American Journal of Botany 89(6): 998–1003. 2002.

THE DEFENSIVE ROLE OF NI HYPERACCUMULATION BY PLANTS: A FIELD EXPERIMENT[1]

SCOTT N. MARTENS[2] AND ROBERT S. BOYD[3,4]

[2]Environmental Science Group, Los Alamos National Laboratory, Los Alamos, New Mexico 87545 USA; and
[3]Department of Biological Sciences, Auburn University, Alabama 36849-5407 USA

Hyperaccumulation of Ni by plants is hypothesized to function as an elemental defense against herbivores and pathogens. Laboratory experiments have documented toxic effects to herbivores consuming high-Ni plant tissues, but this paper reports the first experiment to examine the defensive effectiveness of Ni hyperaccumulation under field conditions. The experiment was conducted at an ultramafic soil site naturally inhabited by the Ni hyperaccumulator *Streptanthus polygaloides* (Brassicaceae). Experimental treatments examined the response of herbivores to hyperaccumulated Ni, using exclosure and insecticide treatments to divide herbivores into groups based primarily upon herbivore size. Three soils (Ni-amended greenhouse soil, unamended greenhouse soil, ultramafic soil), three exclosure treatments (exclosure, control exclosure, no exclosure), and a systemic insecticide treatment were combined in a fractional factorial experimental design. *Streptanthus polygaloides* plants were grown in a greenhouse for 2 mo, transplanted into the field by inserting potted plants into holes dug on the experimental site, and periodically examined for herbivore damage during a 41-d period. Initial surveys showed greater amounts of insect damage to plants with low tissue Ni levels, confirming the defensive effect of Ni against some insect herbivores, but large herbivores (probably vertebrates) later consumed entire plants regardless of plant Ni status. We concluded that Ni was not an effective defense against these large herbivores, probably because their diets mix high-Ni *S. polygaloides* foliage with that of associated non-hyperaccumulating species. We suggest that such dietary dilution is one mechanism whereby some herbivores can circumvent elemental plant defenses.

Key words: Brassicaceae; elemental defense; heavy metals; herbivory; Ni hyperaccumulation; plant defense; *Streptanthus polygaloides*.

Many plant species growing on soils that naturally contain large amounts of metals have elevated concentrations of those metals in their tissues (Brooks, 1987). However, some metal-tolerant plant species, termed ‘‘hyperaccumulators’’ by Brooks et al. (1977), have remarkably elevated metal concentrations in their tissues. Nickel hyperaccumulators are defined by Reeves and Baker (2000) as species containing >1000 μg Ni/g dry mass in the aboveground parts of at least one specimen collected from the wild. At the time of their review, Reeves and Baker (2000) estimated that 418 hyperaccumulator taxa had been discovered, with the majority of these (76%) hyperaccumulating Ni. The remaining taxa hyperaccumulated other metals, such as Zn, Pb, Cd, Cu, Co, Mn, or Se (Reeves and Baker, 2000).

Boyd and Martens (1992) and Boyd (1998) summarized six hypotheses that have been suggested to explain the unusually elevated metal concentrations of hyperaccumulator species. One explanation is that hyperaccumulation is a metal tolerance mechanism (Antonovics, Bradshaw, and Turner, 1971; Baker, 1981, 1987; Kruckeberg and Reeves, 1995). This concept suggests that hyperaccumulators avoid metal toxicity by complexing metals in locations (e.g., cell walls, the central vacuole) away from more metabolically active cellular compartments. A second explanation is the ‘‘disposal hypothesis’’ (Boyd and Martens, 1992), which proposes that hyperaccumulators remove metals from their root zone and either: (1) place them in plant parts that are later shed (Wild, 1978; Ernst, 1972) or burned away by fire (Baker, 1981); or, (2) place them in the epidermis where they are rinsed out by rainfall (Farago and Cole, 1988). Increased drought resistance is a third postulated explanation for metal hyperaccumulation (Severne, 1974; Baker and Walker, 1990; Robertson, 1992). This explanation proposes a water-conserving effect of Ni in epidermal cell walls (Severne, 1974) or suggests that Ni functions as an osmoticum (Baker and Walker, 1990). A fourth explanation is interference with neighboring plants through enrichment of metal in the surface soil under hyperaccumulator plant canopies (Baker and Brooks, 1989; Gabbrielli, Mattioni, and Vergnano, 1991; Wilson and Agnew, 1992; Boyd and Jaffré, in press). A fifth explanation is ‘‘inadvertent uptake’’ (Boyd and Martens, 1992), which views Ni hyperaccumulation as a physiologic by-product of a mechanism that has another adaptive function (Baker and Walker, 1990). Finally, metal hyperaccumulation has been proposed to function defensively against plant enemies such as pathogens and herbivores (Reeves, Brooks, and Macfarlane, 1981; Ernst, 1987; Ernst, Schat, and Verkleij, 1990; Boyd, 1998; Sägner et al., 1998).

There is scant experimental evidence regarding most of the above hypotheses for metal hyperaccumulation. Even the defense hypothesis, which has been tested to a greater extent than all other explanations combined (Boyd, 1998), deserves further experimentation. For example, it has become clear that hyperaccumulated metal does not defend hyperaccumulators against all herbivores or pathogens. A growing number of laboratory studies have shown that high-metal plant tissues are toxic to generalist herbivores (e.g., Boyd and Martens, 1994; Martens and Boyd, 1994; Boyd and Moar, 1999) and pathogens (e.g., Boyd, Shaw, and Martens, 1994; Ghaderian, Lyon, and Baker, 2000) or deter herbivore damage altogether (e.g., Pollard and Baker, 1997). As a consequence, tissues of hyperaccumulator species suffer decreased herbivore damage in

---

[1] Manuscript received 27 September 2001; revision accepted 20 December 2001.

The authors thank C. Boyd and two anonymous reviewers for helpful comments on an earlier version of this manuscript. Portions of this manuscript were written while the second author was Mellon Visiting Scholar at Rancho Santa Ana Botanic Garden at Claremont, California.

[4] Author for reprint requests (e-mail: rboyd@acesag.auburn.edu).

choice experiments using generalist folivores (e.g., Martens and Boyd, 1994; Jhee et al., 1999). However, no plant defense is inviolate (Grubb, 1992), and some herbivores or plant parasites have been documented to attack metal hyperaccumulators despite their elemental defense. These include a parasitic plant (Boyd, Martens, and Davis, 1999), an aphid (Boyd and Martens, 1999), a mirid hemipteran (Schwartz and Wall, 2001), and a virus (Davis, Murphy, and Boyd, 2001). Thus, it is clear that elemental defenses, like other modes of plant defense, can be circumvented (Boyd and Martens, 1998).

The mechanisms whereby elemental plant defenses are circumvented by herbivores are now beginning to be explored. Boyd (1998) suggested three mechanisms of circumvention: (1) avoidance, in which low metal plant parts or cellular compartments are selectively fed upon; (2) dietary dilution, in which high-metal tissues are mixed with low-metal tissues in the diet of a polyphagous herbivore to dilute the overall metal concentration of the diet; and (3) tolerance, in which physiological adaptations allow an herbivore to withstand a high-metal diet. Few studies have explored these mechanisms to date.

To our knowledge, the defense hypothesis has yet to be experimentally tested under field conditions. Field experiments can enhance our understanding of the defense hypothesis by determining if hyperaccumulated metals provide defensive benefits when hyperaccumulators are faced with an array of natural enemies. Such experiments also can determine which plant enemies are able to circumvent elemental plant defenses and which are vulnerable to them. Furthermore, metal hyperaccumulator plants are useful systems for exploring questions of defense and herbivore response because the level of defense in plant tissues can be manipulated by manipulating metal levels in their substrate. Thus, defended and undefended plants can be created and used in experiments examining the defensive efficacy of metal-based defenses. This feature led Boyd (1998) and Pollard (2000) to conclude that hyperaccumulator plants constituted ideal systems for studying plant/herbivore coevolution.

The research reported here is a first attempt to place studies of the defense hypothesis into a field context. Our specific objectives were to determine: (1) if plants with elevated levels of tissue Ni were protected from herbivore damage in the field; (2) what types of naturally occurring herbivores attacked a metal-hyperaccumulating plant species under field conditions; and (3) the susceptibility of different types of herbivores to a metal-based plant defense under field conditions.

MATERIALS AND METHODS

We used plants of the Ni hyperaccumulator *Streptanthus polygaloides* Gray (Brassicaceae). This species grows only on ultramafic soils scattered along the western side of the Sierra Nevada in California (Reeves, Brooks, and Macfarlane, 1981). *Streptanthus polygaloides* is an unusual hyperaccumulator species in that it is a winter annual, whereas almost all other species reported to hyperaccumulate metals are perennials (Brooks, 1987). Nickel concentrations of field-grown plants range from 1100 to 16 400 μg/g dry mass in leaves, stems, roots, flowers, and fruits (Reeves, Brooks, and Macfarlane, 1981). Previous research has shown that plants with relatively low tissue Ni concentrations (about 180 μg Ni/g) can be produced when grown on low-Ni greenhouse soil (e.g., Martens and Boyd, 1994; Boyd and Moar, 1999).

This study was established at an ultramafic site in Nevada County, California that hosted a large natural population of *S. polygaloides*. The site corresponds to that of collection #6732 of Kruckeberg and Reeves (1995) near the town of Washington. The specific location selected for our study was a northeast-facing slope with scattered conifers (*Pinus sabiniana* Douglas, *Calocedrus decurrens* (Torrey) Florin, *Pseudotsuga menziesii* (Mirbel) Franco var. *menziesii*) and shrubs of *Ceanothus* sp. and *Arctostaphylos* sp. at 1330 m elevation. Scattered *S. polygaloides* individuals were located in rocky openings between the woody perennials.

TABLE 1. Description of treatment combinations used in the fractional factorial experimental design of this experiment.

Treatment no.	Exclosure treatment	Soil treatment	Insecticide treatment
1	none	unamended	none
2	control exclosure	unamended	none
3	exclosure	unamended	none
4	none	unamended	Temik
5	exclosure	unamended	Temik
6	none	Ni-amended	none
7	control exclosure	Ni-amended	none
8	exclosure	Ni-amended	none
9	none	Ni-amended	Temik
10	exclosure	Ni-amended	Temik
11	none	ultramafic	none
12	control exclosure	ultramafic	none
13	exclosure	ultramafic	none

Seeds collected from plants growing on other portions of this locality were used to produce experimental plants. Experimental plants were grown in a greenhouse for 2 mo in cylindrical pots, 6.5 cm in diameter and 25 cm in length, that allowed for relatively deep root development. In the spring (1 April 1988), pots, each containing a single plant, were inserted into holes dug in the field so that only a few centimeters of the upper edge of each pot protruded from the ground. Just before plants were placed in the field, biomass samples were harvested from some replicates for analysis of initial Ni concentrations. Aboveground portions of plants growing in the different soil types were dried at about 60°C for several days and analyzed for Ni concentration (analysis described below).

Experimental treatments were designed to examine effects of plant Ni concentration on different groups of herbivores, using exclosure and insecticide treatments to separate herbivores into groups (Table 1). Large herbivores were excluded by cylindrical exclosures made of hardware cloth with 5-mm diameter mesh, about 20 cm tall and 8 cm diameter, anchored to the soil by a pair of small wooden stakes. Exclosure effects on plants other than those caused by herbivore exclusion (e.g., effects due to shading by the exclosure) were partially controlled by including a control exclosure treatment. Control exclosures had two large (5 × 5 cm) holes cut into opposite sides of the exclosures at ground level, allowing most herbivores access to the plants while maintaining the shading effect created by the rest of the exclosure. A pair of small wooden stakes anchored control exclosures to the soil. An insecticide treatment, the systemic insecticide Temik (Rhone-Poulenc Agriculture, Research Triangle Park, North Carolina, USA), was also included in the experimental design. Insecticide was applied to the soil of plants receiving this treatment 4 d prior to placement of plants in the field. The insecticide treatment was included so that, when combined with the exclosure treatment, herbivores of all types and sizes would be deterred from damaging plants. We thus created treatments that allowed for differing combinations of herbivores to attack experimental plants (Table 1) so that comparisons between treatments would demonstrate the relative importance of these different groups of herbivores.

Three soil treatments also were used (Table 1). The ultramafic soil was soil removed from the study site to about 20 cm depth and screened to remove large (>1 cm diameter) rocks. The Ni-amended soil was UC Mix (potting soil) to which powdered $NiCl_2$ was added to yield a Ni concentration of about 800 μg Ni/g. The third soil was unamended UC Mix.

The three soils (Ni-amended greenhouse soil, unamended greenhouse soil, and ultramafic soil), three caging treatments (exclosure, control exclosure, no exclosure) and a systemic insecticide treatment were combined in a fractional factorial experimental design (Table 1). A complete experimental replicate

TABLE 2. Results of ANOVAs for each day that leaf removal data were collected. Values provided are P values for each factor (NS = not statistically significant at $P = 0.05$).

	Factors in fractional factorial ANOVA model			
Day	Insecticide	Exclosure	Soil	Block
6	NS	NS	NS	NS
10	NS	NS	0.04	NS
14	NS	0.009	NS	<0.0001
26	NS	<0.0001	NS	0.0005
41	NS	<0.0001	NS	NS

consisted of 13 treatment combinations, and 11 replicates were established in the field.

Plants remained in the field for 41 d and were watered several times during the experimental period in response to wilting due to apparent water stress. We revisited the site at irregular intervals (6, 10, 14, 26, and 41 d after initiation [DAI]) to examine plants for herbivore damage. Data collected were visual estimates of percentage of leaf damage to each plant due to herbivore activity. Aboveground plant material remaining at 41 DAI was harvested, dried, and divided into 3–5 samples for each soil treatment by combining material from the replicates. These samples were analyzed for Ni concentration as described below.

Plant samples taken at both the start of the experiment and at its completion were analyzed for Ni concentration. One gram of each sample was dry-ashed at 485°C, further oxidized using 1 mol/L HNO_3, and the residue redissolved in 1 mol/L HCl. The solution was then analyzed for Ni using an atomic absorption spectrophotometer (Instrumentation Laboratory IL 251, Lexington, Massachusetts, USA). The effect of soil treatments on plant Ni concentration was evaluated by a random effects model one-way analysis of variance (ANOVA), using Fisher's protected least significant difference (PLSD) test for post-hoc means separations (Abacus Concepts, 1994).

Herbivory data were analyzed by randomized fractional factorial design ANOVAs of the percentage of leaf damage estimates for each date that herbivory data were collected. Leaf damage values were arcsine transformed to better approximate the statistical assumptions underlying analysis of variance (Zar, 1984). When ANOVA indicated a significant treatment effect for factors that contained more than two treatments (i.e., exclosure and soil factors), orthogonal contrasts were used to explore treatment differences.

RESULTS

Plants varied greatly in Ni concentration at the time they were placed into the field. Ni values of aboveground biomass (means ± SE) were: 6500 ± 1200 μg Ni/g dry mass for plants on ultramafic soil ($N = 2$); 6100 ± 780 μg Ni/g dry mass for those from Ni-amended greenhouse soil ($N = 4$); and 120 ± 7.0 μg Ni/g dry mass for plants growing on unamended greenhouse soil ($N = 4$). One-way ANOVA showed that plants grown on these soils differed significantly in Ni concentration ($F_{2,7} = 31.1$, $P = 0.003$). Pairwise comparisons (Fisher's PLSD test, $P \leq 0.05$) showed that plants grown on unamended soil had significantly lower Ni concentrations, whereas plants from ultramafic soil or Ni-amended greenhouse soil had statistically similar Ni levels.

We noted that plant roots had grown through the bottom of the pots by the time the experiment was terminated. This allowed all plants access to Ni in the site's ultramafic soil so that, by the end of the experiment, Ni concentrations of plants from all soil treatments were quite elevated. Ni values of aboveground biomass (means ± SE) were: 8900 ± 200 μg Ni/g dry mass for ultramafic soil plants ($N = 3$); 9100 ± 460 μg Ni/g dry mass for Ni-amended greenhouse soil plants ($N = 5$); and 7000 ± 830 μg Ni/g dry mass for plants growing on unamended greenhouse soil ($N = 5$). One-way ANOVA showed no significant difference in Ni concentration at that time ($F_{2,10} = 3.4$, $P = 0.074$). Plants grown on unamended soil tended to have lower Ni concentrations than plants from the other two soil treatments, but all plants were hyperaccumulating Ni by the end of the experiment.

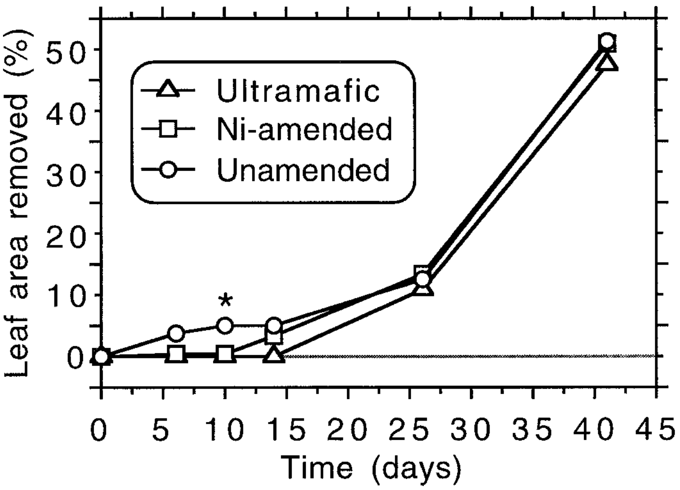

Fig. 1. Soil treatment effects on leaf removal. The asterisk indicates a statistically significant effect of soil treatment at 10 d after initiation (DAI).

Experimental treatments varied in their influence on herbivory through time. Insecticide treatment produced no detectable differences in leaf area removed at any time period (Table 2). Soil treatments resulted in a significant effect only once: at 10 DAI (Table 2). Orthogonal contrasts showed that this effect had two components: there was a significant difference ($P = 0.027$) between the ultramafic soil when compared against the two greenhouse soils (ultramafic soil plants had less damage) and there was a significant contrast ($P = 0.012$) between the two high-metal soils (ultramafic and Ni-amended) and the low-metal unamended soil (plants growing on high-metal soils had less damage).

The influence of soil as a main treatment effect throughout the experiment is summarized in Fig. 1. The low-metal soil was associated with elevated levels of herbivory (relative to other treatments) at 6 DAI and the significant effect at 10 DAI, but this difference disappeared and high levels of herbivore damage were observed for all soil treatments at 26 and 41 DAI.

The major factor influencing leaf damage by herbivores was the exclosure factor, which had significant effects later in the experiment (at 14, 26, and 41 DAI; Table 2). Orthogonal contrasts showed this treatment effect was complex and could be attributed to several components (Table 3). The exclosure treatment almost eliminated herbivore damage, whereas control exclosure and no exclosure treatments were generally ineffective in protecting plants. This is clearly shown in Fig. 2, where values for mean leaf area removed for plants protected by exclosures were well below 5% for the entire study. In contrast, control exclosure and no exclosure plants had >75% of their leaf area removed by the end of the experiment (Fig. 2).

Orthogonal contrasts also showed differences in herbivore damage between exclosed (any type of exclosure) and unexclosed plants (Table 3). There were differences in amount of damage between the two types of exclosures (exclosures and

TABLE 3. Results of ANOVAs for each day that leaf removal data were collected. Values provided are P values for each orthogonal contrast (SAS Proc GLM; NS = not statistically significant at P = 0.05).

	Description of orthogonal contrast		
Day	Any exclosure vs. no exclosure	Exclosure vs. control/no exclosure	Exclosure vs. control exclosure
6	NS	NS	NS
10	NS	NS	NS
14	0.003	NS	0.004
26	0.0001	0.0001	0.0001
41	0.0001	0.0001	0.0001

control exclosures) for days 14, 26, and 41 (Table 3). As seen from Fig. 2, control exclosures had an inhibitory effect on herbivory relative to unexclosed plants. Damage to control exclosure plants was relatively minor until day 41, when many of the unexclosed plants had been eaten (Fig. 2). By the last day of the experiment, however, plants with no exclosures and those with control exclosures had both been damaged to a great degree (Fig. 2).

Observations of herbivore damage during the later part of the experiment (14 DAI and later) indicated that a large herbivore (large in comparison to most insect herbivores) was biting off major portions of plants. This herbivore was small enough to be able to reach through the holes on the sides of the control exclosures to graze on the plants within.

DISCUSSION

Elevated plant Ni levels failed to prevent herbivore attack in this experiment. However, plants grown on ultramafic and Ni-amended soils (and thus having high tissue Ni levels) did show significantly reduced herbivore damage at one point in time (10 DAI; Fig. 1). The herbivores responsible for this damage were judged to be insects, for two reasons. First, damage at this time in the experiment was not influenced by exclosure treatments (10 DAI; Fig. 2), and small insects could easily pass through the wire mesh. Second, the damage patterns we observed were congruent with feeding by folivorous insects. Leaf margins showed evidence of grazing, and small sections of epidermis had been damaged on the other surfaces of the leaves. Thus, this experiment provides some evidence that Ni hyperaccumulation can reduce damage caused by insect herbivores in a field setting. This portion of the results is consistent with laboratory experiments that have demonstrated toxicity of high-Ni *S. polygaloides* tissue to insect folivores (e.g., Martens and Boyd, 1994; Boyd and Moar, 1999).

Two hypotheses may explain why the soil treatment factor did not continue to significantly influence herbivore damage after 10 DAI. Root growth through the bottoms of the pots compromised the soil Ni treatments at some point during the experiment and this could explain why the statistically significant soil treatment effect documented at 10 DAI disappeared at later times. Alternatively, the small differences in levels of insect herbivory observed early in the experiment (up to 10 DAI; Fig. 1) were overwhelmed by the large-scale damage inflicted by vertebrate herbivores from 14 DAI onward (Fig. 2). We cannot determine which of the above hypotheses is correct, but can conclude that a small significant difference in insect herbivory occurred early in this experiment.

Unlike the soil treatments discussed above, the exclosure treatments were not compromised in this experiment and our conclusions for this portion of the experiment are both unequivocal and important. Herbivore attack caused significant and extensive damage to plants unprotected by an exclosure. At least some (and perhaps all) of these plants were hyperaccumulating Ni at this later time in the experiment. This result allows us to make two important points pertinent to the initial objectives of this study: (1) Ni-based defense was not effective against this type of herbivore; and (2) herbivores can inflict extensive damage on metal-defended plants in a field setting.

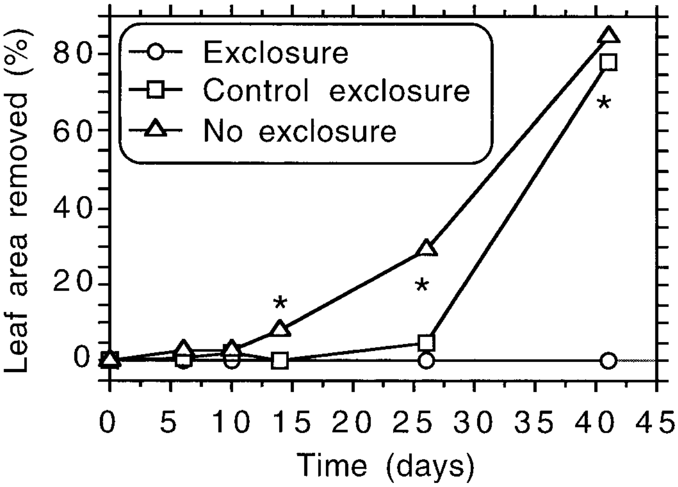

Fig. 2. Exclosure treatment effects on leaf removal. Asterisks indicate statistically significant effects of exclosure treatments at 14, 26, and 41 d after initiation (DAI).

The failure of high levels of tissue Ni to prevent herbivory in the field initially seems to counter the defense hypothesis. However, we suggest that this result stems from the ineffectiveness of Ni against some types of herbivores. Grubb (1992) points out that practically every plant defense can be overcome by certain herbivores. This conclusion was echoed by Belovsky and Schmitz (1994), who pointed out that no type of plant defense guaranteed protection against mammalian herbivores. For elemental defenses like Ni hyperaccumulation, most tests of the defense hypothesis have been controlled laboratory studies (e.g., Martens and Boyd, 1994; Boyd and Martens, 1994; Boyd, Shaw, and Martens, 1994; Pollard and Baker, 1997; Boyd and Moar, 1999) that used readily available herbivores/pathogens. These herbivore/pathogen species probably do not interact with the test plant species in the wild, so that they are useful mainly as ‘‘bioassay’’ organisms (Boyd and Martens, 1994; Boyd, 1998).

This experiment showed that high-Ni tissue can be consumed by some herbivores. Boyd (1998) suggested that there are two ways that this could be achieved without harm to an herbivore: through physiological tolerance of a high-Ni diet or through a mixed diet of high- and low-Ni plant species. Tolerance is an adaptation to high-Ni food and has been reported from invertebrates in metal-polluted areas (Hopkin, 1989). Tolerance also explains how high-metal insects, such as the recently described mirid hemipteran, *Melanotrichus boydi* Schwartz and Wall, are able to feed on hyperaccumulator species. This herbivore species apparently feeds only on *Streptanthus polygaloides* and has a whole-body Ni concentration of almost 800 μg Ni/g dry mass (Schwartz and Wall, 2001).

Apart from tolerance, safe consumption of high-Ni foliage can also be achieved through a mixed diet: the ‘‘diet dilution’’ hypothesis of Boyd (1998). We believe that this hypothesis explains the extensive herbivore damage observed in our experiment. The vertebrate herbivore that attacked plants during the later part of our study was likely large (relative to the size of *S. polygaloides* plants) and probably was polyphagous. Although we did not observe vertebrate herbivores visiting our plots, the damage inflicted on experimental plants suggested that it was a vertebrate herbivore species (probably a rodent or rabbit species). Such an herbivore could consume large portions of single plants, yet, if it also grazed upon other plant species whose tissues contained low Ni concentrations, it could dilute the Ni in its diet to a relatively low level. The herbivore would thus escape harm, but individual *S. polygaloides* plants would be greatly damaged.

Hyperaccumulator plants are of great interest for their promise as phytoextractors of metals from mining or metal-polluted sites (McGrath, 1998; Nicks and Chambers, 1998; Raskin and Ensley, 2000; Terry and Bañuelos, 2000). Our results have implications for the use of hyperaccumulators in these economically valuable activities. First, some polyphagous herbivores can attack crops of hyperaccumulator plants even though these herbivores may lack physiological tolerance of a high-metal diet. They may be able to graze upon hyperaccumulating plants by diluting metal-containing foliage with that of other plant species that do not hyperaccumulate, and thereby may cause significant damage to hyperaccumulator crops. Second, herbivory upon metal-hyperaccumulating crops may result in mobilization of some of the metal. This mobilization may be geographical (movement off-site) or ecological (movement up food chains). By forming part of the diet of potentially wide-ranging polyphagous herbivores, some metal in the hyperaccumulating crop will likely be transported off-site. The extent of this transport probably will be relatively small, but might nonetheless pose a problem in the public’s perception of the desirability of metal phytoextraction from contaminated soils (U.S. Department of Energy, 1994). Mobilization of metal into trophic levels higher than herbivores (i.e., carnivores; see Boyd and Wall, 2001) in food chains may also lead to geographical mobilization. It could also result in biomagnification (Price, Rathcke, and Gentry, 1974) and therefore might lead to toxicity in organisms at higher trophic levels (van Straalen and Ernst, 1991). This phenomenon could slow the implementation of phytoextraction technologies due to regulatory concerns (U.S. Department of Energy, 1994). However, an initial assessment by Boyd and Wall (2001) showed that Ni is not biomagnified in the food chain *S. polygaloides-M. boydi*-arthropod predators. Further exploration of food chains that are based upon hyperaccumulating plants may confirm that biomagnification is not likely to present a problem for phytoextraction technologies.

Finally, herbivore damage to a crop of metal hyperaccumulators can influence the economics of phytoremediation. In our experiment, herbivory greatly reduced the biomass of plants unprotected by an exclosure (at 41 DAI, amount of leaf area removed was about 75% for unprotected plants: Fig. 2). By lowering the yield of metal hyperaccumulating crop species, or by requiring additional herbivore-control treatments for phytoremediation sites, herbivory may change the cost-benefit ratio of this emerging technology. The importance of herbivory to phytoextraction efforts will become apparent as these technologies continue to be applied to diverse field situations.

LITERATURE CITED

ABACUS CONCEPTS. 1994. Survival tools for Statview. Abacus Concepts, Berkeley, California, USA.

ANTONOVICS, J., A. D. BRADSHAW, AND R. G. TURNER. 1971. Heavy metal tolerance in plants. *Advances in Ecological Research* 7: 1–85.

BAKER, A. J. M. 1981. Accumulators and excluders—strategies in the response of plants to heavy metals. *Journal of Plant Nutrition* 3: 643–654.

BAKER, A. J. M. 1987. Metal tolerance. *In* I. H. Rorison, J. P. Grime, R. Hunt, G. A. F. Hendry, and D. H. Lewis [eds.], Frontiers of comparative plant ecology. *New Phytologist* 106 (Supplement).

BAKER, A. J. M., AND R. R. BROOKS. 1989. Terrestrial higher plants which accumulate metallic elements—a review of their distribution, ecology, and phytochemistry. *Biorecovery* 1: 81–126.

BAKER, A. J. M., AND P. L. WALKER. 1990. Ecophysiology of metal uptake by tolerant plants. *In* A. J. Shaw [ed.], Heavy metal tolerance in plants: evolutionary aspects, 155–178. CRC Press, Boca Raton, Florida, USA.

BELOVSKY, G. E., AND O. J. SCHMITZ. 1994. Plant defenses and optimal foraging by mammalian herbivores. *Journal of Mammalogy* 75: 816–832.

BOYD, R. S. 1998. Hyperaccumulation as a plant defensive strategy. *In* R. R. Brooks [ed.], Plants that hyperaccumulate heavy metals, 181–201. CAB International, Oxford, UK.

BOYD, R. S., AND T. JAFFRÉ. In press. Phytoenrichment of soil Ni content by *Sebertia acuminata* in New Caledonia and the concept of elemental allelopathy. *South African Journal of Science*.

BOYD, R. S., AND S. N. MARTENS. 1992. The raison d’etre for metal hyperaccumulation by plants. *In* A. J. M. Baker, J. Proctor, and R. D. Reeves [eds.], The vegetation of ultramafic (serpentine) soils, 279–289. Intercept Limited, Andover, Hampshire, UK.

BOYD, R. S., AND S. N. MARTENS. 1994. Nickel hyperaccumulated by *Thlaspi montanum* var. *montanum* is acutely toxic to an insect herbivore. *Oikos* 70: 21–25.

BOYD, R. S., AND S. N. MARTENS. 1998. The significance of metal hyperaccumulation for biotic interactions. *Chemoecology* 8: 1–7.

BOYD, R. S., AND S. N. MARTENS. 1999. Aphids are unaffected by the elemental defense of the nickel hyperaccumulator *Streptanthus polygaloides* (Brassicaceae). *Chemoecology* 9: 1–7.

BOYD, R. S., S. N. MARTENS, AND M. A. DAVIS. 1999. The nickel hyperaccumulator *Streptanthus polygaloides* (Brassicaceae) is attacked by the parasitic plant *Cuscuta californica* (Cuscutaceae). *Madroño* 46: 92–99.

BOYD, R. S., AND W. J. MOAR. 1999. The defensive function of Ni in plants: response of the polyphagous herbivore *Spodoptera exigua* (Lepidoptera: Noctuidae) to hyperaccumulator and accumulator species of *Streptanthus* (Brassicaceae). *Oecologia* 118: 218–224.

BOYD, R. S., J. SHAW, AND S. N. MARTENS. 1994. Nickel hyperaccumulation defends *Streptanthus polygaloides* (Brassicaceae) against pathogens. *American Journal of Botany* 81: 294–300.

BOYD, R. S., AND M. A. WALL. 2001. Responses of generalist predators fed high-Ni *Melanotrichus boydi* (Heteroptera: Miridae): elemental defense against the third trophic level. *American Midland Naturalist* 146: 186–198.

BROOKS, R. R. 1987. Serpentine and its vegetation: a multidisciplinary approach. Dioscorides Press, Portland, Oregon, USA.

BROOKS, R. R., J. LEE, R. D. REEVES, AND T. JAFFRÉ. 1977. Detection of nickeliferous rocks by analysis of herbarium specimens of indicator plants. *Journal of Geochemical Exploration* 7: 49–57.

DAVIS, M. A., J. F. MURPHY, AND R. S. BOYD. 2001. Nickel increases susceptibility of a nickel hyperaccumulator to *Turnip mosaic virus*. *Journal of Environmental Quality* 30: 85–90.

ERNST, W. H. O. 1972. Ecophysiological studies on heavy metal plants in South Central Africa. *Kirkia* 8: 125–145.

ERNST, W. H. O. 1987. Population differentiation in grassland vegetation. *In* J. Van Andel, J. P. Bakker, and R. W. Snaydon [eds.], Disturbance in grasslands. Causes, effects and processes, 213–228. W. Junk, Dordrecht, The Netherlands.

ERNST, W. H. O., H. SCHAT, AND J. A. C. VERKLEIJ. 1990. Evolutionary biology of metal resistance in *Silene vulgaris*. *Evolutionary Trends in Plants* 4: 45–51.

FARAGO, M. E., AND M. M. COLE. 1988. Nickel and plants. *In* H. Sigel and

A. Sigel [eds.], Metal ions in biological systems, vol. 23, Nickel and its role in biology, 47–90. Marcel Dekker, New York, New York, USA.

GABBRIELLI, R., C. MATTIONI, AND O. VERGNANO. 1991. Accumulation mechanisms and heavy metal tolerance of a nickel hyperaccumulator. *Journal of Plant Nutrition* 14: 1067–1080.

GHADERIAN, S. M., A. J. E. LYON, AND A. J. M. BAKER. 2000. Seedling mortality of metal hyperaccumulator plants resulting from damping-off by *Pythium* spp. *New Phytologist* 146: 219–224.

GRUBB, P. J. 1992. A positive distrust in simplicity—lessons from plant defences and from competition among plants and among animals. *Journal of Ecology* 80: 585–610.

HOPKIN, S. P. 1989. Ecophysiology of metals in terrestrial invertebrates. Elsevier Applied Science, New York, New York, USA.

JHEE, E. M., K. L. DANDRIDGE, A. M. CHRISTY, JR., AND A. J. POLLARD. 1999. Selective herbivory on low-zinc phenotypes of the hyperaccumulator *Thlaspi caerulescens* (Brassicaceae). *Chemoecology* 9: 93–95.

KRUCKEBERG, A. R., AND R. D. REEVES. 1995. Nickel accumulation by serpentine species of *Streptanthus* (Brassicaceae): field and greenhouse studies. *Madroño* 42: 458–469.

MARTENS, S. N., AND R. S. BOYD. 1994. The ecological significance of nickel hyperaccumulation: a plant chemical defense. *Oecologia* 98: 379–384.

MCGRATH, S. P. 1998. Phytoextraction for soil remediation. *In* R. R. Brooks [ed.], Plants that hyperaccumulate heavy metals, 261–287. CAB International, Wallingford, UK.

NICKS, L. J., AND M. F. CHAMBERS. 1998. A pioneering study of the potential for phytomining of nickel. *In* R. R. Brooks [ed.], Plants that hyperaccumulate heavy metals, 313–325. CAB International, Wallingford, UK.

POLLARD, A. J. 2000. Metal hyperaccumulation: a model system for coevolutionary studies. *New Phytologist* 146: 179–181.

POLLARD, A. J., AND A. J. M. BAKER. 1997. Deterrence of herbivory by zinc hyperaccumulation in *Thlaspi caerulescens* (Brassicaceae). *New Phytologist* 135: 655–658.

PRICE, P. W., B. J. RATHCKE, AND D. A. GENTRY. 1974. Lead in terrestrial arthropods: evidence for biological concentration. *Environmental Entomology* 3: 370–372.

RASKIN, I., AND B. D. ENSLEY. 2000. Phytoremediation of toxic metals. John Wiley & Sons, New York, New York, USA.

REEVES, R. D., AND A. J. M. BAKER. 2000. Metal-accumulating plants. *In* I. Raskin and B. D. Ensley [eds.], Phytoremediation of toxic metals: using plants to clean up the environment, 193–229. John Wiley & Sons, New York, New York, USA.

REEVES, R. D., R. R. BROOKS, AND R. M. MACFARLANE. 1981. Nickel uptake by Californian *Streptanthus* and *Caulanthus* with particular reference to the hyperaccumulator *S. polygaloides* Gray (Brassicaceae). *American Journal of Botany* 68: 708–712.

ROBERTSON, A. I. 1992. The relation of nickel toxicity to certain physiological aspects of serpentine ecology: some facts and a new hypothesis. *In* A. J. M. Baker, J. Proctor, and R. D. Reeves [eds.], The vegetation of ultramafic (serpentine) soils, 331–336. Intercept Limited, Andover, Hampshire, UK.

SÄGNER, S., R. KNEER, G. WANNER, J.-P. COSSON, B. DEUS-NEUMANN, AND M. H. ZENK. 1998. Hyperaccumulation, complexation and distribution of nickel in *Sebertia acuminata*. *Phytochemistry* 47: 339–347.

SCHWARTZ, M. D., AND M. A. WALL. 2001. *Melanotrichus boydi*, a new species of plant bug (Heteroptera: Miridae: Orthotylini) restricted to the nickel hyperaccumulator *Streptanthus polygaloides* (Brassicaceae). *Pan-Pacific Entomologist* 77: 39–44.

SEVERNE, B. C. 1974. Nickel hyperaccumulation by *Hybanthus floribundus*. *Nature* 248: 807–808.

TERRY, N., AND G. BAÑUELOS. 2000. Phytoremediation of contaminated soil and water. Lewis, Boca Raton, Florida, USA.

U.S. DEPARTMENT OF ENERGY. 1994. Summary report of a workshop on phytoremediation research needs. DOE/EM-0224.

VAN STRAALEN, N. M., AND W. H. O. ERNST. 1991. Metal biomagnification may endanger species in critical pathways. *Oikos* 62: 255–256.

WILD, H. 1978. The vegetation of heavy metal and other toxic soils. *In* M. J. A. Werger [ed.], Biogeography and ecology of southern Africa, 1301–1332. Dr. W. Junk, The Hague, The Netherlands.

WILSON, J. B., AND A. D. Q. AGNEW. 1992. Positive-feedback switches in plant communities. *Advances in Ecological Research* 23: 263–336.

ZAR, J. H. 1984. Biostatistical analysis. Prentice-Hall, Englewood Cliffs, New Jersey, USA.