive effects. As mentioned above, defense by concentrations below hyperaccumulator levels has been reported for all elements in Table 2, but to greatly varying degrees. For Ni,

there is only one test using an accumulator level (showing no defensive effect) and only one of the six tests using a sub-accumulator level showed a (weak) defensive effect. On the other hand, all of the nine tests in Table 2 that used accumulator concentrations of Se reported defensive effects. Perhaps the best evidence to date showing that defensive enhancement could occur is the field experiment of Freeman et al. (2007), who found accumulator levels of Se (250 mg/kg or less) resulted in significantly greater plant size and survival for *Stanleya pinnata* plants transplanted into their native habitat and exposed to herbivory by the native fauna. Another promising set of experiments using Zn was conducted by Behmer et al. (2005). In that study, artificial diet experiments showed that Zn concentrations in the range of 500–5,000 mg/kg caused feeding aversion in a grasshopper herbivore. Because Zn accumulation is defined as 2,000–10,000 mg/kg (Table 1), Behmer et al. (2005) concluded that enhancement of defensive effects for plants that accumulate Zn could have contributed to the evolution of Zn hyperaccumulation.

Defensive enhancement could be demonstrated experimentally under controlled conditions. Ideally, a population of a plant species that has both genetic variation in element uptake ability and a short generation time could be exposed to a natural enemy. The seeds produced by the population could be collected and re-sowed, perhaps over several generations, all under continued exposure to the natural enemy. At the end of the experiment, an increase in the successive generations' mean element concentrations would demonstrate defensive enhancement. Although it would be tempting to repeat this experiment under field conditions, it may be impossible to recreate the ecological conditions present at the time elevated elemental concentrations may have been selected for by this mechanism. This is because element-tolerant herbivores may now be present. These likely evolved after plants evolved high elemental concentrations: their current presence would preclude re-creating the original ecological conditions under which elevated element concentrations evolved.

Defensive enhancement that leads to element accumulation/hyperaccumulation could also

occur through “joint effects” between plant defenses. Plants often possess multiple defenses against herbivores and these may act differently in concert (joint effects) than each alone. Two positive joint effects between defenses, additivity and synergy, can make them more effective together than each alone (Nelson and Kursar 1999). It is also possible that compounds might interact negatively (resulting in antagonism) so that they are less effective together than each is alone. Joint effects (especially synergy) between plant chemical defenses are receiving increased attention (e.g., Dyer et al. 2003) because they magnify the benefits of each defense. Investigations of synergy between organic defense compounds demonstrate the potential of such studies to explain the evolution of some plant chemical defenses (e.g., Dyer et al. 2003), but such studies are rare in the plant–herbivore interaction literature.

Joint effects may exist between elemental and organic plant defenses. The “joint effects hypothesis” suggests that plant element concentration may act in concert with organic plant defenses to enhance plant defense overall. Boyd (2004) originally speculated that elemental defenses may have joint effects in combination with organic chemical defenses, but this topic is unexplored outside of an initial test by Jhee et al. (2006b). Jhee et al. (2006b) showed additive effects between Ni and several organic defense chemicals (two alkaloids and tannic acid) but their experimental system was unsuited to investigate synergistic effects. As of now, the types of joint effects that may occur between elemental and organic plant defenses are virtually unexplored. Additivity and (especially) synergy are also potentially important to our understanding of how widespread elemental defenses are in plants. These phenomena allow elemental defenses to contribute to plant fitness at concentrations less than expected based on the defensive effects of an element alone. Thus, joint effects may extend the defensive effects of elements to more plant species than otherwise expected and thus broaden the general applicability of elemental defenses to plants.

The concept of joint effects also may help explain the evolution of elemental defenses.

Specifically, organic defenses may have played a role in the evolution of metal accumulation by increasing the defensive effect of elements (through either additivity or synergy). As noted above, defensive enhancement suggests that, once plant tissue element levels are great enough to provide a defensive benefit, natural selection would favor still greater element uptake and storage if the increased element levels resulted in a more effective defense. Joint effects may have contributed to the evolution of element accumulation by lowering the element concentration at which a defensive benefit first accrues to a plant. Thus, a relatively low level of element uptake could be defensively beneficial.

The joint effects hypothesis is a new hypothesis regarding the simultaneous presence of both elemental and organic defenses in plants. An earlier hypothesis, the “tradeoff hypothesis,” suggested that the evolution of elemental hyperaccumulation may have been followed by a reduction of organic defense compounds (Martens and Boyd 1994). The tradeoff hypothesis suggests that evolution of an elemental defense may make an organic defense redundant. Plants that evolve lower levels of organic defenses may still be well protected against natural enemies and so could reduce defense costs. This tradeoff between chemical defenses is more likely to occur if the organic defense is costly and the elemental defense relatively inexpensive. This hypothesis has been tested at two taxonomic scales. Davis and Boyd (2000) compared the level of organic defenses (glucosinolates) between two species of *Streptanthus*, finding that the Ni hyperaccumulator *S. polygaloides* contained a lower level of glucosinolates than the nonhyperaccumulator *S. insignis* subsp. *insignis*. Other studies have examined the tradeoff hypothesis for plants of a single species raised on high or low element soil and thus hyperaccumulating or not. Tolrà et al. (2001) found that *Thlaspi caerulescens* allowed to hyperaccumulate Zn had reduced levels of glucosinolates. On the other hand, Jhee et al. (2006a) found total glucosinolates did not differ between Ni hyperaccumulating and non-hyperaccumulating *Streptanthus polygaloides*, although they did find some differences in specific glucosinolates.

Another example of a tradeoff between elemental and organic defenses is suggested by recent research by Freeman et al. (2005). They showed that Ni hyperaccumulators in the genus *Thlaspi* cannot defend themselves from pathogens using organic molecules because Ni tolerance requires constitutively elevated levels of salicylic acid, an important signal molecule for induced pathogen defense in plants. Freeman et al. (2005) reported that *Thlaspi* plants grown under low Ni conditions were highly susceptible to pathogen attack but not when they were able to hyperaccumulate Ni. They suggest that pathogen defense provided by organic compounds has been replaced by an elemental defense (Ni) in these hyperaccumulators.

The concept of joint effects may be relevant to another feature of some accumulating and hyperaccumulating plants. Some plants accumulate or hyperaccumulate more than one element simultaneously. This phenomenon, co-accumulation, raises the possibility of joint effects between elements, similar to the joint effects discussed above between an element and an organic chemical. Reeves and Baker (2000) list Co and Cu, Zn and Pb, and Zn and Ni as pairs of metals that are sometimes reported as co-accumulated. To my knowledge, only Jhee et al. (2006b) have explored joint effects among elemental defenses. They found additive effects for all three pairs of metals that they investigated: Zn and Ni, Zn and Pb and Zn and Co. Much more investigation is needed to determine how extensive joint effects might be between elements. Furthermore, accumulation of more than two elements may occur in some plants and thus the defensive significance of joint effects may be much more complex than shown by simple pairwise experiments. Finally, it should be kept in mind that co-accumulated elements may have separate defensive functions. For example, Cu may be accumulated for its effectiveness against one enemy (e.g., a pathogen: Boyd and Shaw 2004) and Co for its effectiveness against an herbivore (e.g., a leaf chewing insect: Coleman et al. 2005). Clearly, cases of multiple accumulated elements will require relatively complex experimental designs to test their effectiveness against the multiple natural enemies typically faced by plants.



## Summary: the outlook for plant elemental defense studies

The concept of elemental defense is still relatively new and in need of much experimental investigation. Of the ten elements summarized in Table 1, studies of the defense hypothesis (Table 2) have included only half (As, Cd, Ni, Se and Zn). More elements need to be examined for defensive effects. Research to date has shown cases of defensive effects for all elements tested but results are generally mixed. Continued research is needed to illustrate the benefits and limitations of elemental defenses against the wide variety of natural enemies faced by plants. The available diversity of taxa and growth forms of hyperaccumulators (Whiting et al. 2004) has yet to be tapped for elemental defense studies: most studies to date have focused on a few non-woody taxa from a few plant families (Table 2).

There also is a tremendous need for basic natural history studies in habitats hosting hyperaccumulators (often serpentine habitats) to identify natural enemies, including specialist enemies and the “potential enemies” (referred to above) that may be particularly susceptible to elemental defenses. Experimental tests of herbivore defense should use herbivores with different feeding modes so we can gain a realistic assessment of the limitations of elemental defenses. More tests using other types of natural enemies (pathogens, parasites) also are needed. Finally, investigations need to move from laboratory to field settings so we can understand how elemental defenses behave in the complex matrix of native food webs. New hypotheses regarding the minimum concentrations of elements that may have defensive effects, and regarding possible joint effects of elemental and organic chemical defenses, have the potential to greatly expand the applicability of elemental defenses to plants in nature. They also may provide an evolutionary explanation of how and why elevated element concentrations evolved in plants.

Although elemental defenses were first suggested more than a decade ago (Martens and Boyd 1994), they are only beginning to gain the attention of ecologists other than those studying hyperaccumulators (e.g., Strauss and Zangerl

2002). But the elemental defense concept has relevance to other pure and applied uses of hyperaccumulator plants. Metallomics, a new field that studies biochemistry as assisted by metal ions in cells (Szpunar 2004), can benefit from understanding the ecological functions of metals in whole plant biology (Poschenrieder et al. 2006). Hyperaccumulators are also being investigated for their use as tools (phytoextractors) to clean up metal-contaminated sites (phytoremediation: Pilon-Smits 2005) or to remove metals from natural soils as a mining technology (phytomining: Brooks and Robinson 1998). Before these uses are widely implemented, their ecological impacts on local communities and ecosystems should be explored (Angle and Linacre 2005; Vickerman and Trumble 2003; Boyd 1998). The elemental defense hypothesis illuminates one way by which hyperaccumulator plants can uniquely impact their biotic environments.

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