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The defensive function of Ni in plants: response of the polyphagous herbivore *Spodoptera exigua* (Lepidoptera: Noctuidae) to hyperaccumulator and accumulator species of *Streptanthus* (Brassicaceae)

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Abstract Metals sequestered by plants may defend them against herbivores and/or pathogens. We explored the effect of plant metal content on a polyphagous herbivore, *Spodoptera exigua*. Plant experiments used a Ni hyperaccumulator (*Streptanthus polygaloides*) and two Ni accumulator species (*S. breweri* and *S. tortuosus*). High- and low-Ni plants of each species were produced by growing plants on either Ni-amended or unamended soil. Mean leaf Ni contents for plants grown on Ni-amended soil and control soil, respectively, were: 1500 and 20 mg Ni kg^{-1} for *S. polygaloides*, 40 and 9 mg kg^{-1} for *S. breweri*, and 93 and 0.5 mg kg^{-1} for *S. tortuosus*. Neonate or second-instar *Sp. exigua* larvae were fed high- or low-metal leaves of each plant species, and survival and other parameters were monitored. High-Ni leaves of *S. polygaloides* were acutely toxic, resulting in 96% mortality within 10 days, whereas only 48% of larvae fed low-Ni leaves died. Low- and high-Ni leaves of *S. breweri* did not differ in their effects on larval survival, larval weight, adult weight, and duration of pupation. Leaves of *S. tortuosus* from high-Ni soil did not significantly affect larval survival relative to low-Ni leaves. However, larvae eating high-Ni leaves weighed significantly less and pupation was significantly delayed. Larval feeding experiments using artificial diet amended with Ni demonstrated a toxic threshold at 963 mg Ni kg^{-1} and a sublethal threshold at 535 mg Ni kg^{-1}. Because plant material containing less Ni had detectable sublethal effects, we suggest that Ni interacts with other plant qualities (including secondary defensive compounds) to produce those effects. We conclude that hyperaccumulated Ni is a potent defense against polyphagous folivorous insects, but suggest that the sublethal impacts of the lesser Ni levels found in accumulator plant species may play only a minor defensive role against herbivores.

Key words Serpentine · Elemental defense · Herbivory · Ni hyperaccumulation · Ni accumulation

Introduction

Hyperaccumulators are plants native to metalliferous soils that take up large quantities of metallic elements compared to co-occurring species (Brooks 1987). Baker and Brooks (1989) defined metal hyperaccumulators as plants with tissue concentrations of >1000 mg kg^{-1} dry weight for Ni, Cu, Co, Pb, or Cr, or >10,000 mg kg^{-1} for Zn or Mn. Nickel is the metal most frequently hyperaccumulated: 145 (66%) of the 220 metal hyperaccumulators discussed in the review by Baker and Brooks (1989) were hyperaccumulators of Ni.

The functional explanation for metal hyperaccumulation has received relatively little experimental attention. One hypothesis is that hyperaccumulated metal defends plant tissues against herbivores and/or pathogens (Boyd and Martens 1992). By manipulating soil metal content to produce high- and low-metal plants, Boyd and Martens (1994) demonstrated acute toxicity of high-metal leaves of the Ni hyperaccumulator *Thlaspi montanum* var. *montanum* to the folivore *Pieris rapae* L. Similar results were obtained using the Ni hyperaccumulator *Streptanthus polygaloides* Gray with several species of folivores (Martens and Boyd 1994). Pollard and Baker (1997) used the Zn hyperaccumulator *Thlaspi caerulescens* to demonstrate feeding deterrence due to hyperaccumulated Zn for several invertebrate folivore species. Pathogens also can be affected by elemental defenses. Boyd et al. (1994) demonstrated acute toxicity of hyperaccumulated Ni in *S. polygaloides* to a bacterial pathogen (*Xanthomonas campestris* pv. *campestris*), and inhibition of growth for

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a powdery mildew fungus (*Erysiphe polygoni*). It is now clear that hyperaccumulated metals can have a defensive function against at least some plant enemies (Boyd 1998). These metal-based defenses, termed "elemental defenses" by Boyd and Martens (1992), represent a chemical defense that supplements the more widespread secondary defensive compounds produced by most plants (Boyd 1998).

Evidence supporting the defensive function of hyperaccumulated metals is still limited, as experiments have been conducted using only a small number of folivorous insect species. Herbivores with broad host ranges must be capable of countering a wide variety of plant chemical defenses. Much of the laboratory-based work (e.g., Boyd and Martens 1994; Martens and Boyd 1994; Pollard and Baker 1997) has utilized species of *Pieris* L., which are generalist feeders on cruciferous plants. One objective of this research was to utilize an insect folivore with a very wide host range to determine whether metal hyperaccumulation could defend against a broadly polyphagous insect.

Most plant species growing on metalliferous substrates are not hyperaccumulators, but they usually have elevated metal contents. Reeves (1992) reported that tissue concentrations of 10–100 mg Ni kg^{-1} are common among non-hyperaccumulator species, relatively high concentrations compared to the typical values for Ni (0.3–3.5 mg Ni kg^{-1}) in most plants (Pais and Jones 1997). These serpentine soil species have been termed metal "accumulators" (Baker and Walker 1990). Recent demonstrations of the defensive role of hyperaccumulated metals prompted us to question whether lower metal levels, those below the threshold used to define hyperaccumulation, also may have a defensive function. The question is important, because if these lesser concentrations act defensively, then elemental defenses may be much more widespread and have a greater ecological impact than has been recognized previously. The second objective of this study was to determine if accumulated Ni also might have defensive properties, thus extending research from hyperaccumulator (e.g., Boyd and Martens 1994; Martens and Boyd 1994; Boyd et al. 1994; Pollard and Baker 1997) to accumulator species.

Materials and methods

Study species

Three plant species, all members of the genus *Streptanthus* (Brassicaceae), were selected for these experiments. *S. polygaloides* Gray is a Ni hyperaccumulator endemic to serpentine sites in the western foothills of California's Sierra Nevada. Analyses of field collections by Reeves et al. (1981) have shown >1000 mg Ni kg^{-1} in all plant parts (stems, roots, leaves, flowers, fruits, seeds). Seeds of this species were collected from a population in the Red Hills of Tuolumne County, California (Favre 1987), roughly corresponding to sample 6737 of Kruckeberg and Reeves (1995). Seeds collected from at least 50 individuals were used for our experiments. Kruckeberg and Reeves (1995) reported Ni values ranging from 2430 – 18,600 mg kg^{-1} for their collections of this species. *S. breweri* Gray also is endemic to serpentine soils (Kruckeberg 1984), but does not hyperaccumulate Ni. This species grows in the Coast Ranges of California (Munz and Keck 1968). Field collections of this species by Kruckeberg and Reeves (1995) showed a maximum of 13 mg Ni kg^{-1}, while specimens analyzed by Reeves et al. (1981) contained <9 mg Ni kg^{-1}. Seeds from at least 20 individuals of this species were collected from a population in Napa County, California, in the vicinity of sample 6742 of Kruckeberg and Reeves (1995). The third species, *S. tortuosus* Kell., is more cosmopolitan than the other two species. It can be found on serpentine and non-serpentine substrates in both the Coast Ranges and the Sierra Nevada of California (Munz and Keck 1968). Serpentine soil populations of *S. tortuosus* do not hyperaccumulate Ni. Kruckeberg and Reeves (1995) measured 12 mg Ni kg^{-1}, and serpentine soil specimens analyzed by Reeves et al. (1981) contained no more than 14 mg Ni kg^{-1}. Seeds of this species were collected from a population growing on serpentine soil along Washington Road, Nevada County, California. This site also supports a population of *S. polygaloides*, and approximates the location of sample 6732 of Kruckeberg and Reeves (1995). Seeds collected from approximately 15 individuals were used for our experiments.

The herbivore chosen for these experiments was *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae). Larvae of this insect have a very broad host range (Metcalf and Metcalf 1993) and hence were an appropriate selection as a representative polyphagous insect herbivore. Larvae used for these experiments were obtained from a laboratory colony established from insects collected from Alabama cotton fields and maintained on artificial diet (Chalfant 1975) at 28°C with a light-dark period of 12:12 h.

Foliar feeding trials

Plants were raised from seed in a greenhouse in Auburn, Lee County, Alabama. Two soil mixtures were used: one was a commercial greenhouse soil (ProMix) amended to *c.* 800 mg Ni kg^{-1} dry weight by thoroughly mixing anhydrous $NiCl_2$ (Fisher Scientific) into the soil. This soil was used to grow high-Ni plants, as has been done previously for *S. polygaloides* (e.g., Martens and Boyd 1994). The other soil was unamended ProMix, used to grow corresponding low-Ni plants.

Metal contents of experimental plants were determined from subsamples of leaf material used to feed larvae. Mature leaves were gathered and divided into two replicates each of high-Ni and control *S. polygaloides* and *S. tortuosus* leaves, and three replicates of high-Ni and control *S. breweri* leaves.

Although feeding trials did not occur simultaneously due to inconsistencies in the availability of larvae and plant material, all feeding trials were conducted under similar conditions and in the same location. During feeding trials, larvae were maintained at room temperature and under 24-h illumination. Fresh mature leaves or portions of leaves were provided every 1–2 days in sufficient quantity for larvae to feed ad libitum. Leftover leaf material was removed when fresh material was added. Details for feeding trials using each plant species are provided below.

S. polygaloides

This trial used neonate (0–12 h old) larvae. Five neonates were placed into each of nineteen 5-cm diameter petri plates. Ten of the petri plates were randomly selected to receive high-Ni *S. polygaloides* leaves, and the others received leaves from low-Ni plants. Larval survival was recorded every 3 days during a 9-day period. Surviving larvae were weighed on day 9 of the experiment. The experiment was terminated at that time because very few (4%) of the larvae fed high-Ni plants remained alive.

S. breweri

This trial used larvae *c.* 4 days old (second instar). Neonates were fed low-Ni *S. breweri* leaves from the time of hatching until the experiment was established 4 days later. This was done because 4-day-old larvae are larger and easier to manipulate than neonates and because plant material was relatively limited for this trial. Therefore, we used fewer replicates than in the *S. tortuosus* experiment described below. Four larvae were placed into each of 40 5-cm-diameter petri plates. Twenty of the plates were randomly selected to receive high-Ni leaves. Larvae in the remaining 20 plates were fed low-Ni leaves. Survival was recorded weekly until day 28 of the experiment, when all larvae were weighed. We continued feeding larvae until all either had died or pupated, but did not record date of mortality after day 30 of the experiment. Pupae were monitored daily for emergence, and pupation time and adult fresh weight were recorded.

S. tortuosus

Seven neonates were placed into each of 100 5-cm-diameter petri plates. Larvae in 50 plates were fed leaves of *S. tortuosus* grown on Ni-amended soil and the other larvae were fed control leaves. Survival was monitored at irregular intervals (every 4–9 days) for 2 months, by when all larvae had either died or pupated. Surviving larvae were weighed on day 22 and again upon pupation. Time to pupation (in days) also was recorded for all larvae reaching the pupal stage.

Artificial diet experiment

Artificial diet incorporation bioassays were performed as described by Moar et al. (1995). Diet Ni concentration was manipulated by the addition of $NiCl_2$. Earlier trials caused us to focus on a range of estimated diet Ni concentrations spanning 0–400 mg kg^{-1}. We collected a sample of diet from the highest Ni concentration from three experimental trials. Diet samples were analyzed for Ni content so that we could correct estimated Ni contents to actual (measured) Ni contents in mg Ni kg^{-1} dry weight of diet, using the same analytical procedure used to determine plant Ni contents. Larvae were reared in insect trays containing 24 wells, each about 3 ml in volume. Approximately 1–2 ml of warm diet was poured into each well of the insect trays and allowed to solidify. Two neonate larvae were placed into each well and the top of the tray was covered with mylar film. Trays were incubated at 28°C for 7 days and the survivors were counted. Each replicate contained at least eight Ni concentrations and the experiment was replicated four times. Control mortality was 3%. For two replicates, each surviving larva was weighed to the nearest 1 mg to provide data on sublethal Ni effects. Insect data for one replicate were excluded from LC_{50} analysis due to inconsistent survival patterns. However, those data were included in the overall analysis of the influence of Ni on larval survival, and the diet sample collected for measurement of Ni for that replicate was analyzed and used for determining the correction factor for diet Ni content. Survival and mean weight data for all samples from each run were averaged and expressed as a percentage of the control value for that run.

Tissue and diet elemental analyses

Plant tissue and artificial diet samples were dried at *c.* 60°C for 3 days. Samples were ground, dry-ashed at 485°C, further oxidized with boiling 1 M HNO_3, dissolved in 1 M HCl, and Ni content determined with an atomic absorption spectrophotometer (Instrumentation Laboratory, IL 251).

Data analysis

Nickel concentrations in plant tissue were analyzed by one-way analysis of variance (ANOVA) for each species to determine the effects of soil Ni amendment on plant Ni concentration. Data collected from leaf feeding trials represented either continuous variables (larval weights, survival times) or transition probabilities (survival between stages or to a certain date). Continuous variable data were analyzed by one-way ANOVA. Weight data were log-transformed prior to analysis to better meet the assumptions underlying ANOVA (Zar 1984). Transition probabilities for larvae or pupae from high- and low-Ni leaf treatments were compared using contingency table analysis.

Larval survival and weight data from the artificial diet experiment were analyzed by one-way ANOVA. Data were arcsine square root transformed prior to ANOVA to better satisfy the assumptions of ANOVA (Zar 1984). Post hoc mean separations were performed using Fisher's protected least significant difference (PLSD) test (Abacus Concepts 1992). Insect mortality data also were analyzed using probit analysis (POLO-PC, LeOra Software Inc., 1987) after correction for control mortality to determine LC_{50} values. Data from three replicates, spanning estimated Ni contents of 0–350 mg Ni kg^{-1}, were judged useful for this analysis and contributed to the LC_{50} calculations.

Results

Foliar feeding trials

Elevated soil Ni resulted in elevated plant Ni contents for all *Streptanthus* species. *S. polygaloides* leaves from plants grown on Ni-amended soil contained almost 1500 mg Ni kg^{-1}, approximately 74 times the Ni content of leaves from control soil plants (Table 1). The two accumulator species also contained significantly more Ni

Table 1 Tissue Ni contents of *Streptanthus* leaves used in *Spodoptera exigua* feeding trials expressed as mg Ni kg^{-1} dry weight. ANOVAs test whether leaves from plants of each species growing on Ni-amended soil (Ni+ leaf) differed significantly from leaves of plants of that species growing on unamended soil (control leaf)

	Streptanthus species		
Parameter	*S. polygaloides*	*S. breweri*	*S. tortuosus*
Mean Ni values (SE in parentheses)			
Ni+ leaf (mg kg^{-1})	1480 (37) $n=2$	40 (3.1) $n=3$	93 (19) $n=2$
Control leaf (mg kg^{-1})	20 (2.0) $n=2$	9.0 (4.2) $n=3$	0.50 (0.50) $n=2$
One-way ANOVA results			
df	1,2	1,4	1,2
F	1600	35.6	23.5
P	0.0006	0.0040	0.040

when grown on Ni-amended soil. *S. breweri* leaves contained about 4 times as much Ni, and *S. tortuosus* leaves 180 times as much Ni, than leaves of the corresponding species grown on control soil (Table 1).

Survival of *Sp. exigua* larvae was markedly reduced by a diet of high-Ni *S. polygaloides* leaves (Fig. 1). Contingency table analysis confirmed that survival at 9 days was significantly affected by leaf type ($\chi^2 = 25.8$, $df = 1$, $P < 0.0001$). Survival of larvae fed *S. breweri* and *S. tortuosus* leaves did not vary greatly between Ni+ and control leaves (Fig. 1). Contingency table analysis of survival data at the end of each feeding trial confirmed the lack of statistically significant survival differences (larvae fed *S. breweri* at 30 days: $\chi^2 = 0.476$, $df = 1$, $P = 0.49$; larvae fed *S. tortuosus* at 59 days: $\chi^2 = 1.69$, $df = 1$, $P = 0.19$).

Because of the rapid death of larvae fed high-Ni *S. polygaloides* leaves, few other data were collected from that experiment. Larvae fed high-Ni *S. polygaloides* grew slowly compared to those fed control leaves. Weight data taken at day 9 of the experiment showed a mean weight (±SE) of 35±3 mg ($n = 27$) for larvae fed control leaves. The two larvae still alive at that time that had eaten leaves from plants grown on Ni-amended soil each weighed less than 1 mg.

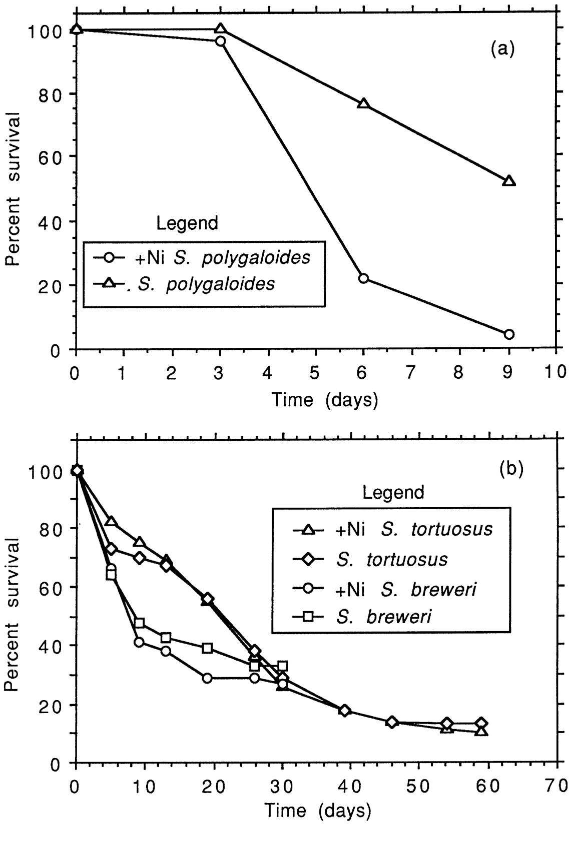

Fig. 1a, b Survival of *Spodoptera exigua* larvae feeding upon leaves of *Streptanthus* species grown on Ni-amended (800 mg Ni kg^{-1} dry weight) and unamended greenhouse soils. **a** Survival of larvae fed *S. polygaloides* (Ni hyperaccumulator) leaves ($n = 50$ for control leaves and $n = 45$ for Ni+ leaves). **b** Survival of larvae fed *S. breweri* or *S. tortuosus* (Ni accumulators) leaves. $n = 80$ for *S. breweri*, $n = 350$ for *S. tortuosus*

Several other parameters were analyzed for the *S. breweri* experiment, including larval weight, transition probabilities between developmental stages (larva/pupa/adult), adult weight, and duration of pupation (Table 2). None were significantly affected by leaf type (Table 2). Larvae fed control leaves weighed 1.6 times as much after 28 days, but the effect was not statistically significant (ANOVA of log weight: $F = 2.9$, $df = 1$, 47, $P = 0.094$). Results for log mean adult weight and duration of pupation showed no significant effects of leaf type (respective ANOVAs: $F = 0.64$, $df = 1{,}18$, $P = 0.44$; and $F = 0.019$, $df = 1{,}18$, $P = 0.89$). Analysis of transition probabilities (as proportion of larvae progressing from one life-history stage to another) revealed a near significant difference for the pupa-to-adult transition, which was 1.9 times greater for larvae fed control leaves ($\chi^2 = 3.68$, $df = 1$, $P = 0.055$). Probabilities for larva-to-adult and larva-to-pupa transitions also were higher for control leaves, but were not statistically significant (respective contingency table analyses: $\chi^2 = 2.06$, $df = 1$, $P = 0.15$; $\chi^2 = 0.379$, $df = 1$, $P = 0.54$).

Some of the experimental parameters from the *S. tortuosus* experiment did show statistically significant differences (Table 3). Mean larval weight was 1.9-fold higher for larvae fed control leaves compared to larvae feeding on Ni+ leaves. This difference was statistically significant (ANOVA: $F = 88$, $df = 1$, 338, $P < 0.0001$). Time to pupation also varied significantly, averaging 39 days for larvae fed Ni+ leaves but only 35 days for larvae fed control leaves (Table 3, ANOVA: $F = 5.6$, $df = 1$, 76, $P = 0.020$). Mean pupal weight and transition probabilities showed no significant differences (Table 3, pupal weight ANOVA: $F = 0.070$, $df = 1$, 79, $P = 0.93$; larva-to-pupa transition: $\chi^2 = 2.4$, $df = 1$, $P = 0.12$; pupa-to-adult transition: $\chi^2 = 0.11$, $df = 1$, $P = 0.74$; larva-to-adult transition: $\chi^2 = 1.2$, $df = 1$, $P = 0.27$).

Artificial diet

Calculated Ni concentrations in artificial diet differed greatly from measured concentrations. This was probably due to a difference between our estimated wet-weight to dry-weight conversion value for artificial diet and the actual value. Measured concentrations averaged 4.28 times the estimated concentrations, so that the range of Ni concentrations used in the experiments was 0–1710 mg Ni kg^{-1}, instead of the estimated 0–400 mg Ni kg^{-1}.

Nickel in artificial diet significantly affected larval survival (ANOVA of arc-sine transformed data: $F = 20$, $df = 16{,}34$, $P < 0.0001$). Nearly all larvae survived on diets containing up to 500 mg Ni kg^{-1}, but mortality increased to about 80% at 640–1070 mg Ni kg^{-1}, and nearly all died at >1500 mg Ni kg^{-1} (Fig. 2). Relative to the control, the first statistically significant decrease in survival was detected at 963 mg Ni kg^{-1} (Fisher's PLSD

Table 2 Summary of data (mean, SE in parentheses) from *Sp. exigua* larvae fed Ni+ or control leaves of *S. breweri*. The *P* value is the result of either one-way ANOVA or contingency table analysis to test whether larvae responded equally to leaves from plants growing on Ni-amended soil (Ni+ leaf) and plants growing on unamended soil (control leaf)

Experimental parameter	Ni+ leaf	Control leaf	*P*
Mean larval weight at 28 days (mg)	49 (8.4) $n=23$	77 (11) $n=26$	0.094[a]
Survival: larva to pupa (%)	16 $n=80$	20 $n=80$	0.38[b]
Survival: pupa to adult (%)	46 $n=13$	87 $n=15$	0.055[b]
Survival: larva to adult (%)	8.8 $n=80$	16 $n=80$	0.15[b]
Mean adult weight (mg)	29 (3.8) $n=7$	31 (1.7) $n=13$	0.44[a]
Duration of pupation (days)	14 (0.51) $n=7$	14 (0.38) $n=13$	0.89[a]

[a] ANOVA result
[b] Contingency table analysis result

Table 3 Summary of data from *Sp. exigua* larvae fed Ni+ or control leaves of *S. tortuosus*. The *P* value is the result of either one-way ANOVA or contingency table analysis to test whether larvae responded equally to leaves from plants growing on Ni-amended soil (Ni+ leaf) and plants growing on unamended soil (control leaf)

Experimental parameter	Ni+ leaf	Control leaf	*P*-value
Mean larval weight at 22 days (mg)	29 (1.5) $n=170$	55 (2.5) $n=170$	<0.0001[a]
Survival: larva to pupa (%)	9.7 $n=350$	13 $n=350$	0.12[b]
Survival: pupa to adult (%)	74 $n=34$	70 $n=47$	0.74[b]
Survival: larva to adult (%)	7.1 $n=350$	9.4 $n=350$	0.27[b]
Mean pupal weight (mg)	38 (1.5) $n=34$	47 (1.6) $n=47$	0.93[a]
Time to pupation (days)	39 (1.1) $n=33$	35 (1.1) $n=45$	0.020[a]

[a] ANOVA result
[b] Contingency table analysis result

test, $\alpha=0.05$). Probit analysis for survival data based on estimated Ni concentrations yielded a LC_{50} value (±95% confidence interval) of 228 (208–248) mg Ni kg^{-1} ($n=852$, slope $\pm$ SE $=8.07\pm0.525$). For corrected concentrations, the LC_{50} was 976 (890–1061) mg Ni kg^{-1}.

Nickel also significantly affected mean larval weight (ANOVA of arcsine transformed data: $F=17$, $df=16, 34$, $P<0.0001$). Mean weight declined slightly as Ni level (corrected concentrations) increased from 0 to 430 mg kg^{-1}, and then declined more sharply from 535 to 1070 mg kg^{-1} to a relatively low value that remained fairly constant through 1500 mg kg^{-1} (Fig. 2). Statistically significant decreases in mean larval weight (relative to the control) occurred for concentrations of 535 mg kg^{-1} Ni or greater (Fisher's PLSD test, $\alpha=0.05$).

Discussion

S. polygaloides plants that hyperaccumulated Ni were acutely toxic to *Sp. exigua* larvae. Despite the ability of *Sp. exigua* to counter a wide variety of plant defenses and hence maintain a broad host range (Metcalf and Metcalf 1993), hyperaccumulated Ni proved an insurmountable barrier to larval survival. We speculate that *Spodoptera*'s counterdefense mechanisms were unsuccessful due to the nature of elemental plant defenses (such as Ni), which cannot be chemically degraded. However, despite the failure of *Spodoptera* to circumvent this Ni-based defense, we strongly suspect that elemental defenses can be countered in several ways. Boyd and Martens (1998) suggested that diet dilution, tissue feeding specialization, and physiological metal tolerance are tactics that might allow herbivores to successfully attack metal hyperaccumulators. We are currently surveying insect species native to habitats occupied by metal hyperaccumulating plant species in order to discover herbivores that have adopted one or more of these evolutionary strategies.

We also conclude that the Ni in *S. polygaloides* leaves was probably responsible for their toxic effect on *Sp. exigua* larvae. We found rapid mortality when larvae ate high-Ni (1500 mg kg^{-1}) leaves. Results of the artificial diet experiment showed that the Ni content of these leaves was alone sufficient to explain this mortality, as almost all larvae fed artificial diet containing >1500 mg kg^{-1} Ni had died after 7 days.

Results from this and our other experimental work regarding the toxicity of Ni to insect folivores suggest that 1000 mg Ni kg^{-1} is a general toxic threshold. Earlier work with *Pieris rapae* fed Ni-amended artificial diet showed acute mortality at 1000 mg Ni kg^{-1} (Martens

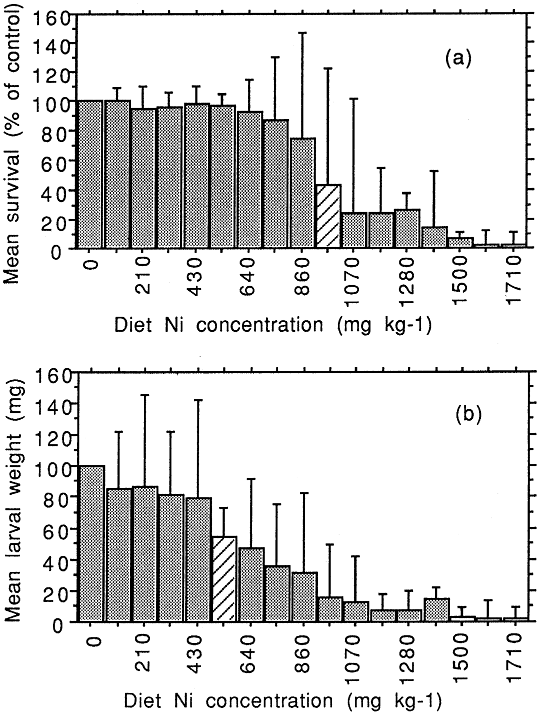

Fig. 2 **a** Survival after 7 days and **b** mean larval weight after 7 days for *Spodoptera exigua* larvae fed artificial diet amended with Ni. For survival data $n=3$, whereas $n=2$ for weight data. *Vertical bars* represent 95% confidence intervals. Diet Ni concentration (x-axis) is expressed on a dry weight basis to allow for comparison with measurements of Ni concentration in food plants (Table 1). The *hatched bar* in each graph represents the lowest Ni concentration that causes a significant increase in mortality (963 mg kg^{-1}) or decrease in mean larval weight (535 mg kg^{-1}) compared to control (0 mg Ni kg^{-1}) diet

and Boyd 1994). Our results using *Sp. exigua*, along with more comprehensive experiments and improved data analysis, showed an LC_{50} of 976 mg Ni kg^{-1}. The fact that the definition of Ni hyperaccumulation has been established at about this same concentration, 1000 mg Ni kg^{-1} (Brooks 1987; Reeves 1992), is suggestive. We speculate that plants capable of accumulating metal to this extent (or greater) achieve a level of protection from herbivores that is strongly favored by natural selection. Supporting experimental evidence is provided by Martens and Boyd (1994), who demonstrated the selective value of Ni hyperaccumulation when plants were grown under conditions of intense herbivory. They created an artificial mixed population of Ni-hyperaccumulating and non-hyperaccumulating *S. polygaloides* (by manipulating soil Ni content) and allowed larvae of *P. rapae* to feed upon the mixed population. Feeding damage and mortality were much higher among non-hyperaccumulating *S. polygaloides* plants.

Plant chemical defenses either can poison herbivores or simply deter them from attacking. Pollard and Baker (1997) reported that concentrated Zn in leaves of the Zn hyperaccumulator *Thlaspi caerulescens* deterred herbivory. The most striking case was for larvae of *P. brassicae*, which refused to eat high-Zn leaves. Such extreme deterrence was not seen in our experiment involving Ni and *Sp. exigua*, as we observed feeding by larvae offered either high- or low-Ni *S. polygaloides* leaves.

Results with the two accumulator *Streptanthus* species showed some sublethal effects of Ni+ leaves, but these effects were subtle compared to the rapid death of larvae fed high-Ni *S. polygaloides* leaves. These sublethal effects raise two questions: (1) what is the cause of these effects (is it elevated Ni?), and (2) are these subtle effects ecologically significant? We will address each of these questions in turn.

The cause of the sublethal effects cannot be determined from our experiments, but Ni levels seem unlikely to be the sole cause. That Ni played a role is suggested by our data. When grown on high-Ni soil, *S. tortuosus* leaves contained twice as much Ni as *S. breweri* leaves (93 and 40 mg Ni kg^{-1}, respectively). Sublethal effects, in this case reduced larval weights and extended pupation times, were documented for larvae fed high-Ni *S. tortuosus* leaves. Similar trends were present for larvae fed *S. breweri* leaves, although they were not statistically significant. We should note that, because of differences in the sample sizes used in our experiments, the experiment using *S. tortuosus* had greater statistical power than the experiment using *S. breweri*. This may explain the lack of statistical significance in the *S. breweri* experiment.

Results from the artificial diet experiment show that the Ni levels we measured in *S. tortuosus* and *S. breweri* do not fully explain the sublethal effects we documented. Levels of Ni needed to cause decreased larval growth in the artificial diet experiment were 535 mg kg^{-1} or greater, yet leaves of the accumulator plants growing on Ni-amended soil averaged no more than 100 mg kg^{-1}. It is possible that nutritional features of the artificial diet (that cause it to differ from *Streptanthus* leaves) may have resulted in greater Ni tolerance by the *Sp. exigua* larvae feeding upon it. In addition, we suspect that longer-term artificial diet experiments may show significant sublethal effects at lower Ni concentrations, but such experiments have not yet been conducted. For our experiments, however, we suggest that the Ni in the accumulator plant species may have interacted with another factor, possibly secondary defensive compounds, to produce the sublethal effects noted here. Members of the Brassicaceae often produce glucosinolates (Howe and Westley 1988), and these compounds are known from *Streptanthus* (Rodman et al. 1981). Currently, interactions between elemental and other chemical plant defenses appear to be unexplored (Boyd 1998).

Perhaps the more important question is not causal but one of ecological consequence. Unfortunately, this is a difficult question because sublethal defenses are themselves paradoxical (Clancy and Price 1987). For example, slowed herbivore growth may result in prolonged exposure to predators and thus be a positive effect of a sublethal plant defense (e.g., Haggstrom and Larsson 1995; Benrey and Denno 1997). On the other hand, a sublethal defense may cause increased damage by stimulating an herbivore to increase its feeding rate to compensate for negative physiological effects of the sublethal defense on the herbivore (e.g., Price et al. 1980). In the case of metal accumulator plants, we have shown that sublethal effects can occur in a highly controlled setting. Future studies should determine if these sublethal effects have selective value under natural conditions. At this point, however, we can conclude that accumulator plants are not nearly as well defended against polyphagous insect herbivores as are Ni hyperaccumulators such as *S. polygaloides*.

Finally, if the sublethal effects reported here for Ni accumulators have an impact on herbivores and their host choices, our results may have implications for the evolution of Ni hyperaccumulation. Data from larvae fed *S. tortuosus* leaves suggest that Ni accumulation may have some selective value. This implies that increased Ni uptake and sequestration abilities may be selected by herbivore pressure. Pollard and Baker (1997) demonstrated some heritability of Zn hyperaccumulation ability in the Zn hyperaccumulator *Thlaspi caerulescens*. Since the features required to hyperaccumulate Ni are most likely heritable, herbivory may act as a selective force that, by favoring survival of plants with higher Ni contents, favors the evolution of Ni hyperaccumulation. We suggest that Ni hyperaccumulation may have evolved through step-wise elevation of metal contents that proved advantageous due to lower herbivore damage inflicted upon individuals containing higher levels of metals.

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References

Abacus Concepts (1992) StatView. Abacus Concepts, Berkeley

Baker AJM, Brooks RR (1989) Terrestrial higher plants which hyperaccumulate metallic elements – a review of their distribution, ecology and phytochemistry. Biorecovery 1:81–126

Baker AJM, Walker PL (1990) Ecophysiology of metal uptake by tolerant plants. In: Shaw AJ (ed) Heavy metal tolerance in plants: evolutionary aspects. CRC, Boca Raton, pp 155–177

Benrey B, Denno RF (1997) The slow-growth-high-mortality hypothesis: a test using the cabbage butterfly. Ecology 78:987–999

Boyd RS (1998) Hyperaccumulation as a plant defensive strategy. In: Brooks RR (ed) Plants that hyperaccumulate heavy metals. CAB International, Wallingford, pp 181–201

Boyd RS, Martens SN (1992) The raison d'etre of metal hyperaccumulation by plants. In: Baker AJM, Proctor J, Reeves RD (eds) The vegetation of ultramafic (serpentine) soils. Intercept, Andover, pp 279–289

Boyd RS, Martens SN (1994) Nickel hyperaccumulated by *Thlaspi montanum* var. *montanum* is acutely toxic to an insect herbivore. Oikos 70:21–25

Boyd RS, Martens SN (1998) The significance of metal hyperaccumulation for biotic interactions. Chemoecology 8:1–7

Boyd RS, Shaw J, Martens SN (1994) Nickel hyperaccumulation defends *Streptanthus polygaloides* (Brassicaceae) against pathogens. Am J Bot 81:294–300

Brooks RR (1987) Serpentine and its vegetation: A multidisciplinary approach. Dioscorides, Portland

Chalfant RB (1975) A simplified technique for rearing the lesser cornstalk borer (Lepidoptera: Phyciditae). J Ga Entomol Soc 10:32–33

Clancy KM, Price PW (1987) Rapid herbivore growth enhances enemy attack: Sublethal plant defenses remain a paradox. Ecology 68:733–737

Favre RM (1987) A management plan for rare plants in the Red Hills of Tuolumne County, California. In: Elias TS (ed) Conservation and management of rare and endangered plants. California Native Plant Society, Sacramento, pp 425–427

Haggstrom H, Larsson S (1995) Slow larval growth on a suboptimal willow results in high predation mortality in the leaf beetle *Galerucella lineola*. Oecologia 104:308–315

Howe HF, Westley LC (1988) Ecological relationships of plants and animals. Oxford University Press, Oxford

Kruckeberg AR (1984) California serpentines: flora, vegetation, geology, soils, and management problems. University of California Press, Berkeley

Kruckeberg AR, Reeves RD (1995) Nickel accumulation by serpentine species of *Streptanthus* (Brassicaceae): field and greenhouse studies. Madroño 42:458–469

Martens SN, Boyd RS (1994) The ecological significance of nickel hyperaccumulation: a plant chemical defense. Oecologia 98:379–384

Metcalf RL, Metcalf RA (1993) Destructive and useful insects: their habits and control. McGraw-Hill, New York

Moar WJ, Pustazi-Carey M, Mack TP (1995) Toxicity of purified proteins and the HD-1 strain from *Bacillus thuringiensis* against lesser cornstalk borer (Lepidoptera: Pyralidae). J Econ Entomol 88:606–609

Munz PA, Keck DD (1968) A California flora. University of California Press, Berkeley

Pais I, Jones JB Jr (1997) The handbook of trace elements. St. Lucie, Boca Raton

Pollard AJ, Baker AJM (1997) Deterrence of herbivory by zinc hyperaccumulation in *Thlaspi caerulescens* (Brassicaceae). New Phytol 135:655–658

Price PW, Bouton CE, Gross P, McPheron BA, Thompson JN, Weis AE (1980) Interactions among three trophic levels: influence of plants on interactions between insect herbivores and natural enemies. Annu Rev Ecol Syst 11:41–65

Reeves RD (1992) The hyperaccumulation of nickel by serpentine plants. In: Baker AJM, Proctor J, Reeves RD (eds) The vegetation of ultramafic (serpentine) soils. Intercept, Andover, 253–277

Reeves RD, Brooks RR, Macfarlane RM (1981) Nickel uptake by Californian *Streptanthus* and *Caulanthus* with particular reference to the hyperaccumulator *S. polygaloides* Gray (Brassicaceae). Am J Bot 68:708–712

Rodman JE, Kruckeberg AR, Al-Shahbaz IA (1981) Chemotaxonomic diversity and complexity in seed glucosinolates of *Caulanthus* and *Streptanthus* (Cruciferae). Syst Bot 6:197–222

Zar JH (1984) Biostatistical analysis. Prentice-Hall, Englewood Cliffs