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NICKEL HYPERACCUMULATION BY *THLASPI MONTANUM* VAR. *MONTANUM* (BRASSICACEAE): A CONSTITUTIVE TRAIT[1]

ROBERT S. BOYD[2] AND SCOTT N. MARTENS

Department of Botany and Microbiology and Alabama Agricultural Experiment Station,
Auburn University, Alabama 36849-5407; and
Department of Land, Air, and Water Resources University of California, Davis, California 95616

Adaptations to particular stresses may occur only in populations experiencing those stresses or may be widespread within a species. Nickel hyperaccumulation is viewed as an adaptation to high-Ni (serpentine) soils, but few studies have determined if hyperaccumulation ability is restricted to populations from high-Ni soils or if it is a constitutive trait found in populations on both high- and low-Ni soils. We compared mineral element concentrations of *Thlaspi montanum* var. *montanum* plants grown on normal and high-Ni greenhouse soils to address this question. Seed sources were from four populations (two serpentine, two non-serpentine) in Oregon and northern California, USA. Plants from all populations were able to hyperaccumulate Ni, showing Ni hyperaccumulation to be a constitutive trait in this species. Populations differed in their ability to extract some elements (e.g., Ca, Mg, P) from greenhouse soils. We noted a negative correlation between tissue concentrations of Ni and Zn. We suggest that the ability to hyperaccumulate Ni has adaptive value to populations growing on non-serpentine soil. This adaptive value may be a consequence of metal-based plant defense against herbivores/pathogens, metal-based interference against neighboring plant species, or an efficient nutrient scavenging system. We suggest that the Ni hyperaccumulation ability of *T. montanum* var. *montanum* may be an inadvertent consequence of an efficient nutrient (possibly Zn or Ca) uptake system.

Key words: adaptation; Brassicaceae; metal hyperaccumulation; nickel; serpentine soil; *Thlaspi montanum* var. *montanum.*

Many traits of organisms have been interpreted as adaptations to specific environmental conditions (selective pressures). It is not surprising to find such adaptations in populations that continually or periodically experience a given selective pressure. However, it is more intriguing to find populations that are far removed in time and/or space from a given selective pressure that possess the adaptation to it. If the adaptation is generally possessed by all members of the species it may be termed a constitutional (or constitutive) trait (Reeves and Baker, 1984) of the species. These ''pre-adapted'' populations may be explained in three ways: (1) by high rates of gene flow from populations experiencing the selective pressure, (2) by the low cost of the adaptation (resulting in neutral selective value), or (3) by multiple adaptive functions of a single trait (Verkleij and Schat, 1990; Meharg, Cumbes, and Macnair, 1993).

Plants growing on metalliferous soils often accumulate metallic elements, resulting in elevated tissue metal concentrations relative to plants on normal soils (Brooks, 1987). However, some metalliferous-soil species have remarkably high levels of metals in their tissues. These species, termed hyperaccumulators, may contain large amounts of Co, Cr, Cu, Mn, Pb, Ni or Zn (Brooks, 1987). Hyperaccumulators of Co, Cr, Cu, Pb, or Ni have concentrations of >1000 ppm dry mass, whereas hyperaccumulators of Mn or Zn are defined as those containing >10 000 ppm dry mass (Baker and Brooks, 1989).

Many hyperaccumulator species are endemic to metalliferous soils, so that it is relatively unusual to find a species that is capable of hyperaccumulation which also has populations growing on non-metalliferous substrates (Brooks, 1987). Reeves and Baker (1984) found that both serpentine and non-serpentine (in this case, calcareous) soil populations of the European Ni and Zn hyperaccumulator *Thlaspi goesingense* Halacsy (Brassicaceae) were capable of metal hyperaccumulation. *Thlaspi goesingense* therefore possessed a 'constitutional' metal uptake mechanism that was apparent only when plants grew on metalliferous soil. Reeves and Baker (1984) concluded that hyperaccumulation was not a microevolutionary response to metalliferous soils, but had wider ecological significance. The exact nature of this ecological significance is relatively unexplored.

Whether metal hyperaccumulation ability is restricted to populations found only on metalliferous soil has important implications for hypotheses dealing with the evolution and function of metal hyperaccumulation. For example, widespread constitutive metal hyperaccumulation may suggest that metal hyperaccumulation has an adaptive function on non-metalliferous soils. This function may differ from the defensive role that has been demonstrated for Ni hyperaccumulated by plants growing on metalliferous soil (Boyd and Martens, 1994; Boyd, Shaw, and Martens, 1994; Martens and Boyd, 1994). To our knowledge, the only evaluations to date of the constitutive nature of metal hyperaccumulation are the abovementioned research of Reeves and Baker (1984) with *T. goesingense* and more recent work with *T. caerulescens*

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[2] Author for correspondence and reprint requests.

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There are few known Ni hyperaccumulating species from continental North America, and most of them are endemic to serpentine soils (Reeves, MacFarlane, and Brooks, 1983; Reeves, 1988). One species, *Thlaspi montanum* L. var. *montanum*, is widespread in western North America, occurring in mountain ranges from Washington and Montana to northern Mexico (Holmgren, 1971). Some populations in California and Oregon occur on serpentine soils, whereas others are on non-mineralized substrates. Reeves (1988) reported that serpentine soil populations hyperaccumulate Ni, whereas non-serpentine populations do not. In this study, we investigate the elemental composition of populations of the Ni-hyperaccumulator *Thlaspi montanum* var. *montanum* when grown on high- and low-Ni substrates. Seeds were obtained from populations growing on serpentine and non-serpentine soils. Our objective was to examine the element uptake abilities of these populations, including their ability to hyperaccumulate Ni.

MATERIALS AND METHODS

Serpentine populations of *Thlaspi montanum* var. *montanum* were located 31 km apart in Siskiyou County, California, USA. One was collected from Baldy Mountain (1700 m elevation), located 10 km west of Happy Camp; the second was from Lake Mountain (2100 m), 11 km west of Scott Bar. Both sites are mapped as ultramafic rocks (partly to completely serpentinized) surrounded by larger expanses of metasedimentary rocks (Wagner and Saucedo, 1987).

Non-serpentine populations were located in the Warner Mountains of northeastern California and the Strawberry Range in north-central Oregon, USA, ~240 km east and 460 km NE (respectively) from the serpentine soil populations. The Warner population was found ca. 0.5 km southwest of Squaw Peak, Modoc County, at an elevation of 2500 m. The Strawberry population was ~0.8 km SSE of Strawberry Mountain, Grant County, at 2500 m elevation. The Warner site is mapped as andesite (Gay and Aune, 1958). The Strawberry Mountain site is mapped by Walker and MacLeod (1991) as various volcanic rocks (basalt, basaltic andesite, and andesite) with a small occurrence of ultramafic rocks within 5 km.

Seeds and aboveground plant biomass of *Thlaspi montanum* var. *montanum* were collected in July 1989 from the four populations. Soil was also collected by removing samples (to 10 cm depth) from at least three locations within each population. Soil samples were composited to form two samples for analysis from each collection site.

Soil samples were double-acid extracted using 20 mL of extractant (0.05 *N* HCl/0.025 *N* H_2SO_4) shaken with 5 g of dry soil for 5 min. The extract was analyzed for Ca, K, Mg, P, Cu, Fe, Mn, Zn, Mo, Al, Co, Cr, and Pb using an inductively-coupled argon plasma emission spectrometer (Jarrell-Ash ICAP 9000, Jarrell-Ash, Franklin, Massachusetts). Nickel was determined by a separate analysis of the extract using an atomic absorption spectrophotometer. Soil pH was measured with a pH meter, using a soil/water paste (1:1 ratio, v:v) for each sample. Random effects model one-way analysis of variance (ANOVA) was used to compare pH and levels of extractable soil elements between sites. Fisher's PLSD test was used for post-hoc means separations (Abacus Concepts, 1992).

Field-collected plant material was combined into two samples from each population for elemental analysis. Samples (0.5 g) were dry-ashed at 450°C, dissolved in 10 mL of 1 *N* HNO_3/1 *N* HCl, diluted to 100 mL with water and analyzed for Ca, K, Mg, P, Cu, Fe, Mn, and Zn using an inductively-coupled argon plasma spectrometer (Jarrell-Ash ICAP 9000). Nickel was determined by a separate procedure in which the sample was dry-ashed and acid-extracted as described above, and the Ni concentration determined using an atomic absorption spectrophotometer. Resulting Ni and other elemental contents were compared between populations by a random effects model one-way ANOVA. Post-hoc mean separations utilized Fisher's PLSD test (Abacus Concepts, 1992).

Plants were grown on greenhouse soil (ProMix) from seeds collected in the field. Greenhouse soil was either amended to ~700 ppm dry mass Ni by adding $NiCl_2$ or left unamended. Seeds were planted in 10 cm diameter pots filled with soil and topped with a layer of perlite. Plants were grown ~4 mo in a greenhouse in Auburn, Lee County, Alabama under a 16/8 h light/dark photoperiod and 30°/22°C thermoperiod. We also planted several pots of seeds from the Warner population on Zn-enriched soil, to which $ZnCl_2$ was added to yield a final concentration of ~700 ppm dry mass Zn. Due to limited seed supply, other populations were not planted on Zn-enriched soil.

Harvested biomass was combined into two replicates of each treatment to obtain adequate sample quantities for elemental analyses. Greenhouse-grown biomass samples were dry-ashed and analyzed as described above for field-collected samples. Data for the four populations grown on Ni-amended and unamended soils were analyzed by a random effects model two-way ANOVA, with site and soil type as main effects. An interaction term was not included in the ANOVA model to conserve statistical power. An additional ANOVA was performed on data from the Warner population. We used a random effects model one-way ANOVA to examine the effect on elemental concentrations of the three greenhouse soils used for this population (Ni-amended, Zn-amended, and unamended). Post-hoc mean separations for all significant ANOVAs utilized Fisher's PLSD test (Abacus Concepts, 1992).

RESULTS

Comparison of field-collected soils showed a number of significant differences, some of which differed consistently between serpentine and non-serpentine sites (Table 1). The serpentine soils had lower Ca and K levels and higher amounts of two metals, Co and Ni. Of these four elements, the soils differed most markedly in Ni, which was at least 332-fold higher in serpentine soils (83 ppm vs. 0.25 ppm; Table 1). There was also an evident trend for the Ca/Mg ratios, even though the means for the Warner (non-serpentine) and Lake (serpentine) populations were not significantly different (Table 1). The non-serpentine soils had mean Ca/Mg ratios >1, whereas these ratios for serpentine soils were <1. Other elements in Table 1 either failed to show consistent differences between soil types (e.g., Fe, Mn), or did not differ significantly between sites (e.g., P, Cu, etc.).

Elemental composition of field-collected plant material differed significantly between populations for some elements. Nickel contents of plants from serpentine sites were significantly higher than for plants from non-serpentine sites, showing on average a 14-fold difference (3600 vs. 250 ppm; Table 2). Calcium and P values also differed between populations, in that plants from non-serpentine sites had higher concentrations of both elements (Table 2). The Ca/Mg ratio showed an intermediate relationship, but non-serpentine plants tended to have higher Ca/Mg ratios than serpentine plants (Table 2). Concentrations of other elements listed in Table 2 did not differ significantly between populations.

Both collection site (population) and greenhouse soil type had significant effects on elemental composition of greenhouse-grown plants. Populations varied in composition for six elements (Fe, Ca, K, Mg, P, and Mn; Table

TABLE 1. Characterization of soils from the collection sites. Mean elemental concentrations are ppm (mg/Kg soil). Standard errors are in parentheses ($N = 2$). Means with the same superscript are not significantly different (Fisher's PLSD test, ∝ = 0.05).

	Nonserpentine sites		Serpentine sites	
Soil character	Strawberry	Warner	Lake	Baldy
Characters differing significantly (ANOVA, $P \leq 0.05$) among collection sites				
Ni	0.086 (0.014)^{a}	0.25 (0.090)^{a}	130 (18)^{b}	83 (21)^{b}
Ca	2300 (430)^{a}	3100 (30)^{a}	750 (110)^{b}	440 (220)^{b}
K	220 (64)^{a}	250 (13)^{a}	83 (5.5)^{b}	26 (5.0)^{b}
Co	0.30 (0.075)^{a}	0.66 (0.096)^{a}	5.5 (1.0)^{b}	3.9 (0.36)^{b}
Fe	12 (2.0)^{a}	33 (3.2)^{b}	45 (5.8)^{b}	32 (3.1)^{b}
Mn	39 (2.5)^{a}	27 (2.5)^{a}	83 (16)^{b}	40 (9.2)^{a}
Mg	250 (92)^{a}	650 (9.9)^{a}	1100 (130)^{b}	740 (140)^{a,b}
Cr	0.61 (0.12)^{a}	0.85 (0.013)^{a}	0.90 (0.087)^{a}	1.4 (0)^{b}
Ca/Mg ratio	10 (2.2)^{a}	4.9 (0.12)^{b}	0.72 (0.19)^{b,c}	0.56 (0.19)^{c}
Characters not differing significantly (ANOVA, $P > 0.05$) among collection sites				
pH	6.3 (0.15)	6.4 (0.10)	6.3 (0.10)	6.6 (0.10)
P	94 (70)	42 (1.9)	16 (2.5)	8.1 (0.29)
Cu	0.62 (0.17)	0.96 (0.059)	0.99 (0.037)	1.2 (0.16)
Zn	1.4 (0.27)	1.1 (0.19)	3.2 (0.68)	2.2 (0.54)
Mo	0.65 (0.24)	0.37 (0.0020)	0.32 (0.023)	0.23 (0.033)
Al	870 (380)	370 (1.9)	210 (1.4)	170 (21)
Pb	2.3 (0.75)	1.6 (0)	1.4 (0.15)	1.6 (0.30)

3). Mean separations for each of these elements showed a general trend for serpentine populations to have lower concentrations of these elements. For example, in all cases the lowest mean concentration of each of these elements occurred in plants from a serpentine population, and (with the exception of P) the highest mean concentration occurred in plants from a non-serpentine population (Table 3).

Greenhouse soil treatment (Ni-amended or unamended) resulted in significant effects on plant Fe, Zn, and Ni contents (Table 3). Iron and Zn concentrations were lower in plants growing on Ni-amended soil, whereas Ni concentrations were dramatically increased on Ni-amended soil. The magnitudes of these effects were quite different. For example, compare elemental concentrations between plants grown on unamended and Ni-amended soil. The decrease in Fe was most extreme for the Lake population plants (96 vs. 46 ppm, or 52%; Table 3). The decrease in Zn was most extreme for the Strawberry population plants (900 vs. 310 ppm, or 66%; Table 3). In contrast, the increase in Ni when plants were grown on Ni-amended soil was tremendous. The smallest increase was 55-fold for the Warner population, whereas the largest was 350-fold for the Lake population (Table 3).

Elemental analysis of plants from the Warner population grown on all three soil treatments (Ni-amended, Zn-amended, and unamended) showed a number of significant differences between these soil types (Table 4). Manganese was significantly decreased in plants grown on Zn-amended soil, whereas K and Mg were significantly increased. Zinc levels were lowest in plants grown on Ni-amended soil, intermediate (showing a 2.3-fold increase) in unamended soil, and highest in Zn-amended soil, showing a 14-fold increase over Ni-amended soil. Nickel was highest for plants from Ni-amended soil, reaching levels at least 55-fold that of plants growing on other soil

TABLE 2. Elemental analysis of *Thlaspi montanum* var. *montanum* field-collected material. Data are means ($N = 2$), with SE in parentheses, except for the Baldy location where $N = 1$ for all determinations other than Ni. Means with the same superscript are not significantly different (Fisher's PLSD test, ∝ = 0.05).

	Nonserpentine populations		Serpentine populations	
Element	Strawberry	Warner	Lake	Baldy
Elements differing significantly (ANOVA, $P \leq 0.05$) among populations				
Ni (ppm)	33 (25)^{a}	470 (30)^{a}	4300 (950)^{b}	2900 (50)^{b}
Ca (%)	1.2 (1.2)^{a}	0.85 (0.066)^{a}	0.34 (0.090)^{b}	0.30^{b}
P (%)	0.15 (0.0013)^{a}	0.16 (0.00075)^{a}	0.11 (0.0062)^{b}	0.069^{b}
Ca/Mg ratio	5.6 (0.86)^{a}	4.4 (0.24)^{a,b}	1.2 (0.63)^{c}	1.3^{b,c}
Elements not differing significantly (ANOVA, $P > 0.05$) among populations				
K (%)	0.95 (0.21)	0.99 (0.025)	0.82 (0.00050)	0.44
Mg (%)	0.22 (0.012)	0.20 (0.026)	0.34 (0.10)	0.22
Fe (ppm)	300 (35)	480 (100)	440 (280)	91
Zn (ppm)	130 (31)	320 (110)	110 (11)	26
Mn (ppm)	56 (7.0)	37 (5.2)	27 (4.8)	15
Co (ppm)	4.2 (2.1)	4.9 (1.4)	11 (1.4)	7.5
Cr (ppm)	1.6 (1.0)	0.85 (0.14)	2.6 (1.1)	3.0
Pb (ppm)	6.7 (4.3)	5.6 (0.47)	15 (1.8)	8.9
Cu (ppm)	5.5 (1.4)	4.1 (0.65)	4.2 (0.43)	1.7

TABLE 3. Elemental analyses of *Thlaspi montanum* var. *montanum* grown on Ni-amended and unamended greenhouse soil. Data are means (N = 2), with SE in parentheses. Elements are grouped according to the results of the two-way ANOVA testing for the significant influence of population and greenhouse soil treatment on mean element concentration. Statistical grouping is Fisher's PLSD test results for the population factor only, where different letters denote significantly different means (∝ = 0.05).

		Nonserpentine populations		Serpentine populations	
Element	Greenhouse soil treatment	Strawberry	Warner	Lake	Baldy
Greenhouse soil treatment significant					
Ni (ppm)	Ni-amended	3000 (500)	3300 (200)	3500 (3.0)	2200 (320)
	Unamended	18 (8.0)	60 (30)	10 (0)	10 (0)
Zn (ppm)	Ni-amended	310 (11)	560 (73)	560 (140)	390 (120)
	Unamended	900 (51)	1300 (260)	770 (170)	390 (27)
Population and greenhouse soil treatment significant					
Fe (ppm)	Ni-amended	73 (1.0)	91 (1.0)	46 (2.0)	42 (0)
	Unamended	80 (14)	120 (35)	96 (2.0)	45 (3.0)
	Statistical grouping	b,c	c	a,b	a
Population significant					
Ca (%)	Ni-amended	1.8 (0.16)	1.9 (0.050)	1.1 (0.012)	1.2 (0.10)
	Unamended	2.2 (0.35)	1.8 (0.020)	1.8 (0.28)	1.4 (0.17)
	Statistical grouping	c	b,c	a,b	a
K (%)	Ni-amended	1.5 (0.14)	2.9 (0.18)	1.0 (0.065)	1.1 (0.24)
	Unamended	1.5 (0.085)	1.4 (0.33)	1.5 (0.26)	1.1 (0.085)
	Statistical grouping	a,b	b	a	a
Ca (%)	Ni-amended	1.8 (0.16)	1.9 (0.050)	1.1 (0.012)	1.2 (0.10)
	Unamended	2.2 (0.35)	1.8 (0.020)	1.8 (0.28)	1.4 (0.17)
	Statistical grouping	c	b,c	a,b	a
K (%)	Ni-amended	1.5 (0.14)	2.9 (0.18)	1.0 (0.065)	1.1 (0.24)
	Unamended	1.5 (0.085)	1.4 (0.33)	1.5 (0.26)	1.1 (0.085)
	Statistical grouping	a,b	b	a	a
Mg (%)	Ni-amended	0.30 (0.020)	0.38 (0)	0.18 (0.020)	0.11 (0)
	Unamended	0.29 (0.010)	0.34 (0.0050)	0.29 (0.070)	0.14 (0)
	Statistical grouping	a,b	a	b	c
P (%)	Ni-amended	0.23 (0.010)	0.28 (0.025)	0.26 (0.020)	0.17 (0.015)
	Unamended	0.27 (0.015)	0.26 (0.010)	0.30 (0.035)	0.19 (0.015)
	Statistical grouping	a	a	a	b
Mn (ppm)	Ni-amended	150 (2.0)	190 (10.)	97 (11)	110 (17)
	Unamended	170 (14)	200 (9.0)	150 (3.0)	71 (17)
	Statistical grouping	b	c	a	a
None significant					
Cu (ppm)	Ni-amended	2.0 (0)	4.0 (0)	3.0 (1.0)	5.0 (1.0)
	Unamended	6.0 (0)	4.0 (0)	3.0 (1.0)	4.0 (0)

TABLE 4. Elemental analyses of *Thlaspi montanum* var. *montanum* from a nonserpentine seed source (Warner Mts.) grown on Ni-amended, Zn-amended, and unamended greenhouse soils. Data are means (SE in parentheses; N = 2). Means with the same superscript are not significantly different (Fisher's PLSD test, ∝ = 0.05).

	Greenhouse soil treatment		
Element	Unamended	Ni-amended	Zn-amended
Elements significantly affected (ANOVA, $P \leq 0.05$) by soil treatment			
Mn (ppm)	200 (9.0)^a	190 (10.0)^a	80 (4.0)^b
Mg (%)	0.34 (0.0050)^a	0.38 (0)^a	0.44 (0.020)^b
K (%)	1.4 (0.0033)^a	2.9 (0.18)^b	2.6 (0.15)^b
Zn (ppm)	1300 (260)^a	560 (73)^b	7600 (10)^c
Ni (ppm)	60 (30)^a	3300 (200)^b	9.0 (1.0)^a
Elements not significantly affected by soil treatment (ANOVA, $P > 0.05$)			
Ca (%)	1.8 (0.020)	1.9 (0.050)	2.3 (0.12)
P (%)	0.26 (0.010)	0.27 (0.025)	0.24 (0.050)
Fe (ppm)	120 (35)	91 (1.0)	87 (1.0)
Cu (ppm)	4 (0)	4 (0)	4 (0)

types. Plants from Zn-amended and unamended soils did not differ significantly from each other in Ni content.

DISCUSSION

The analysis of field-collected soils from this study highlights a number of typical serpentine soil features. The two serpentine soils were low in Ca, K, P, and had lower Ca/Mg ratios, whereas Co, Zn, and Ni levels were significantly higher than in the non-serpentine (in this case, volcanic) soils. These differences are typical for most serpentine soils (Proctor and Woodell, 1975). Chromium and magnesium levels of soils in our study did not vary significantly, although they are often higher in serpentine soils (Proctor and Woodell, 1975).

We also observed that plants from serpentine populations tended to have lower levels of several elements than plants from non-serpentine populations when grown on the same greenhouse soil type. Three of these elements (Ca, K, and P) also showed lower extractable levels in the serpentine soils, as noted above. We suggest that the lower elemental levels in greenhouse-grown plants may be explained by lower nutrient uptake abilities of populations from serpentine soils. Due to the lower nutrient concentrations of serpentine soils (Proctor and Woodell, 1975), uptake systems for these plants may be saturated at relatively low soil nutrient levels (Chapin, 1980).

Studies of constitutive metal tolerances in plants suggest several possible explanations for the maintenance of Ni hyperaccumulation ability in populations on non-serpentine soil (Verkleij and Schat, 1990; Meharg, Cumbes, and Macnair, 1993). These may be summarized as: (1) gene flow from populations on metalliferous soil to those on non-metalliferous soil, (2) low or no cost of Ni hyperaccumulation ability, and (3) existence of an adaptive advantage to hyperaccumulation ability on non-metalliferous soil. We will discuss each of these explanations in turn below.

Gene flow from nearby populations on metalliferous soils may maintain the ability of populations from non-metalliferous soils to hyperaccumulate Ni. In the case of *T. montanum* var. *montanum*, gene flow from nearby serpentine populations is unlikely. The nearest ultramafic rocks to the Strawberry Mt. site are only 4.2 km distant (Walker and MacLeod, 1991), but we do not know if they support a population of *T. montanum* var. *montanum*. However, Kruckeberg (1984) reports no ultramafic rocks are exposed in Modoc County, California, where the Warner Mts. are located. According to Jennings (1977), the closest ultrabasic rocks to the Warner Mts. population are 164 km to the west. Because *T. montanum* var. *montanum* is restricted to mountain ranges (Holmgren, 1971) and would be absent from the extensive intervening valleys, gene flow between the Warner Mt. population and a serpentine soil population is a highly unlikely event. We conclude that gene flow cannot explain the existence of Ni hyperaccumulation ability in this population.

The second potential explanation for constitutive Ni hyperaccumulation ability depends on the cost of the adaptation(s) that allow *T. montanum* var. *montanum* to hyperaccumulate Ni. The argument is that, if the adaptation(s) involved have little or no cost, their presence would not be deleterious for populations on non-metalliferous soils and there will be little or no selection against plants which possess that ability. Costs of adaptations are often difficult to assess, and the costs of metal hyperaccumulation have not been rigorously examined. However, Boyd and Martens (1994) and Martens and Boyd (1994) suggest that Ni hyperaccumulation may be inexpensive relative to other plant chemical defenses. Even if Ni hyperaccumulation has no cost, it is viewed as a fairly complex adaptation (Reeves, 1992), so that genetic drift would probably result in inactivation without adequate reinforcement by natural selection.

Finally, Ni hyperaccumulation ability may be maintained on non-serpentine soils by positive selective value on those soils. The phenomenon of metal hyperaccumulation by plants has a number of postulated explanations. These were summarized by Boyd and Martens (1992) as: (1) tolerance or disposal of metal from the plant, (2) drought resistance, (3) interference with neighboring plants, (4) inadvertent uptake, and (5) defense against herbivores and/or pathogens. All of these explanations, with the exception of the inadvertent uptake hypothesis, assign positive selective value to high metal levels in hyperaccumulators growing on metalliferous soil.

The above-listed benefits of hyperaccumulated metals might not occur for plants growing on non-metalliferous soils. Certainly, tolerance or disposal of metal would be unnecessary in such a case. The drought resistance explanation has not been tested in metal hyperaccumulating plants growing on high-metal soils. However, if the degree of drought resistance is positively correlated with metal content, then plants growing on low-metal soils (and therefore having low metal contents) would also have low levels of drought resistance. Interference with neighboring plants has been proposed as operating by causing accumulation of metal in the soil near the hyperaccumulating species that poisons less metal-tolerant species (Boyd and Martens, 1992). This function cannot be eliminated for populations from non-metalliferous soil, especially given the very high (for a non-serpentine population) Ni level in leaves of the Warner Mts. population (470 ppm). However, we did not observe strong spatial segregation of *T. montanum* var. *montanum* from other plant species growing in association with that population. In fact, we noticed that many *T. montanum* var. *montanum* individuals were growing under the canopies of small shrubs. This interference explanation therefore seems unlikely to have positive selective value on non-serpentine soil.

The defense hypothesis has received support from several recent studies that have shown that Ni hyperaccumulation poisons attacking pathogens (Boyd, Shaw, and Martens, 1994) and herbivores (Boyd and Martens, 1994; Martens and Boyd, 1994). On non-metalliferous soils this defense may not function because plant metal concentrations may be below toxic levels. For example, we found that *T. montanum* var. *montanum* plants grown on low-Ni greenhouse soil averaged low (10–60 ppm Ni) tissue metal contents (Table 3). Experiments with artificial diet amended with Ni showed that the crucifer folivore *Pieris rapae* was unaffected by 500 ppm Ni, but suffered complete mortality at 1000 ppm (Boyd and Martens, 1994; Martens and Boyd, 1994). However, we cannot unequivocally discard the defense hypothesis for populations on non-metalliferous soils, especially since wild-collected plants of one of the populations we examined (Warner Mts.) had a high Ni content (470 ppm). This level of metal may be high enough to injure pathogens or herbivores, even if it does not result in acute toxicity. Boyd, Shaw, and Martens (1994) found that growth of the bacterial pathogen *Xanthomonas campestris* pv. *campestris* was inhibited in nutrient medium at 400 ppm Ni. Ernst (1987) reported that Cu concentrations of 400 ppm in *Silene cucubalis* (= *S. vulgaris*) growing on metalliferous soils were toxic to larvae of a noctuid moth. Both these reports suggest that metals may have a defensive effect at levels less than those used to define hyperaccumulation (1000 ppm for both Ni and Cu).

The inadvertent uptake hypothesis suggests that Ni hyperaccumulation is the consequence of an effective nutrient-uptake mechanism that also can concentrate Ni when Ni is available in the soil (Boyd and Martens, 1992). We know of no direct evidence supporting this hypothesis as an explanation for metal hyperaccumulation, but suggest that the existence of constitutive Ni hyperaccumulation supports this idea. It has been reported that Ni hyperaccumulators have relatively high levels of

Zn. For example, Reeves, MacFarlane, and Brooks (1983) demonstrated high Zn levels in field-collected plants. Furthermore, Ni uptake in Ni hyperaccumulators is negatively correlated with uptake of other metals, notably Zn. In this study, we found that elevated Ni levels in plants grown on Ni-amended soil corresponded to decreased levels of both Zn and Fe (Table 3). We have not found this Ni/Fe relationship before, but the Ni/Zn antagonism has been reported for *T. montanum* var. *montanum* by Boyd and Martens (1994), for the annual Ni-hyperaccumulator *Streptanthus polygaloides* Gray (Brassicaceae) by Boyd, Shaw, and Martens (1994) and Martens and Boyd (1994), and for the Ni/Zn hyperaccumulator *Thlaspi caerulescens* from Europe and the southeast Asian Ni/Zn hyperaccumulator *Dichapetalum gelonioides* (A. J. M. Baker, University of Sheffield, unpublished data). This relationship suggests that Ni and (at least) Zn are in competition for sites in a common uptake system.

Other studies of both Ni hyperaccumulators and non-hyperaccumulators show that various divalent cations compete with Ni in uptake processes, implying common uptake/transport systems. Gabbrielli, Mattioni, and Vergnano (1991), working with the Ni-hyperaccumulator *Alyssum bertolonii* Desv. (Brassicaceae), concluded that Ni, Zn, and Co were subject to a common uptake system. Work with non-hyperaccumulators has shown similar relationships between some divalent metals. Cataldo, Garland, and Wildung (1978), working with soybean *(Glycine max* (L.) Merr.) seedlings, and Korner, Møller, and Jensen (1987), working with barley (*Hordeum vulgare* L.), concluded that Ni, Cu, and Zn shared a common carrier site.

Common to all of these studies is a relationship between Ni and Zn uptake. We suggest that hyperaccumulation ability, on more fertile, non-metalliferous soils, functions as an efficient uptake mechanism for Zn and possibly other mineral elements. On less fertile nickelliferous soils, this uptake mechanism leads to enhanced uptake of Ni and, if proper complexation (e.g., Lee et al., 1978; Homer et al., 1991) and storage (e.g., Vazquez et al., 1992) abilities are present, Ni hyperaccumulation.

It has been suggested that, in Ni hyperaccumulators, there is a positive relationship between Ca and Ni uptake. Studies of *Berkheya coddii* Roessl. (Asteraceae) from South African serpentines indicate that addition of Ca to serpentine soils enhances Ni uptake (A. W. Howes, University of Natal, unpublished data). These studies also indicated that Ca addition stimulated Ca port production by *Berkheya* roots and that Ni uptake occurred through these Ca ports. It has also been pointed out that Ca/Mg ratios of Ni hyperaccumulating species are often unusually elevated relative to other serpentine species (R. D. Reeves, Massey University, unpublished data). In this study, we found that the Ca/Mg ratio of plants grown on greenhouse soil varied with population, but was not affected by soil Ni content (Table 3). Differences in plant Ca/Mg ratio did not correlate with the soil type of these populations in the field (Table 3).

The inadvertent uptake hypothesis also is supported by recent research with the metal-tolerant plant *Holcus lanatus* (L.). Meharg, Cumbes, and Macnair (1993) investigated the constitutive tolerance of *H. lanatus* to arsenate-polluted sites and found arsenate-tolerant plants were present on both arsenate-polluted and unpolluted sites. They argued that arsenate and P shared a common high affinity uptake system in this species, and that arsenate tolerance stemmed from suppression of this system. This high affinity uptake system had low selective value in nutrient-poor habitats because P uptake in such habitats was diffusion limited rather than uptake limited. Therefore, maintenance of the presumably costly high affinity uptake system would be selected against. As a result, populations in nutrient-poor habitats had suppressed high affinity uptake systems. The inadvertent result was that, when faced with high levels of arsenate in the soil, they took up little and hence were pre-adapted to habitats with high levels of arsenate (Meharg, Cumbes, and Macnair, 1993).

The evidence is not available to allow a definitive conclusion regarding the above hypotheses dealing with the adaptive value of Ni hyperaccumulation ability on low-metal soils. An essential step to resolving this question is testing more metal hyperaccumulators for constitutive hyperaccumulation ability to determine how widespread the phenomenon is. Westerburgh (1994) reported constitutive tolerance to serpentine soil for a non-hyperaccumulating species. Several Ni-hyperaccumulating plant taxa have now been reported from both serpentine and non-serpentine substrates in various parts of the world (e.g., Brooks, Reeves, and Baker, 1992; Morrey et al., 1992; Roberts, 1992). Tests of the constitutive nature of Ni hyperaccumulation in these species would be valuable for resolving these competing hypotheses.

Finally, we speculate that if hyperaccumulation ability has adaptive value on non-metalliferous soil, then ‘‘latent hyperaccumulators’’ may exist. Latent hyperaccumulators are species that naturally grow only on non-metalliferous soil but, if grown on metalliferous soil, can hyperaccumulate metal (Boyd and Martens, 1992). We further suggest that one useful clue for the discovery of latent hyperaccumulators would be elevated tissue metal content of populations growing on non-metalliferous soil. For example, Ni concentrations of plants on ‘normal’ (non-metalliferous) soils usually do not exceed 10 ppm (Reeves, Brooks, and MacFarlane, 1981). However, Reeves, MacFarlane, and Brooks (1983) reported that Ni contents of non-serpentine populations of *Thlaspi montanum* var. *montanum* ranged from 3 to 345 ppm. They concluded that these populations were displaying a predisposition for Ni hyperaccumulation—a conclusion confirmed by the research reported here. We therefore suggest that other species with such a predisposition be tested to see if some might be latent Ni hyperaccumulators.

LITERATURE CITED

ABACUS CONCEPTS. 1992. StatView. Abacus Concepts, Berkeley, CA.

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

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 ecology of ultramafic (serpentine) soils, 279–289. Intercept, Andover.

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

———, J. SHAW, AND S. N. MARTENS. 1994. Nickel hyperaccumulation as a defense against plant pathogens. *American Journal of Botany* 81: 294–300.

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

———, R. D. REEVES, AND A. J. M. BAKER 1992. The serpentine vegetation of Goias State, Brazil. *In* A. J. M. Baker, J. Proctor, and R. D. Reeves [eds.], The ecology of ultramafic (serpentine) soils, 67–81. Intercept, Andover.

CATALDO, D. A., T. R. GARLAND, AND R. E. WILDUNG. 1978. Nickel in plants. **I**. Uptake kinetics using intact soybean seedlings. *Plant Physiology* 62: 563–565.

CHAPIN, F. S. III. 1980. The mineral nutrition of wild plants. *Annual Review of Ecology and Systematics* 11: 233–260.

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.

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.

GAY, T. E. AND Q. A. AUNE. 1958. Geologic Map of California: Alturas Sheet. Division of Mines, California Department of Natural Resources.

HOLMGREN, P. K. 1971. A biosystematic study of North American *Thlaspi montanum* and its allies. *Memoirs of the New York Botanic Garden* 21: 1–106.

HOMER, F. A., R. D. REEVES, R. R. BROOKS, AND A. J. M. BAKER. 1991. Characterization of the nickel-rich extract from the nickel hyperaccumulator *Dichapetalum gelonioides. Phytochemistry (Oxford)* 30: 2141–2145.

JENNINGS, C. W. 1977. Geologic map of California. California Geological Data Map Series, Division of Mines and Geology, California Department of Natural Resources.

KORNER, L. E., I. M. MØLLER, AND P. JENSÉN. 1987. Effects of Ca^{2+} and other divalent cations on uptake of Ni^{2+} by excised barley roots. *Physiologia Plantarum* 71: 49–54.

KRUCKEBERG, A. R. 1984. California serpentines: flora, vegetation, geology, soils, and management problems. University of California Press, Berkeley, CA.

LEE, J., R. D. REEVES, R. R. BROOKS, AND T. JAFFRÉ. 1978. The relation between nickel and citric acid in some nickel-accumulating plants. *Phytochemistry (Oxford)* 17: 1033–1035.

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

MEHARG, A. A., Q. J. CUMBES, AND M. R. MACNAIR. 1993. Pre-adaptation of Yorkshire fog, *Holcus lanatus* L. (Poaceae) to arsenate tolerance. *Evolution* 47: 313–316.

MORREY, D. R., K. BALKWILL, M.-J. BALKWILL, AND S. WILLIAMSON. 1992. A review of some studies of the serpentine flora of southern Africa. *In* A. J. M. Baker, J. Proctor, and R. D. Reeves [eds.], The ecology of ultramafic (serpentine) soils, 147–157. Intercept, Andover.

PROCTOR J., AND S. R. J. WOODELL. 1975. The ecology of serpentine soils. *Advances in Ecological Research* 9: 255–366.

REEVES, R. D. 1988. Nickel and zinc accumulation by species of *Thlaspi* L., *Cochlearia* L., and other genera of the Brassicaceae. *Taxon* 37: 309–318.

———. 1992. The hyperaccumulation of nickel by serpentine plants. *In* A. J. M. Baker, J. Proctor, and R. D. Reeves [eds.], The ecology of ultramafic (serpentine) soils, 253–277. Intercept, Andover.

———, AND J. M. BAKER. 1984. Studies on metal uptake by plants from serpentine and non-serpentine populations of *Thlaspi goesingense* Halacsy (Cruciferae). *New Phytologist* 98: 191–204.

———, 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.

———, R. M. MACFARLANE, AND R. R. BROOKS. 1983. Accumulation of nickel and zinc by western North American genera containing serpentine-tolerant species. *American Journal of Botany* 70: 1297–1303.

ROBERTS, B. A. 1992. Ecology of serpentinized areas, Newfoundland, Canada. *In* B. A. Roberts and J. Proctor [eds.], The ecology of areas with serpentinized rocks: a world view, 75–113. Kluwer, Dordrecht, The Netherlands.

VAZQUEZ, M. D., J. BARCELO, CH. POSCHENREIDER, J. MADICO, P. HATTON, A. J. M. BAKER, AND G. H. COPE. 1992. Localization of zinc and cadmium in *Thlaspi caerulescens* (Brassicaceae), a metallophyte that can hyperaccumulate both metals. *Journal of Plant Physiology* 140: 350–355.

VERKLEIJ, J. A. C., AND H. SCHAT. 1990. Mechanisms of metal tolerance in higher plants. *In* A. J. Shaw [ed.], Heavy metal tolerance in plants: evolutionary aspects, 180–193. CRC Press, Boca Raton, Florida.

WAGNER, D. L., AND G. J. SAUCEDO. 1987. Geologic map of the Weed Quadrangle. Regional geologic map series, map no. 4A. Division of Mines, California Department of Natural Resources.

WALKER, G. W., AND N. S. MACLEOD. 1991. Geologic map of Oregon. U. S. Geological Survey.

WESTERBURGH, A. 1994. Serpentine and non-serpentine *Silene dioica* plants do not differ in nickel tolerance. *Plant and Soil* 167: 279–303.