

## ORIGINAL PAPER

Scott N. Martens · Robert S. Boyd

## The ecological significance of nickel hyperaccumulation: a plant chemical defense

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**Abstract** Nickel hyperaccumulating plants have more than 1000 mg Ni kg<sup>-1</sup> dry weight when grown on nickel-bearing soils. We hypothesized that Ni hyperaccumulation could serve as a chemical defense against herbivores. In feeding experiments with potential insect herbivores and Ni hyperaccumulating plants, only those insects fed leaves from plants grown on non-nickel-bearing soil survived or showed a weight gain. Among chemical parameters measured, only Ni content of plants was sufficient to explain this result. When subjected to herbivory by lepidopteran larvae, plants grown on Ni-amended soil showed greater survival and yield than plants on unamended soil. Ni hyperaccumulation may be an effective plant chemical defense against herbivores because of its high lethality, apparent low cost, and broad spectrum of toxicity.

**Key words** Serpentine · *Streptanthus* · Herbivory  
Heavy metals

### Introduction

Unusually high nickel accumulation (>1%) in plant tissue was first described in 1948 (Minguzzi and Vergnano 1948). Brooks and others (see Brooks 1987), seeking botanical indicators of Ni deposits world-wide, have since analyzed more than 20000 specimens. More than 170 plant taxa have been found that have more than 1000 mg

Ni kg<sup>-1</sup> dry tissue (Brooks 1987; Brooks et al. 1990). The term “hyperaccumulator” is applied to these plants to distinguish them from others which have elevated Ni levels (but <1000 mg kg<sup>-1</sup>) when grown on nickel-containing soils (Jaffre et al. 1976; Brooks et al. 1977).

Ni hyperaccumulators are taxonomically diverse and occur in at least 22 families over six superorders of higher plants, although nearly half are in the family Brassicaceae (Brooks 1987). Ecologically, though found on six continents, Ni hyperaccumulators consistently occur on soils derived from serpentine or other nickel-bearing ultramafic parent materials. Most Ni hyperaccumulating species are endemic to serpentine soils but some also occur on non-nickel-bearing soils. However, Ni hyperaccumulation is unusual among species that occur on serpentine soils. In California, only 3 of about 215 taxa endemic to serpentine soils hyperaccumulate Ni (Reeves et al. 1983; Kruckeberg 1984).

The ecological role of Ni hyperaccumulation has not been experimentally investigated (Boyd and Martens 1992), but it has been speculated to be a means of nickel tolerance (Brooks 1987), drought resistance (Severne 1974), or a competitive strategy (Baker and Brooks 1989). Reeves et al. (1983) suggested that high levels of Ni might protect hyperaccumulators against insect or fungal attack. Given the general biological toxicity of Ni (Nieboer et al. 1988) this hypothesis seems plausible.

We hypothesized that Ni hyperaccumulation would be an effective defense against herbivores which attempt to use a hyperaccumulator as a primary host plant. Because Ni hyperaccumulators can be grown on non-nickel-bearing soils and hence contain little Ni, plant material differing substantially only in Ni content can be produced, allowing us to experimentally evaluate the ecological significance of Ni hyperaccumulation. We used *Streptanthus polygaloides* Gray. (Brassicaceae), a Ni hyperaccumulating annual plant endemic to serpentine soils in the foothills of the Sierra Nevada, California, in experiments to evaluate our hypothesis.

S. N. Martens  
Department of Botany, University of California,  
Davis, CA 95616-8537, USA

R. S. Boyd  
Department of Botany and Microbiology,  
and Alabama Agricultural Experiment Station,  
Auburn University,  
Auburn, AL 36849-5407, USA

S. N. Martens (✉)  
Environmental Science Group, EES-15, MS J495,  
Los Alamos National Laboratory,  
Los Alamos, NM 87545, USA

## Materials and methods

In feeding experiments we used three putative insect herbivores of *Streptanthus polygaloides*: larvae of *Pieris rapae* and *Euchloe hyantis hyantis* (Lepidoptera:Pieridae), and grasshoppers (Orthoptera:Acrididae). *P. rapae* is a semi-cosmopolitan species (although introduced to North America), whose larval food plants are primarily cultivated and weedy Brassicaceae (Garth and Tilden 1986). *P. rapae* larvae (3 days old) used in experiments were reared on broccoli in a greenhouse from eggs provided by local volunteers.

*Euchloe hyantis hyantis* is a California native pierid whose larval food plants are native, non-weedy Brassicaceae (Garth and Tilden 1986). *E. hyantis* larvae were collected from *Streptanthus tortuosus* on granitic substrate near Norden, Calif., about 40 km east of Washington, Calif.

Grasshoppers were collected from a serpentine site near Washington, Nevada County, Calif. but were too immature to be specifically identified by specialists at the California Department of Food and Agriculture.

During feeding experiments, insects were enclosed (larvae in 6-cm-diameter petri dishes; grasshoppers in 250-ml rectangular plastic bottles) with leaves and a moist sponge. Insects were fed one of three types of *S. polygaloides* leaves: from plants grown in Ni-amended, unamended, or serpentine soils.

For all feeding experiments, *S. polygaloides* plants were greenhouse-grown from seed in a soilless potting mix (UC Mix) or mix amended with 1000 mg Ni (as  $\text{NiCl}_2$ )  $\text{kg}^{-1}$  soil. Wild plants (including roots) were collected from serpentine soils near Washington, Nevada County, Calif., sealed in plastic bags, and transported on ice to the University of California, Davis. Plants were kept with their roots in water and leaves were removed as needed for the duration of the experiment.

In feeding experiments to determine larval food preference, *P. rapae* larvae were placed in 6-cm-diameter petri dishes with equivalently sized high-Ni and low-Ni leaves. The percentage of leaf area offered that was eaten was assessed 8 h after a larva began to feed.

Plant samples for elemental analyses were dry-ashed in a muffle furnace, the ash dissolved in  $\text{HCl}/\text{HNO}_3$ , and analyzed by inductively-coupled argon plasma spectrometry, except that Ni was analyzed by graphite furnace absorption spectroscopy. Total nitrogen was determined using a LECO CHN-600 analyzer. The percentage moisture content was determined on individual leaves dried at 45°C for 4 days. Data were analyzed by one-way analysis of variance, with multiple comparisons by Fisher's protected least significant difference test.

The effect of Ni on larval survival was determined with synthetic diet (Bio-Serv, Frenchtown, N.J.) that consisted of ground cabbage in an agar base with added vitamins and minerals. Ni was added as  $\text{NiCl}_2$  at 0, 100, 500, and 1000 mg Ni  $\text{kg}^{-1}$  dry weight of diet. Chloride itself is not toxic as we have reared larvae to pupation on as much as 4%  $\text{CaCl}_2$  added to artificial diet. Diet was poured into 6-cm-diameter petri dishes and solidified. Larvae were placed in dishes ( $n=8$  for each treatment) which were then capped.

In an experiment to examine plant fitness, 20 pots of each soil type (unamended or Ni-amended) with one *S. polygaloides* plant per pot were placed in a randomized rectangular array in a 1×1×1 m nylon mesh cage. About 20 adult female *P. rapae* caught in Davis, Calif. were introduced into the cage. Adults were allowed to oviposit at will for 3 days, when the number of ovipositions (eggs plus newly hatched larvae) per plant were counted. Larvae that hatched from the oviposited eggs were allowed to migrate at will among plants for 21 days until harvest.

Yield of aboveground biomass of *S. polygaloides* in Ni-amended, unamended, and serpentine soils was assessed to estimate cost of Ni hyperaccumulation. Ni-amended potting mix was prepared as described above. Serpentine soil was obtained from near Washington, Nevada County, Calif. Plants were grown from seed in pots (as for the feeding experiments above) in the greenhouse for 6 weeks, then transferred outside for an additional 12 weeks. Each

pot was caged with 6-mm mesh hardware cloth when outside. Plants were then harvested, dried at 105°C, and weighed.

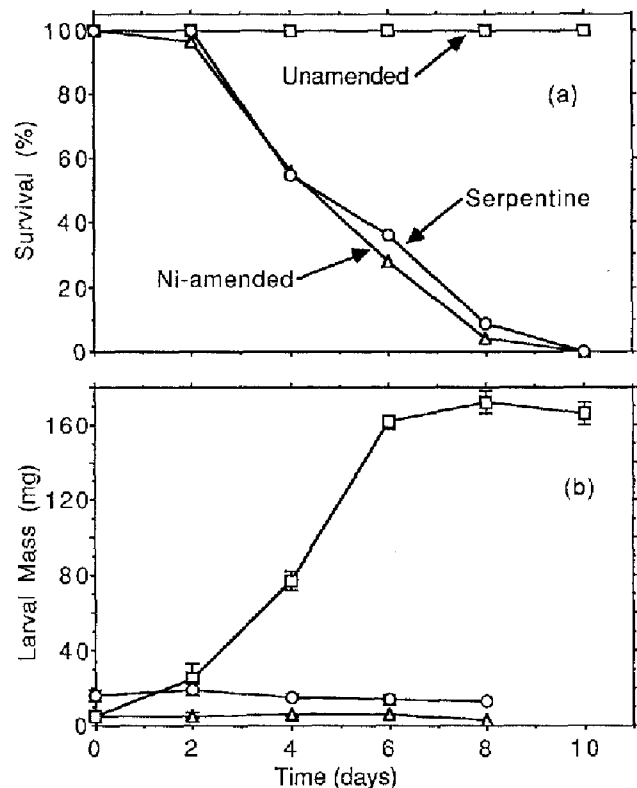
## Results

No larvae of *Pieris rapae* survived when fed leaves from the Ni-containing treatments, whereas all larvae fed leaves grown in unamended soil survived to pupation (Fig. 1a). Larvae fed leaves grown in Ni-containing soil lost weight during the experimental period (Fig. 1b) before death.

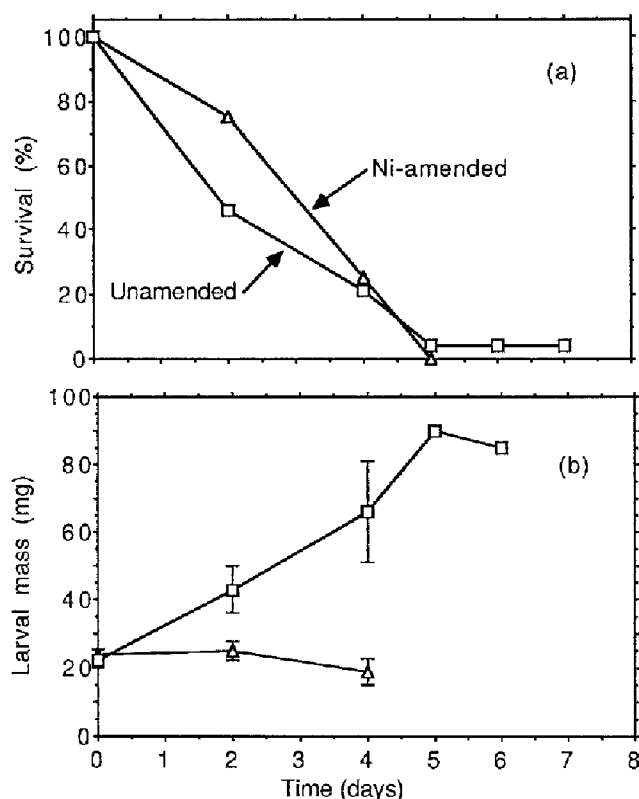
*Euchloe hyantis* larvae fed Ni-amended or unamended leaves showed equally poor survival (Fig. 2a) because of parasitoid infection. However, only larvae fed unamended leaves gained weight through the experimental period (Fig. 2b). Parasitoids of *E. hyantis* collected at this site have been reported to be *Cotesia* sp. (Hymenoptera:Bracconidae) and an unidentified ichneumonid (Kurban and Courtney 1987).

Similar results were obtained with grasshoppers collected from a serpentine site with *S. polygaloides*. Only grasshoppers fed leaves from plants grown in unamended soil survived (Fig. 3a) and gained weight (Fig. 3b).

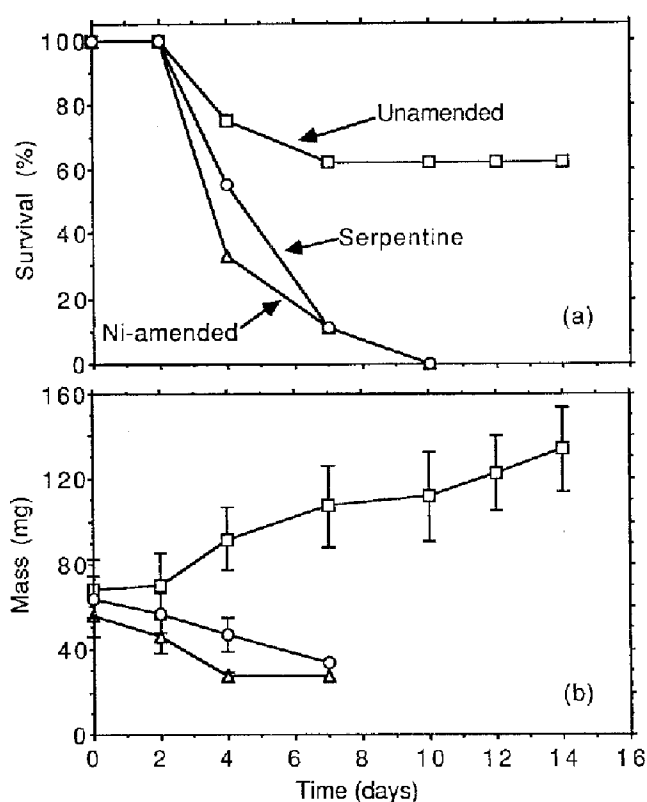
Individuals of all three insect species were observed to ingest the leaves offered regardless of Ni content so



**Fig. 1** a Survival of *Pieris rapae* larvae when fed only leaves of *Streptanthus polygaloides* grown in Ni-amended soil (△), unamended soil (□), or serpentine soil (○), which naturally contains Ni. b Average mass of the *P. rapae* larvae during the same period of feeding. We used 18 replicates each for Ni-amended and unamended treatments, and 11 replicates for the serpentine treatment



**Fig. 2** **a** Survival of *Euchloe hyantis hyantis* larvae when fed only leaves of *Streptanthus polygaloides* grown in Ni-amended soil ( $\Delta$ ) or unamended soil ( $\square$ ). **b** Average mass of the *E. hyantis hyantis* larvae during the same period of feeding



**Fig. 3** **a** Survival of grasshoppers when fed only leaves of *Streptanthus polygaloides* grown in Ni-amended soil ( $\Delta$ ), unamended soil ( $\square$ ), or serpentine soil ( $\circ$ ), which naturally contains Ni. **b** Average mass of the grasshoppers during the same period of feeding. We used 8, 9, and 9 replicates in the unamended, Ni-amended, and serpentine-grown treatments, respectively

**Table 1** Analysis of aboveground portions of *Streptanthus polygaloides* grown under greenhouse conditions (Ni-amended and unamended potting mix) or from field-collected plants (serpentine). Data are means (SD), in  $\text{mg kg}^{-1}$  except where specified.  $n = 5$  for greenhouse-grown and  $n = 2$  for serpentine plants (except for moisture,  $n = 5$ ). Means for the same parameter that differ significantly ( $P < 0.05$ ) are denoted by different superscripts

Parameter measured	Soil type		
	Unamended	Ni-amended	Serpentine
Aluminum	63 (28) <sup>a</sup>	38 (22) <sup>a</sup>	80 (6.5) <sup>a</sup>
Barium	100 (35) <sup>a</sup>	120 (35) <sup>a</sup>	140 (47) <sup>a</sup>
Chromium	8.2 (0.42) <sup>a</sup>	9.1 (0.73) <sup>a</sup>	9.5 (2.2) <sup>a</sup>
Copper	13 (1.5) <sup>a</sup>	10 (7.0) <sup>a</sup>	14 (7.6) <sup>a</sup>
Iron	190 (24) <sup>a</sup>	190 (42) <sup>a</sup>	242 (0) <sup>a</sup>
Nitrogen (%)	4.3 (0.97) <sup>a</sup>	3.0 (1.3) <sup>a</sup>	2.4 (0.32) <sup>a</sup>
Boron	130 (28) <sup>a</sup>	110 (25) <sup>a</sup>	55 (8.8) <sup>b</sup>
Cobalt	15 (6.6) <sup>a</sup>	20 (5.0) <sup>a</sup>	42 (27) <sup>b</sup>
Lead	12 (4.2) <sup>a</sup>	42 (9.9) <sup>a</sup>	120 (120) <sup>b</sup>
Manganese	350 (89) <sup>a</sup>	280 (30) <sup>a</sup>	54 (5.4) <sup>b</sup>
Molybdenum	7.8 (1.5) <sup>a</sup>	8.1 (2.2) <sup>a</sup>	2.8 (0.48) <sup>b</sup>
Silicon	230 (60) <sup>a</sup>	200 (36) <sup>a</sup>	350 (100) <sup>b</sup>
Magnesium (%)	0.54 (0.057) <sup>a</sup>	0.68 (0.056) <sup>b</sup>	0.76 (0.16) <sup>b</sup>
Nickel	180 (180) <sup>a</sup>	7400 (2000) <sup>b</sup>	7000 (1400) <sup>b</sup>
Zinc	160 (36) <sup>a</sup>	90 (20) <sup>b</sup>	130 (37) <sup>a,b</sup>
Calcium (%)	2.3 (0.35) <sup>a</sup>	2.9 (0.30) <sup>b</sup>	0.75 (0.24) <sup>c</sup>
Moisture (%)	88 (1.4) <sup>a</sup>	91 (0.37) <sup>b</sup>	83 (2.0) <sup>c</sup>
Phosphorus (%)	0.80 (0.11) <sup>a</sup>	0.55 (0.099) <sup>b</sup>	0.20 (0.064) <sup>c</sup>
Potassium (%)	3.3 (0.13) <sup>a</sup>	2.5 (0.39) <sup>b</sup>	1.6 (0.092) <sup>c</sup>

that mortality was not due simply to feeding deterrence. An experiment with *P. rapae* larvae given a choice of Ni-amended or unamended leaves showed that larvae generally preferred unamended leaves (Wilcoxon signed rank test,  $P < 0.01$ ). However, larvae consumed a signifi-

cant proportion of Ni-amended leaves ( $22.9\% \pm 4.84$  leaf area offered, mean  $\pm$  SE) relative to unamended leaves ( $37.6\% \pm 5.93$ ).

Concentrations of 18 elements and percent moisture in each leaf type (Table 1) indicate that only Ni and Mg

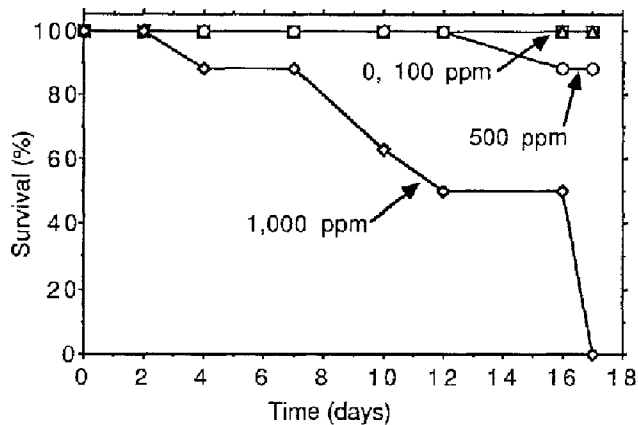


Fig. 4 Survival of *Pieris rapae* larvae fed synthetic diet containing Ni at 0, 100, 500 or 1000 mg Ni kg<sup>-1</sup> diet

showed a pattern consistent with the results of the feeding experiments. However, only Ni had differences among the treatments of sufficient magnitude (180 mg Ni kg<sup>-1</sup> dry wt in the unamended treatment versus 7000 and 7400 in the serpentine and amended treatments respectively) to produce the observed toxicity.

To demonstrate that Ni toxicity alone was sufficient to account for the lethal response observed, we fed *P. rapae* larvae a synthetic diet amended with up to 1000 mg Ni kg<sup>-1</sup> dry wt (Fig. 4). No larvae survived in treatments with Ni at 1000 mg Ni kg<sup>-1</sup> dry wt (Fig. 4), the defined threshold level for hyperaccumulation. Ni levels in leaves which were toxic in the feeding experiment were more than 7 times this concentration.

To examine the consequences for plant fitness, we let *P. rapae* females oviposit on caged *S. polygaloides* plants grown in Ni-amended or unamended soil (Table 2). No oviposition preference between Ni-amended and unamended plants was detected when plant height was used as a covariate (analysis of covariance,  $F = 0.83$ ,  $P = 0.37$ ). After 21 days of herbivory, Ni-amended plants showed much greater survival and dry weight compared to plants grown in unamended soil, many of which were almost completely defoliated. The adaptive value of Ni hyperaccumulation is demonstrated by the greater fitness of Ni-amended plants versus unamended plants.

In a separate experiment without herbivory, a gross examination of the cost of nickel hyperaccumulation was made by comparing the aboveground yield of *S. polygaloides* grown in nickel-amended and unamended me-

dia as well as their native serpentine soil. Yield (mg dry weight per pot) differed among the treatments [unamended soil,  $919 \pm 193$  mg (mean  $\pm$  95% C.I.); Ni-amended,  $1388 \pm 219$  mg; serpentine soil,  $875 \pm 255$  mg; ANOVA,  $F = 7.66$ ,  $P = 0.001$ ]. However, a comparison using orthogonal contrasts of yield in nickel-rich soil (Ni-amended and serpentine treatments) versus unamended soil showed a non-significant difference (contrast value = 425;  $P = 0.10$ ). Lower biomass in the serpentine treatment relative to the Ni-amended treatment is likely due to the lower fertility of the serpentine soil as compared to the potting mix. These results indicate that there was no apparent cost of Ni hyperaccumulation as measured by aboveground biomass.

## Discussion

We have shown that hyperaccumulated Ni can clearly function as a plant defense and increase plant fitness. It is a unique kind of plant defense – an elemental defense – in which an element absorbed from the soil renders the plant toxic. In this respect it is similar to Se (Rosenfeld and Beath 1964) and F accumulation (Oelrichs and McEwan 1961) by plants. However, unlike Se and F, Ni is not incorporated into an active primary metabolite (Reeves 1992). In accumulator plants, Se is incorporated into seleno-amino acids (Brown and Shrift 1982), and F is combined into fluoroacetate (Morrison and Peters 1954). After ingestion by an herbivore these altered metabolites could disrupt metabolic systems. When incorporated into proteins seleno-amino acids render them dysfunctional (Brown and Shrift 1982), and fluoroacetate disrupts the tricarboxylic acid cycle (Morrison and Peters 1954). In contrast, Ni is not incorporated into any specific compound, but is likely sequestered with organic acids in vacuoles (Reeves 1992). As a consequence, free Ni is probably released after ingestion and toxicity could result from the non-specific interactions of Ni with many metabolic processes (Borovik 1989). Ni hyperaccumulation appears to occur in far more species (Brooks 1987) than Se accumulation (Group I type; Rosenfeld and Beath 1964) or fluoroacetate production (Twigg and King 1991). The potential selective advantage of an elemental defense has only been experimentally demonstrated for Ni hyperaccumulation (this study).

Defense by Ni hyperaccumulation, as with other defenses (Coley et al. 1985), can be examined in terms of

**Table 2** Effect of Ni hyperaccumulation on plant fitness when plants are exposed to insect herbivory. *S. polygaloides* plants were greenhouse-grown from seed in pots of Ni-amended or unamended soil. Data are means  $\pm$  SD for 20 replicates

Parameter measured	Soil type		
	Unamended	Ni-amended	<i>P</i>
Initial plant height (cm)	6.3 $\pm$ 1.6	9.1 $\pm$ 2.5	0.0004
Ovipositions per plant	10.8 $\pm$ 4.9	18.7 $\pm$ 9.0	0.0025
Plant dry weight, 21 days (mg)	53 $\pm$ 23	167 $\pm$ 30	ns <sup>a</sup>
Plant survival, 21 days (%)	35	83	<0.0001

<sup>a</sup> ns when plant height was used as a covariate, ANCOVA,  $F = 0.83$ ,  $P = 0.37$

its benefits and costs. Ni hyperaccumulation may provide a novel alternative to C- or N-based chemical defenses because of its low cost and large benefit. The potential benefits of Ni hyperaccumulation are large, as demonstrated above by the increased fitness of Ni-containing plants in a mixed population of Ni- and non-Ni-containing plants exposed to herbivores. Since Ni is a general biological toxicant, Ni hyperaccumulation may also be effective against bacterial and fungal plant pathogens (Reeves et al. 1983). A broad-spectrum defense would have even greater selective value (Futuyma 1983).

Ni hyperaccumulation apparently has relatively low costs. We, and others (Reeves and Baker 1984), have observed no decrease in plant yield of Ni hyperaccumulators grown under controlled conditions on nickel-bearing soil as compared to non-nickel-bearing soil. Ni-based defense incurs no construction cost for the primary chemical (Ni), and probably has low maintenance costs because Ni in hyperaccumulators is usually complexed with a small organic acid molecule (e.g., citric acid: Lee et al. 1978; malic acid: Gabbrielli et al. 1991). However, the costs of Ni uptake and transport are unknown in *S. polygaloides* (and other hyperaccumulators) because these processes are poorly understood (Reeves 1992).

The ability of herbivores to evolve counteradaptations to Ni-based defense is unknown. The elemental nature of Ni means that it cannot be catabolized by a consumer, as occurs for some C- or N-based defenses (Krieger et al. 1971). In order to avoid toxicity, a consumer of Ni must efficiently complex and excrete it or compartmentalize it (Hopkin 1989). An alternative may simply be to consume a mixed diet. Our experiment showed that a dose of 500 mg kg<sup>-1</sup> in synthetic diet was not acutely toxic but 1000 mg kg<sup>-1</sup> was toxic (Fig. 4).

However, there are reports of apparent Ni tolerance in other organisms. Schlegel et al. (1991) have found Ni-resistant bacteria associated with the roots of Ni hyperaccumulators. Wild (1975) reported that *Catamerus* beetles on serpentine in Zimbabwe contained >2000 mg kg<sup>-1</sup> Ni and Cr, although much of the metal was probably in the gut contents (Hopkin 1989). Other Ni-adapted organisms may exist which can counter Ni-based defenses (Klerks 1989), perhaps by use of currently understood mechanisms of metal tolerance, e.g., metallothioneins (Hopkin 1989). Whether Ni tolerant herbivores exist in natural serpentine vegetation has yet to be demonstrated.

The novelty of Ni hyperaccumulation, in contrast to biochemical defenses, may in itself be a disincentive to the evolution of counteradaptations by herbivores. An infrequently encountered plant defense may lessen the probability that an herbivore would evolve an anti-defense mechanism. At present there is a lack of appropriate data to fully evaluate the predominance of tissues high in hyperaccumulated metal, but usually only a small percentage of plant taxa on serpentine soils hyperaccumulate Ni. Brooks (1987) found that about 9% of forest species in New Caledonia hyperaccumulated Ni. On Mt. Bloomfield (Palawan) in the Phillipines, Baker and Proctor (1988) found that only 5% of the taxa were nickel

hyperaccumulators. Less than 2% of the 215 plant taxa endemic to serpentine in California are nickel hyperaccumulators (Reeves et al. 1983; Kruckeberg 1984). This percentage is reduced ten-fold when all taxa that occur on Californian serpentine are considered. However, despite the small contribution of nickel hyperaccumulators to serpentine floras they may be locally dominant (Baker and Brooks 1989).

Despite our demonstration of the defensive role of hyperaccumulated nickel, defense may be only one function for which nickel hyperaccumulation has evolved (Boyd and Martens 1992). Baker and Proctor (1988) suggested that nickel hyperaccumulation was a primitive mechanism of metal tolerance because it occurs in relatively primitive groups of tropical plants. We have speculated that nickel hyperaccumulators (or their ancestors) were pre-adapted for metal tolerance and hyperaccumulation (Boyd and Martens 1992). Support for this idea is offered by the constitutive abilities of *Thlaspi goesingense* (Reeves and Baker 1984) and *Thlaspi montanum* var. *montanum* (our unpublished results) to hyperaccumulate nickel; i.e., plants grown from seeds collected far from serpentine soils have the ability to hyperaccumulate nickel. Further, this pre-adaptation might be a form of an efficient metal nutrient acquisition mechanism, which leads us to speculate that there may exist species which we would call latent hyperaccumulators – species which possess this pre-adaptation but have not colonized metal-rich soils (Boyd and Martens 1992).

Elements other than Ni are hyperaccumulated by plants, although Ni hyperaccumulation is currently the most widespread and best characterized type of hyperaccumulation. We postulate that other hyperaccumulated elements, such as Co, Cu, Mn, Pb, and Zn (Baker and Brooks 1989), may also serve as elemental defenses much as Ni does in *S. polygaloides* (Ernst 1987; Ernst et al. 1990; our unpublished data).

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