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EXTENDING THE ELEMENTAL DEFENSE HYPOTHESIS: DIETARY METAL CONCENTRATIONS BELOW HYPERACCUMULATOR LEVELS COULD HARM HERBIVORES

CHRISTINA M. COLEMAN,[1] ROBERT S. BOYD,[1,*] and MICKY D. EUBANKS[2]

[1]*Department of Biological Sciences, Auburn University, AL 36849, USA*
[2]*Department of Entomology and Plant Pathology, Auburn University, AL 36849, USA*

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Abstract—Previous work has shown that hyperaccumulator levels of some metals can defend plants against herbivores, but the possibility of defense by metal concentrations at accumulator or normal levels is unexplored. This study tested the hypothesis that metals can defend plants at low concentrations. We determined the relative toxicities of eight metals commonly acquired by plants: Cd, Co, Cr, Cu, Mn, Ni, Pb, and Zn. Larvae of the diamondback moth (*Plutella xylostella*), a representative crucifer specialist, were fed with artificial diet amended with concentrations of metal varying from 2 to 3,000 μg/g. Different concentration ranges were used for each of the eight metals, and larval survival at 10–14 days was calculated for each concentration. All metals were toxic to diamondback moth larvae at hyperaccumulator levels. All metals, however, were also toxic to larvae at accumulator concentrations, far below those found in hyperaccumulating plants. Five metals (Cd, Mn, Ni, Pb, and Zn) were toxic below accumulator levels, Cd and Pb were toxic near the concentration ranges of normal plants, and Zn was toxic at a concentration within the normal range. Our results indicate that uptake of certain metals may provide a defensive benefit for plants, and that elemental defenses may be effective at concentrations far lower than previously hypothesized. This study implies that elemental defenses are more widespread in plants than previously believed, and that the ecological consequences of even low levels of metal accumulation need to be explored.

Key Words—Accumulator, diamondback moth, elemental defense, hyperaccumulator, metal, metal toxicity, *Plutella xylostella.*

* To whom correspondence should be addressed. E-mail: boydrob@auburn.edu

INTRODUCTION

The concentrations of metals in the aboveground tissues of plants can be used to divide them into three somewhat arbitrary categories: normal plants, accumulators, and hyperaccumulators. These categories are defined in a relative fashion for each metal (Reeves and Baker, 2000), with normal plants having relatively low metal concentrations and accumulator and hyperaccumulator plants having increasingly extreme metal concentrations. According to Reeves and Baker (2000), normal plants typically contain low concentrations (generally <5 μg/g on a dry mass basis in aboveground parts) of several metals, including Cd (0.1–3 μg/g), Co (0.03–2 μg/g), Cr (0.2–5 μg/g), and Pb (0.1–5 μg/g). Normal concentrations of Ni and Cu can be somewhat greater: 5–25 μg Cu/g and 1–10 μg Ni/g. Still greater concentrations of Mn or Zn are typical, with normal concentrations of both metals ranging from 20 to 400 μg/g. Accumulator plants typically grow on mineralized soils and contain greater concentrations of metals (Baker, 1981; Macnair, 2003). Minimum accumulator concentrations, signifying unusually elevated metal concentrations, are 20 μg/g for Cd and Co, 50 μg for Cr/g, 100 μg/g for Ni, Cu, or Pb, and 2000 μg/g for Mn and Zn (Reeves and Baker, 2000).

Hyperaccumulators have exceptionally high concentrations of metals in their tissues (Brooks et al., 1977). These plants are almost exclusively found on mineralized or ultramafic soils (Brooks, 1987, 1998; Reeves and Baker, 2000; Boyd et al., 2004) that, in part because of their high metal concentrations, are unfavorable environments for most plants (Sieghardt, 1990; Reeves and Baker, 2000; Prasad and Strzalka, 2002). However, a number of species (about 400) actively take up and concentrate (hyperaccumulate) one or more of these metals (Baker et al., 2000; Reeves and Baker, 2000). Minimum tissue concentrations used to define hyperaccumulators of the above metals are as follows: 100 μg/g for Cd; 1000 μg/g for Co, Cu, Cr, Ni, and Pb; and 10,000 μg/g for Mn and Zn (Brooks, 1987; Pollard and Baker, 1997).

We know of no comprehensive study that has compiled the numbers of species in hyperaccumulator and accumulator categories for each metal. Pollard et al. (2002) showed that Ni levels among *Alyssum* species form a bimodal distribution with relatively few accumulator species, but noted also that the data were incomplete. Most surveys of plant metal concentrations are designed to discover hyperaccumulator species and, therefore, target taxa and geographic areas likely to yield them (e.g., Reeves et al., 1999). Researchers also may not proceed with detailed analyses of species that do not seem promising as hyperaccumulator candidates in initial sample screenings (e.g., Reeves et al., 1999). Thus, it seems likely that the number of accumulator species in the literature is underreported. For example, a recent survey of Cuban ultramafic species by Reeves et al. (1999) examined specimens from 277 species and documented 50 taxa of Ni hyperaccumulators and 25 of Ni accumulators.

The toxicity of hyperaccumulators to herbivores has been addressed by an increasing (but still relatively small) number of experiments. Much of this research has focused on determining the advantages a plant may receive from hyperaccumulation of an element (Boyd, 2004). Martens and Boyd (1994) suggested that hyperaccumulation may serve as an "elemental defense" against herbivory. Experiments with Ni (Boyd and Martens, 1994; Martens and Boyd, 1994; Boyd and Moar, 1999; Davis and Boyd, 2000; Boyd et al., 2002), Se (Hanson et al., 2003, 2004), Cu (Ernst, 1987), and, in some cases, Zn (Pollard and Baker, 1997; Jhee et al., 1999; Behmer et al., 2005) have supported this defense hypothesis. Other experiments have shown that not all herbivores are negatively affected by hyperaccumulator metal concentrations (e.g., Boyd and Martens, 1999), especially for Zn (Huitson and Macnair, 2003; Noret et al., 2005).

The above experiments deal with only a few of the elements hyperaccumulated by plants, targeting Ni, Zn, Cu, or Se hyperaccumulators. While Reeves and Baker (2000) reported that Ni is the most commonly hyperaccumulated metal, with 318 taxa (78% of known hyperaccumulators), a number of plants hyperaccumulate other metals: to date, there are 26 Co hyperaccumulators, 35 Cu hyperaccumulators, 14 Pb hyperaccumulators, and 16 Zn hyperaccumulators (Brooks et al., 1998; Reeves and Baker, 2000). There are also at least 11 species of Mn hyperaccumulators and at least one Cd hyperaccumulator (Brooks et al., 1998; Reeves and Baker, 2000). The protective function of these elements in plants has yet to be explored.

Although herbivore feeding experiments can demonstrate the toxicity of metals at hyperaccumulator concentrations, they do not show the minimum plant tissue metal concentration that can produce a negative or toxic effect (Boyd and Martens, 1998). Our experiment was, therefore, designed to investigate the toxic thresholds of Ni and Zn, as well as six other hyperaccumulated metals (Cd, Co, Cr, Cu, Mn, and Pb). Our goals were twofold: (1) to determine whether these metals are toxic to herbivores at hyperaccumulator levels, thus extending the defense hypothesis to other hyperaccumulated metals; and (2) to determine whether any of these metals could serve as elemental defenses at concentrations within the ranges of accumulator or normal plants, thus extending the defense hypothesis to new categories of plant species.

METHODS AND MATERIALS

Experiment Rationale. Our experiment used a representative herbivore raised on artificial diet amended with a range of metal concentrations. We used larvae of the diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), a folivore that attacks plants of the Brassicaceae (Talekar and Shelton, 1993), a family that contains about 25% of all hyperaccumulator taxa

(Reeves and Baker, 2000). Lepidopteran larvae have been shown to be important herbivores of wild-growing species of *Streptanthus* (Brassicaceae) plants and can easily be responsible for the death of a plant. For example, Shapiro (1981a) reported that *Pieris sisymbrii* (Lepidoptera: Pieridae) is a specialist herbivore of several *Streptanthus* species, including *S. glandulosus* and *S. breweri*, both of which are found on ultramafic soils in California. He also noted that some populations of *S. polygaloides* may be attacked by *P. sisymbrii* (Shapiro, 1981b), and that a single *P. sisymbrii* oviposition on a plant of *S. tortuosus* could be effectively lethal if the resulting larva destroys the plant's leaves and fruits (Shapiro, 1981c).

Advantages of using DBM also include its short generation time, small size, ease of culture on artificial diet, and the commercial availability of a standardized artificial diet. Its short generation time (about 25 d from egg to adult for our colony) allowed us to rapidly assess survival at the completion of the larval stage (about 11–12 d). Use of artificial diet provided us with direct control over the concentrations of metal that the insects consumed. Experiments that have varied soil metal concentrations to obtain different plant metal concentrations have reported difficulties obtaining consistency within a treatment (Boyd and Moar, 1999), have had problems generating subhyperaccumulator concentrations of a metal within a plant species (Boyd and Moar, 1999), or have found it difficult to use a single plant species to accumulate the range of metals that we desired to study (e.g., Boyd and Davis, 2001).

Experimental Colony. The DBM colony used was established using eggs obtained from a colony at Cornell University (Harvey, 2002). Founder moths from the Cornell colony were collected from a field in Geneva, NY, USA. After the colony was established in Auburn, it was supplemented with wild individuals collected from Auburn, AL, USA. Artificial diet for the colony was obtained from Bio-Serv® (Frenchtown, NJ, USA). The diet's exact ingredients are proprietary information, but wheat germ and cabbage leaf powder are two of the main ingredients (Carpenter and Bloem, 2002). The diet was used to maintain the colony following a procedure similar to that of Shelton and Collins (2000).

Our colony maintenance protocol was as follows. Sheets of DBM eggs obtained from the established colony were sterilized in 10% bleach solution for 20 sec, rinsed in water for 1 min, and allowed to dry. Dried egg sheets were cut into strips, containing about 300–400 eggs per strip, and each strip was placed in a 250-ml paperboard cup with about 1 cm of congealed artificial diet covering its bottom. The cups were placed in an incubator (37°C, ~30–50% relative humidity) until they hatched and the first instar larvae began to feed (~60 hr). Empty egg sheets were then removed, and larvae were allowed to feed until pupation (~11–12 d after hatching). Pupae were then placed in screen cages where adults could emerge to mate and lay eggs (~4 d from pupation to

emergence). Eggs were collected on scored aluminum foil sheets that had been dipped in sterilized collard juice (an oviposition stimulant) (Shelton and Collins, 2000). Peak oviposition occurred between 2 and 6 d after adult emergence. Eggs collected from this time period had the greatest vitality; eggs collected after this time period produced larvae with 25–50% lower survival (from egg to adult) than average for the colony. Consistent with the Cornell colony, mean survival of larvae from egg to pupa was at least 50% (Shelton and Collins, 2000).

Artificial Diets Containing Metal. Artificial diet was amended with varying concentrations of the eight metals studied by using stock solutions of metal chloride salts (obtained from Sigma-Aldrich, St. Louis, MO, USA). These amended diets were used to determine metal toxicity by feeding different sets of DBM larvae different concentrations of a given metal. Experimental concentrations selected were based on preliminary results, and we attempted to include at least one nontoxic dose of each metal chloride salt. Five to seven concentrations were tested for each of the eight metals. For each concentration, 100 ml of diet were made to produce the concentrations listed in Table 1. Diet was distributed into 30-ml plastic cups to give 14 replicates of each

TABLE 1. METAL CONCENTRATIONS IN ARTIFICIAL DIET[a]

Metal	Units	Concentration						
Cd	mM $CdCl_2$	0	0.018	0.044	0.070	0.088	0.132	
	μg Cd/g	0	7.50	19.0	31.5	38.5	56.5	
Co	mM $CoCl_2$	0	0.022	0.066	0.088	0.176	0.264	
	μg Co/g	0	2.8	9.0	12.3	25.0	40.0	
Cr	mM $CrCl_2$	0	0.22	0.44	0.88	1.76	3.52	
	μg Cr/g	0	24.0	53.0	106	210	418	
Cu	mM $CuCl_2$	0	0.88	1.32	1.76	2.64	3.52	4.4
	μg Cu/g	7[b]	130	195	280	400	500	660
Mn	mM $MnCl_2$	0	4.4	8.8	17.6	26.4	35.2	
	μg Mn/g	15[b]	350	680	1370	2000	2750	
Ni	mM $NiCl_2$	0	0.22	0.44	0.88	1.76	3.08	
	μg Ni/g	0	20.0	35.0	70.0	136	245	
Pb	mM $PbCl_2$	0	0.044	0.088	0.220	0.308	0.440	
	μg Pb/g	2[b]	15.0	30.0	100	165	250	
Zn	mM $ZnCl_2$	0	0.22	0.44	0.55	1.10		
	μg Zn/g	30[b]	140	275	350	700		
Ca/Cl	mM $CaCl_2$	0	1	20	30	40		
	μg Ca/g	5900[b]	6900	7840	8540	9500		
	μg Cl/g	0	1660	3330	4570	6250		

[a] mM data, as millimoles of metal chloride per liter, calculated from dilutions of stock solutions; μg/g data from elemental analysis of diet samples. Fourteen replicates were used for each concentration of each metal.

[b] Amounts of these elements were present in the original diet mix (Mn, Zn, Cu, and Ca) or result from contamination during sample preparation for ICAP analysis (Pb).

concentration, with each cup receiving about 2–3 ml. A 30-ml diet sample of each metal concentration was reserved for elemental analysis.

Use of chloride salts introduced the potential for chloride toxicity to affect the results. Thus, we created an experiment using diet amended with $CaCl_2$ to serve as a partial control. For this test, a range of $CaCl_2$ concentrations was used that included the highest molar concentration of chloride from all metals tested (Table 1). If the $CaCl_2$ amended diet did not cause significant larval mortality, whereas diets amended with other metals did, then we could conclude that chloride toxicity did not significantly influence our results.

Elemental Analysis. Elemental analysis of dried diet samples was used to determine metal concentrations expressed as parts per million (μg/g) of metal in each batch of diet. Diet was analyzed using inductively coupled argon plasma spectroscopy (ICAP) (Jarrell-Ash ICAP 9000: Genesis Laboratory Systems, Grand Junction, CO, USA) for Cd, Co, Cr, Cu, Mn, and Pb and atomic absorption spectroscopy (AA) (Instrumental Laboratory, IL 215) for Ni and Zn. For both processes, the 30-ml preserved sample of diet were dried at ~60°C for 5 d and ground to a fine powder. The AA samples were wet-ashed with nitric and perchloric acids, dissolved in HCl, diluted with deionized water, and analyzed. The ICAP samples were dry-ashed in a muffle furnace, oxidized with boiling nitric acid, dissolved in HCl, diluted with deionized water, and analyzed.

Metal Toxicity Tests. Each set of concentrations for a given metal was tested for toxicity to DBM larvae using eggs collected from a single cage containing adults that were 2–4 d posteclosion. Eggs were collected at the completion of a 24-hr period and sterilized as described above. After egg sheets dried, they were cut into strips that were divided among the 70–100 cups (of five to seven metal concentrations) that composed a single metal toxicity test. Between 60 and 100 eggs were placed in each cup of diet, yielding 30–50 first instars per cup.

Cups were placed into an incubator with the same temperature and humidity conditions used for the colony. Egg sheets were removed from cups after larvae hatched and had begun to feed (60–72 hr after the initial egg sheet was collected and sterilized). When egg sheets were removed, an initial count was made of first instars present in each cup. The number of larvae alive was then counted at 2- or 3-d intervals. When larvae began to pupate (generally 10–12 d after eggs were collected), the number of pupae plus larvae alive at that time was used to represent survival in each cup, and survival was expressed as the percentage of initial first instar larvae. Counting for all cups within a metal set was terminated when adults started to emerge in the control cups, usually 14–17 d after the eggs were collected.

Statistical Analysis. Survival data for each metal were analyzed to determine each metal's minimum toxic concentration. Survival values were arcsine square root transformed to satisfy the normality assumption (Zar, 1996) underlying one-way analysis of variance (ANOVA), and ANOVA (StatView:

Abacus Concepts, 1998) was used to determine if metal concentration significantly affected survival at $\alpha \leq 0.05$. For each metal, Fisher's protected least significant difference (PLSD) test was used to determine which concentrations significantly decreased survival relative to the control treatment at $\alpha \leq 0.05$ (Abacus Concepts, 1998).

RESULTS

Toxic effects of metal treatments on DBM larvae were due to metals and not to chloride toxicity. The test for chloride toxicity showed that concentrations of

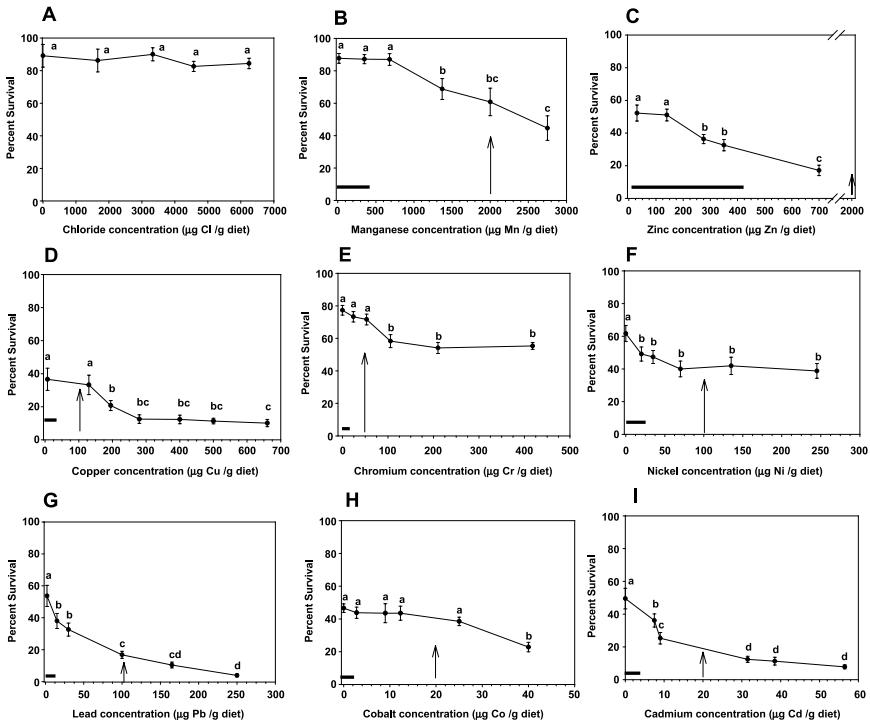

FIG. 1. Mean survival of DBM on $CaCl_2$ (A), $MnCl_2$ (B), $ZnCl_2$ (C), $CuCl_2$ (D), $CrCl_2$ (E), $NiCl_2$ (F), $PbCl_2$ (G), $CoCl_2$ (H), and $CdCl_2$ (I) amended diets. Bars represent the standard error of each mean. Means with the same letter are not significantly different (Fisher's PLSD test at $\alpha \leq 0.05$). A horizontal line denotes the normal concentration range for each metal in plant tissues (dry mass basis), and a vertical arrow shows the minimum concentration for accumulator plant status (Reeves and Baker, 2000).

TABLE 2. ANOVA RESULTS OF LARVAL MORTALITY DATA FROM THE EXPERIMENT FOR EACH METAL

Metal	*F*	*df*	*P* value
Cd	21	5, 78	<0.001
Co	5	6, 90	<0.001
Cr	10	5, 78	<0.001
Cu	7.7	6, 75	<0.001
Mn	4.7	5, 84	<0.001
Ni	3.4	5, 71	0.008
Pb	27	5, 70	<0.001
Zn	14	4, 76	<0.001

$CaCl_2$, which at their greatest exceeded the highest concentration of chloride of any other metal used (Mn: Table 1), produced no significant toxic effects (ANOVA: $F_{8,102} = 0.794$, $P = 0.609$) (Figure 1a). The mean survival for all experimental controls (no metal added to diet) was about 60%, a value above the minimum average survival of insects within the colony. These two control systems (chloride test and no-metal diet) showed that the main variable affecting larval survival was the addition of metals.

All metals affected larval survival at some concentration (Table 2). A concentration of 1,370 μg Mn/g produced the first significant decrease in survival for that metal (Figure 1b). For Zn, survival decreased at 275 μg Zn/g (Figure 1c). The lowest dose of Cu that produced significant toxicity was 195 μg Cu/g (Figure 1d), with that for Cr at 106 μg Cr/g (Figure 1e).

TABLE 3. NORMAL RANGE, MINIMUM ACCUMULATOR LEVEL, MINIMUM HYPERACCUMULATOR LEVEL, AND LEVEL TOXIC TO DBM LARVAE FOR THE METALS USED IN THIS EXPERIMENT[a]

Metal	Normal range	Minimum accumulator level	Minimum hyperaccumulator level	Minimum toxic level to DBM
Cd	0.1–3	20	100	7.5 (0.018 mM)
Co	0.03–2	20	1,000	40 (0.264 mM)
Cr	0.2–5	50	1,000	106 (0.88 mM)
Cu	5–25	100	1,000	195 (1.3 mM)
Mn	20–400	2,000	10,000	1,370 (17.6 mM)
Ni	1–10	100	1,000	20 (0.22 mM)
Pb	0.1–5	100	1,000	15 (0.044 mM)
Zn	20–400	2,000	10,000	275 (0.44 mM)

[a] Normal range, minimum accumulator level, and minimum hyperaccumulator level refer to tissue concentrations in field-collected plants and follow Reeves and Baker (2000). All values are expressed in μg/g (dry mass basis) with toxic levels also expressed parenthetically in mM wet diet so that metal toxicities can be compared.

We did not determine the toxic thresholds for Ni and Pb, as the lowest dose tested for each, 20 μg Ni/g and 15 μg Pb/g, showed significantly toxic effects (Figure 1f and g). For Co, 40 μg/g significantly decreased DBM survival (Figure 1h). The lowest dose of Cd tested, 7.5 μg/g, significantly decreased DBM survival (Figure 1i). Minimum toxic doses for each metal are summarized in Table 3, along with the normal range, minimum accumulator threshold, and minimum hyperaccumulator threshold for these metals.

DISCUSSION

Minimum toxic concentrations (in wet diet) of metals to DBM larvae varied greatly. Most toxic were Cd and Pb (<0.05 mM; Table 3) followed by Ni and Co at <0.3 mM. Zinc and Cr were toxic at <1 mM, with Cu toxic at 1.3 mM and Mn as least toxic at 17.6 mM (Table 3). The ranking of these metals in terms of their toxicity to DBM was not surprising, as essential metals such as Mn and Cu are generally less toxic than nonessential metals such as Cd and Pb (Yasutake and Hirayama, 2002). We note that we did not differentiate between toxic (where metal in diet caused a toxic effect on larvae) and antifeedant (where metal in diet caused larvae to stop feeding and starve) effects of dietary treatments and, thus, cannot address the mechanisms whereby these metals affected DBM larvae. Studies that address these questions (e.g., Behmer et al., 2005) are valuable extensions of the work reported here. However, regardless of the mechanism(s) involved, our experiments still allow us to make conclusions about the effectiveness of these metals as elemental defenses.

We conclude that hyperaccumulator concentrations of all metals tested are toxic to DBM. Table 3 shows that toxic levels were far below hyperaccumulator thresholds, in every case <20% of the minimum value used to define hyperaccumulation. Thus, for the representative crucifer herbivore used in our study (DBM), hyperaccumulation of any of the metals we examined would cause plant tissues to be toxic. Because these results show that metal hyperaccumulation could protect plants via toxic effects on a folivore, they support the defense hypothesis of metal hyperaccumulation. These results also support other studies that have shown toxic effects of hyperaccumulator species of Ni (e.g., Boyd and Martens, 1994; Martens and Boyd, 1994; Boyd and Moar, 1999; Boyd et al., 2002) and Zn (Pollard and Baker, 1997; Jhee et al., 1999; Behmer et al., 2005) to most folivores tested. Furthermore, our results extend the defense hypothesis to hyperaccumulated metals that have not yet been experimentally tested *in planta* (Cd, Co, Cr, Cu, Mn, and Pb) and suggest that, at least for DBM, metal hyperaccumulation would be a potent plant chemical defense. We should note that metal toxicity may depend on other constituents of

the diet, and further experimentation *in planta* is needed to confirm our results. In regard to this, recent research on the defensive effects of Zn hyperaccumulation (Behmer et al., 2005), using both Zn-amended artificial diet and plants containing varying levels of Zn, has shown that defensive effects occurred at similar Zn concentrations in both experimental situations.

All of the metals we studied were also toxic to DBM at concentrations in the accumulator range. Toxic levels extended into the accumulator range for Co, Cr, and Cu and below that range for Cd, Mn, Ni, Pb, and Zn (Table 3). Thus, for these metals, accumulator concentrations in plant tissues could also constitute elemental defenses. Five metals (Cd, Mn, Ni, Pb, and Zn) could have defensive effects at concentrations below accumulator levels. One of these metals (Zn) was toxic at a concentration within the normal range of plant tissues (Table 3). The other four were toxic at concentrations between the upper limit of the normal range and the start of accumulator levels. We, therefore, conclude that, in the case of Cd, Zn, Ni, and Pb, concentrations below the accumulator level may be toxic to some folivores and act as elemental defenses.

While the matrix within which a herbivore ingests a metal may serve to decrease that metal's toxicity, the reverse is also possible. Metals in plants may act in combination with other defense compounds in ways that may contribute to their effectiveness at low concentrations. This would result in the possibility that even lower concentrations of metals than shown by our study may contribute to plant defense. Our experiments determined the toxicity of single metals added to artificial diet. Under natural conditions, a single metal is only one chemical component of a particular plant's tissues. Some plants hyperaccumulate or accumulate more than one metal (Reeves and Baker, 2000). Furthermore, plants usually contain one or more of a wide variety of organic (or secondary) chemicals that also may be toxic to some herbivores. For example, members of the Brassicaceae, a family which contains many Ni hyperaccumulator species (Reeves and Baker, 2000), commonly produce glucosinolates that have defensive effects against some herbivores (Bodnaryk, 1992). Combinations of chemicals may act together, either additively or synergistically (Dyer et al., 2003), to be more toxic to a herbivore than each is alone. If this is the case with elemental defenses, as suggested by Boyd (2004), then concentrations less than those used in our single-metal studies could contribute to defensive effects against herbivores when combined with other plant chemicals. By this reasoning, the minimum toxic concentrations we detected may not be the least quantity of each metal that can have defensive value to a plant. Combination effects may decrease the effective defensive threshold of metals to levels even less than those shown in our study. A recent study by Jhee (2004) has shown just such combination effects for Zn paired with Cd, Ni, and Pb, and for Ni paired with some organic defense chemicals (tannic acid and

alkaloids). These considerations imply that elemental defenses are more important than heretofore recognized.

A defensive effect of metals at levels below hyperaccumulator concentrations is also notable because it suggests a mechanism whereby hyperaccumulation may have evolved. Boyd (2004) proposed that natural selection, driven by a defensive effect of metals against herbivores and/or pathogens, could have caused a stepwise magnification of metal levels in plants culminating in hyperaccumulation. Our study supports this hypothesis by showing that accumulation can itself have selective value against herbivory, leading to the survival of more toxic variants. Recent genetic studies of hyperaccumulation, summarized by Pollard et al. (2002), have demonstrated the existence of quantitative genetic variation in hyperaccumulation ability. Action of natural selection on similar variation in levels of accumulation could have resulted in the evolution of hyperaccumulation.

In summary, we suggest that elemental defenses may be more widespread among plants than previously suspected and may be more ecologically important than previously thought (Boyd, 2004). Our results show that plant species that accumulate or hyperaccumulate the metals that we studied will be toxic to DBM. These results also suggest that elemental defenses may be more widespread geographically than previously supposed. Most hyperaccumulator and many accumulator plant species are found on mineralized soils (Reeves and Baker, 2000), but the low concentration at which some metals (e.g., Zn; Figure 1c and Table 3) can be an effective defense suggests that some species growing on less mineralized soils may also be defended by metals. Our study extends the elemental defense hypothesis to plants other than hyperaccumulators, and suggests that hundreds or even thousands of additional plant species may be protected by elemental defenses.

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