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Aphids are unaffected by the elemental defence of the nickel hyperaccumulator *Streptanthus polygaloides* (Brassicaceae)

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Summary. Nickel hyperaccumulation, resulting in plant Ni contents of >1000 mg kg^{-1} dry mass, has been shown to defend plants against folivorous herbivores. We determined whether this elemental defence tactic protected hyperaccumulating plants from attack by a phloem-feeding herbivore. We used the pea aphid, *Acyrthosiphon pisum*, and the Ni-hyperaccumulating plant *Streptanthus polygaloides*. Aphids were allowed to colonize mixed arrays of *S. polygaloides* in which plants either were hyperaccumulating Ni, not hyperaccumulating Ni and treated with a systemic insecticide, or not hyperaccumulating Ni. Aphid numbers g^{-1} dry mass of plant biomass were lowest for the insecticide treatment, intermediate for low-Ni plants, and highest for plants hyperaccumulating Ni. Artificial liquid aphid diet, amended with varying levels of Ni, resulted in decreased aphid survival at 2500 mg kg^{-1} Ni dry mass (or 5.03 m*M* Ni). We concluded that Ni levels in the phloem of hyperaccumulating plants of *S. polygaloides* were <5.03 m*M* and, as a result, were not effective in defending plants against aphid attack.

Key words. Aphid – elemental defences – *Streptanthus polygaloides* – nickel – heavy metals

Introduction

Plants are defended against herbivores and pathogens by a wide variety of secondary compounds (Levin 1976; Berenbaum & Seigler 1993; Smith 1996). These chemical defences are almost always synthesized by a plant's own biochemical machinery (Berenbaum 1995). However, some plants, termed hyperaccumulators (Brooks 1987), extract from the soil and store in their tissues unusually high levels of some elements. Hyperaccumulators of metals are defined as plants containing more than 1000 mg kg^{-1} dry mass of Ni, Cu, Co, or Pb, or more than 10,000 mg kg^{-1} of Zn or Mn (Baker & Brooks 1989).

The function of hyperaccumulated metals is relatively unexplored. Boyd & Martens (1992) reviewed five explanations for metal hyperaccumulation but focused on the hypothesis that hyperaccumulated metals deter attack by herbivores or pathogens, thus serving as "elemental defences" (Boyd & Martens 1992). Recent experimental work with Ni-hyperaccumulating plants (see review by Boyd 1998) has supported this hypothesis. Elemental defences differ from the more common secondary chemical defences of plants in two important ways: 1) elemental defences are extracted from the soil rather than synthesized within the plant; and, 2) herbivores or pathogens that encounter elemental defences cannot detoxify them via chemical degradation, due to the elemental nature of these defences (Martens & Boyd 1994).

No plant defensive tactic is completely effective against all plant enemies (Grubb 1992; Belovsky & Schmitz 1994), and this is probably the case for elemental defences. In a recent review, Martens & Boyd (1998) predicted that metal-based defences may be ineffective against some herbivores or pathogens. They suggested that circumvention of elemental defences could occur by three mechanisms: diet dilution, metal tolerance of the herbivore/pathogen, or avoidance of the metal during feeding. Diet dilution occurs when a generalist herbivore consumes a small amount of hyperaccumulator plant tissue mixed with a large amount of low-metal food from other plant species. In this way, the total metal dose in the diet would be lowered to a non-toxic level. Metal tolerance is the ability of an herbivore/pathogen to consume a diet high in metals without ill effect, presumably as a result of physiologic adaptation (Hopkin 1989). Avoidance is selective feeding upon tissues or cells within the plant that are low in metals and hence are relatively undefended.

To our knowledge, previous tests of the defensive effectiveness of hyperaccumulated metals have focused on folivores such as *Pieris rapae* [L.] (Boyd & Martens 1994; Martens & Boyd 1994) or slugs and locusts (Pollard & Baker 1997). These herbivores consume entire leaves and do not demonstrate feeding specialization that might result in the avoidance of elemental defences. In contrast, aphids are plant ectoparasites that tap phloem tissue to obtain sugar-rich phloem fluid (Dixon 1985). If phloem fluid of hyperaccumulators contains only small amounts of metals, then aphids may avoid the metal-based defence and successfully feed on metal-hyperaccumulating plants.

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This study explores the effectiveness of hyperaccumulated Ni as a defence against attack by aphids. The specific questions addressed in this paper are:

1) Do aphids exhibit a preference when presented with a mixed population of Ni-hyperaccumulating and non-hyperaccumulating plants of the same species?
2) What is the minimum dietary Ni concentration required to reduce aphid survival?
3) Do aphids feeding on high-Ni plants contain elevated levels of Ni, compared to aphids feeding on low-Ni plants?

Methods

Experimental Organisms: We used the Ni hyperaccumulator *Streptanthus polygaloides* Gray as the test plant species. This winter annual is endemic to serpentine areas in the western foothills of the Sierra Nevada in California, USA (Reeves *et al*. 1981). Nickel hyperaccumulation is present in all populations of this species that have been examined, and Ni is present at concentrations of more than 1000 mg kg^{-1} dry mass in all plant organs tested (Reeves *et al*. 1981).

The Ni content of *S. polygaloides* plants can be manipulated experimentally by controlling the metal content of the soil on which plants are grown. Prior experiments (*e.g*., Martens & Boyd 1994; Boyd, Shaw & Martens 1994) have shown that aboveground plant Ni contents of *S. polygaloides* can be elevated up to 270-fold by growing plants on soil amended with $NiCl_2$, making this species suitable for experimentally manipulating plant Ni content. Seeds for the experiments reported here were collected from plants growing in the U. S. Bureau of Land Management's Red Hills Management Area in Tuolumne County, California.

The pea aphid (*Acyrthosiphon pisum* [Harris]; Homoptera: Aphididae) was used as the experimental aphid species. This species was selected due to its availability as a pest in glasshouses at Auburn University, Auburn, Alabama, USA, and for its ability to utilize *S. polygaloides* as a host. A population of *A. pisum* was maintained in a glasshouse at Auburn University by rearing them on young broccoli (*Brassica oleracea* L.) plants. This colony was used to provide insects for the experiments reported here.

Preference Experiment: *Streptanthus polygaloides* plants were raised from seed in pots 6.5 cm in diameter and 25 cm in height. Two soils were used: one was a commercial glasshouse soil (ProMix®) amended to *ca*. 800 mg Ni kg^{-1} dry mass by thoroughly mixing powdered $NiCl_2$ (Fisher Scientific) into the soil (''amended'' soil treatment). The second soil was ProMix (''unamended'' soil treatment). The pots were arranged in an alternating pattern in rectangular arrays and placed in a glasshouse in Auburn, Alabama. Plants were grown under a 12 hr photoperiod (natural fall-season sunlight extended by fluorescent light at night) at a temperature of *ca*. 20°C. Five weeks after sowing, half of the unamended soil pots were randomly selected for treatment with the systemic insecticide, Temik®. Temik was added at the maximum labeled rate (22.5 kg ha^{-1}) to the soil of each selected pot. Temik treatment was used so that we could compare the defensive effectiveness of Ni to a non-elemental ''defence'' of known effectiveness. In final condition, each array contained five pots of each treatment (Ni-amended soil, unamended soil + Temik, unamended soil). A total of eight arrays were used for this experiment.

Aphids from a colony maintained on young broccoli plants in the same glasshouse were transferred to the experimental arrays when plants were 8 wk old. In total, several hundred aphids (forming a mixture of all available ages) were scattered over the arrays and the resulting aphid populations were allowed to develop for three weeks. After that time, we harvested the above-ground plant material from each pot and counted the number of aphids present on each pot's plant material. Plant biomass from each pot was dried for several days at *ca*. 60°C and weighed.

Biomass and aphid count data were expressed as a mean per pot for each treatment within an array. Aphid count per unit biomass was used as the dependent variable in a randomized complete block analysis of variance (ANOVA). Soil type (Ni-amended, unamended, unamended + Temik) was the treatment variable while the array in which each pot was located was used for the blocking factor in the ANOVA. Aphid counts were expressed per unit dry mass of plant biomass because we observed plant biomass differences between the soil treatments used in this experiment. We suspected that aphid population size would correlate positively with plant size and, by scaling population size to biomass per pot, sought to thereby remove the influence of plant size on the results of this analysis. Multiple comparisons of means utilized Fisher's Protected Least Significant Difference (PLSD) test (Abacus Concepts 1992).

Plant biomass samples from the arrays were analyzed for elemental composition. All plant material for each soil treatment in each array was combined and ground. A subsample of the combined material was dry-ashed at 485°C, further oxidized with boiling 1 *N* HNO_3, dissolved in 1 *N* HCl, and analyzed for Ca, K, Mg, P, Cu, Fe, Co, Cr, Mo, Al, Pb, Mn, and Zn using an inductively-coupled argon plasma spectrometer (Jarrell-Ash, ICAP 9000). Nickel was determined by using the same extract with an atomic absorption spectrophotometer (Instrumentation Laboratory, IL 251). Elemental concentrations were analyzed by separate one-way ANOVAs and PLSD tests for each element to determine the effects of soil treatments.

No-choice feeding experiment: Seeds of *S. polygaloides* were sown into 15-cm diameter pots filled with one of three treatments of ProMix potting soil. Soil treatments were 1000 mg Ni kg^{-1} soil, 1000 mg Ca kg^{-1} soil, and unamended soil. Amended soils were produced by thoroughly mixing powdered $NiCl_2$ or $CaCl_2$ into the potting mix. The Ca-amended soil was included as a partial control, in case the addition of chloride anions affected plant characteristics or aphid survival and reproduction. Plants were grown under a 12 hr photoperiod (natural winter-season sunlight extended by fluorescent light at night) in a glasshouse at Auburn University at *ca*. 20°C for 8–12 wk.

Small cages were used to isolate test aphids on a portion of the stem of the inflorescence of flowering *S. polygaloides* plants. Flowering plants were used because their habit of bolting while flowering allowed access to relatively long sections of the elongating stem. Cages were hinged plastic boxes *ca*. 2 cm on a side. One side of each cage had a hole (*ca*. 1 cm diameter) cut into it that was covered with screen of very fine mesh to allow air to enter the cage. Aphids were placed inside a cage, the cage was closed over a portion of stem, the sites where the stem entered and exited the cage were sealed with modeling clay to prevent escape of the aphids, and the cage was affixed to a small stake to prevent the inflorescence from bending due to the mass of the cage.

Ten pots of flowering *S. polygaloides* for each soil treatment were selected for this experiment. A cage was placed on the stem of one plant in each pot, three aphids were placed in each cage, and the number of aphids present in the cage was counted after they were allowed to feed and reproduce for 4 d. Aphid counts were analyzed by Analysis of Covariance (ANCOVA), using above-ground plant dry mass per pot as a covariate to account for possible differences in plant vigour. After experiments were concluded, above-ground plant material from all pots of each soil treatment was harvested and divided into two composite samples for each soil treatment. Samples were dried at 60°C and analyzed for Ni and other elements as described above.

Artificial diet experiment: Plants growing on high-Ni soil may differ in characteristics other than Ni content, as has been shown by earlier experiments with *S. polygaloides* (Boyd *et al*. 1994; Martens & Boyd 1994). Thus, an effect of Ni-hyperaccumulating plants on aphids may be due to factors other than Ni. In order to determine the toxicity threshold of Ni to *A. pisum*, aphids were fed artificial diets containing Ni concentrations ranging from 0–1000 mg kg^{-1} dry mass. Artificial pea aphid diet was purchased from Bio-Serv Inc. (Frenchtown, NJ, USA). Diet was amended with $NiCl_2$ to determine the minimum Ni concentration required to reduce aphid survival, and amended with $CaCl_2$ as a partial control to determine if there was a significant effect of either chloride concentration or total added solute concentration. Nickel concentrations used were 0, 1000, 2500, 5000, 10,000, and 15,000 mg Ni kg^{-1} dry mass and Ca concentrations used were 0, 2500, 5000, 10,000, and 15,000 mg Ca kg^{-1} dry mass. Samples of diet from each concentration were dried, ground, and analyzed for elemental composition in the same manner described above for plant material.

Element	Soil treatment		
	Low-Ni	Low-Ni + Temik	High-Ni
Ni (mg kg^{-1})	68[a] (13)	53[a] (10)	6000[b] (130)
Co (mg kg^{-1})	0.74[a] (0.16)	0.66[a] (0.04)	5.9[b] (0.06)
Pb (mg kg^{-1})	1.9[a] (0)	1.6[a] (0.30)	15[b] (1.6)
Zn (mg kg^{-1})	740[a] (74)	610[a] (23)	600[a] (13)
Cu (mg kg^{-1})	9.0[a] (0.11)	7.7[a] (0.11)	9.4[a] (0.54)
Fe (mg kg^{-1})	91[a] (4.6)	85[a] (1.2)	87[a] (2.3)
Mn (mg kg^{-1})	200[a] (58)	190[a] (47)	150[a] (6.3)
Ca (%)	2.4[a] (0.26)	2.0[a] (0.02)	2.7[a] (0.10)
K (%)	5.0[a] (0.02)	5.0[a] (0.18)	4.4[a] (0.46)
Mg (mg kg^{-1})	1500[a] (200)	1300[a] (12)	2200[a] (160)
P (mg kg^{-1})	8900[a] (800)	8300[a] (60)	8500[a] (800)
Cr (mg kg^{-1})	2.2[a] (0.14)	2.0[a] (0)	2.6[a] (0.28)
Mo (mg kg^{-1})	4.8[a] (0.53)	4.0[a] (0.41)	3.7[a] (0.46)
Al (mg kg^{-1})	170[a] (4.9)	140[a] (59)	160[a] (40)

Table 1 Elemental concentrations in above-ground biomass of plants from the choice experiment. Treatments were low-Ni soil, low-Ni soil + Temik, and high-Ni soil. Data are means (SE) with N = 2. Mean values for an element that differ significantly ($P < 0.05$; Fisher's PLSD test) between treatments for which an overall significant treatment effect was determined (one-way ANOVA, $P < 0.05$) are denoted by differing superscripts

Aphids were fed artificial diet following a method similar to that of Mittler & Kleinjan (1970), wherein diet was encapsulated by stretched Parafilm® that the aphids then punctured with their stylets to feed. In our experiments, small aphid cages were made from a 2-cm long section of 3-cm diameter PVC pipe. A piece of fine-mesh screen was glued to one end. Aphids were placed into the cage and a piece of Parafilm was stretched over the unscreened end. Several ml of diet were placed on the center of the Parafilm, and a second Parafilm layer was stretched over the first. We included an additional treatment, de-ionized water, in an attempt to determine if aphid mortality resulted from starvation or toxicity of the diet.

This experiment was conducted seven times, using four or six aphids in each cage. The number of aphids per cage varied between runs but was consistent for all cages during each run. Aphid survival was noted after 3 d. Survival data were expressed as a decimal fraction, log-transformed to better satisfy the assumptions behind ANOVA (Zar 1984), and data for each cation (Ni or Ca) were analyzed separately by one-way ANOVA and Fisher's PLSD test (Abacus Concepts 1992).

Aphid elemental analysis: Aphids feeding on high-Ni plants may themselves contain elevated levels of Ni. To test this possibility, we made mass collections of *A. pisum* that were feeding upon *S. polygaloides* plants growing on either high- or low-Ni glasshouse soil (ProMix). Plants were growing in a glasshouse different from the one in which our preference experiment was performed. Aphids were not intentionally released onto plants, but represented a pest outbreak in the glasshouse. Most of the plants were about 12 wk old, and were beginning to bolt to flower. Aphids were scraped from host plants, using a scalpel, into glass containers and frozen at −5°C. Collections were made periodically over a period of several weeks and, when sufficient insects had been collected, were combined into two samples of aphids: one from high-Ni plants and the second from low-Ni plants. Each sample comprised many thousands of aphids. Even so, the dry mass of each sample was barely adequate for elemental analysis.

The aphids were analyzed for element composition in a manner similar to that used for plant material. After thawing, aphid collections were weighed. They were then dried to constant mass at 60°C, dry-ashed at 485°C, further oxidized with boiling 1 *N* HNO_3, dissolved in 1 *N* HCl, and analyzed for Ca, K, Mg, P, Cu, Fe, Co, Cr, Pb, Mn, and Zn using an inductively-coupled argon plasma spectrometer (Jarrell-Ash, ICAP 9000). Nickel was determined from the same dilute acid solution using an atomic absorption spectrophotometer (Instrumentation Laboratory, IL 251).

Results

Preference Experiment: Aphid counts per unit biomass were significantly affected by the three treatments (unamended soil, unamended soil + Temik, and Ni-amended soil). The treatment effect in the ANOVA was highly significant ($F = 44.3$, df = 2, 14, $P = 0.0001$) and Fisher's PLSD test revealed that the mean for each treatment differed significantly from the others (at $P <$ 0.003 or less). Plants treated with Temik had the lowest aphid numbers, with a mean of 83.0 (SE = 15.0) aphids g^{-1} plant dry mass. Mean plant dry mass per pot was largest for this treatment (0.83 g, SE = 0.075). Plants not treated with Temik and growing on unamended soil had more than four times as many aphids, 376 (SE = 26.4) aphids g^{-1}, as the Temik-treated plants. Mean plant biomass per pot (0.73 g, SE = 0.51) was similar to that for the Temik treatment. The highest aphid number was observed for plants growing in Ni-amended soil (561 aphids g^{-1}; SE = 60.9). This latter value was 6.7-fold that of the Temik treatment, and almost 50% greater than that of the unamended soil treatment. Plants growing on high-Ni soil were much smaller, with a mean biomass of 0.50 g (SE = 0.058).

Concentrations of most elements in plants from this experiment were unaffected by treatments (Table 1). Of the 14 elements analyzed, only three metals (Ni, Co, and Pb) differed among treatments. All these metals were significantly elevated in plants growing on Ni-amended soil, but the concentration of Ni was elevated to the greatest extent (Table 1). The mean metal concentration of plants growing on Ni-amended soil, compared to unamended soil, was 88-fold greater for Ni and *ca* 8-fold greater for Co and Pb.

No-choice feeding experiment: Fecundity of aphids forced to feed upon plants growing on soil amended with $NiCl_2$, $CaCl_2$, or with no amendment was not significantly affected by soil treatments and the consequent variation in plant nickel content (Table 2). Mean aphid counts per cage after 4 d (SE in parentheses; N = 10) were: 10.6 (0.792) for plants growing on soil amended with $CaCl_2$; 11.3 (0.916) for plants growing on unamended potting mix; and 11.5 (1.17) for plants growing on $NiCl_2$-amended soil. ANCOVA, using aboveground plant dry mass per pot as a covariate to account for possible differences in plant vigour, re-

vealed no significant soil treatment effect ($F = 0.805$, df = 2, $P = 0.459$). The ANCOVA also showed no significant variation due to plant biomass per pot ($F = 0.115$, df = 1, $P = 0.737$).

Artificial diet experiment: Elevated Ni concentrations in artificial diet significantly decreased aphid survival. ANOVA of log-transformed survival data showed a significant effect of concentration ($F = 22.7$, df = 6, 175, $P < 0.0001$). Pairwise comparisons, using Fisher's PLSD test, showed that Ni concentrations of 2500 mg kg^{-1} or greater resulted in a significant decrease in aphid survival relative to unamended artificial diet (Fig. 1). Water and 15,000 mg Ni kg^{-1} diet had the lowest aphid survival of all treatments, significantly lower than that for 10,000 mg Ni kg^{-1} diet (Fig. 1). The similarly low survival of aphids provided with water or 15,000 mg Ni kg^{-1} diet indicates that the aphids may have refused to feed upon the 15,000 mg Ni kg^{-1} diet and died of starvation. Conversely, the significantly lower survival of aphids provided with diet of 2500 mg Ni kg^{-1} or 5000 mg Ni kg^{-1} (relative to diet lacking added Ni) implies that these aphids were able to feed and that the resultant mortality was due to the added Ni.

Calcium concentration also significantly decreased aphid survival, but only at relatively high Ca levels. The ANOVA of log-transformed data showed a significant concentration effect ($F = 26.5$, df = 5, 143, $P < 0.0001$). Multiple comparisons (Fisher's PLSD test, $P \leq 0.05$) revealed that aphid survival was significantly decreased at Ca concentrations of 15,000 mg kg^{-1}, relative to the survival rate of aphids feeding on unamended diet (Fig. 1). Water and 15,000 mg Ca kg^{-1} diet both resulted in very low aphid survival (<13%, Fig. 1), again indicating that aphids provided with highly amended diet may have starved to death.

Analysis of diet elemental concentrations revealed that measured concentrations of both Ni and Ca were higher than calculated concentrations. For Ni, linear regression of calculated versus actual Ni contents resulted in a R^2 of 1, showing excellent linearity of the relationship. The regression equation revealed that actual Ni values were 1.28 × calculated values. For Ca, a similar linear regression procedure gave an R^2 of 0.99. The regression equation showed that actual Ca values were 1.14 × calculated values. Thus, measured concentrations of both cations were higher than the calculated values displayed in Figure 1. However, the two regressions were similar, so that comparisons between Ni and Ca levels based upon calculated concentrations are probably valid for measured concentrations as well.

The $CaCl_2$ and $NiCl_2$ sources used to amend diets contained small amounts of other elements as contami-

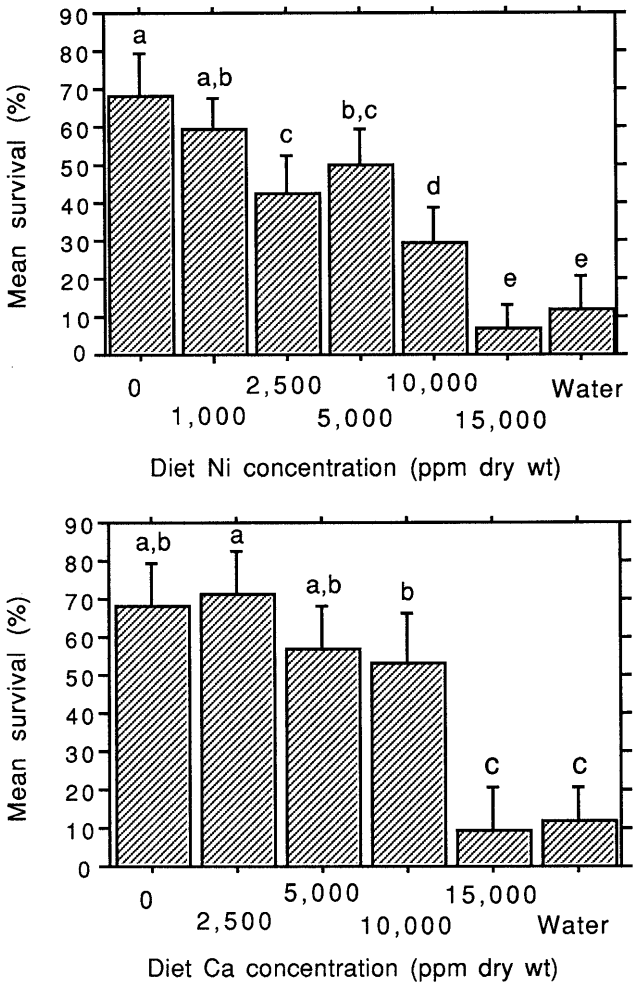

Fig. 1 Effect of calculated Ni and Ca concentrations on survival of aphids feeding on artificial diet. For each cation, bars with differing letters denote means that are statistically different (Fisher's PLSD test, $P \leq 0.05$). Cation concentrations are expressed as ppm (=mg kg^{-1}) dry mass for ease of comparison to Ni hyperaccumulator literature. They may be converted to m*M* by multiplying by 2.01×10^{-3} for Ni and by multiplying by 2.87×10^{-3} for Ca. Measured Ni values in diets are 1.28 × the calculated value, and measured Ca values in diets are 1.14 × the calculated value

Table 2 Elemental concentrations of the commercial aphid diet used in this study, expressed on a dry mass basis. Ca and Ni concentrations are presented only from those diet samples that were unamended with $CaCl_2$ or $NiCl_2$, respectively

Element	Mean concentration	SE	N
K (%)	1.3	0.041	9
P (mg kg^{-1})	5500	200	9
Mg (mg kg^{-1})	2900	150	9
Ca (mg kg^{-1})	380	17	6
Zn (mg kg^{-1})	47	7.7	9
Fe (mg kg^{-1})	36	4.4	9
Pb (mg kg^{-1})	13	4.8	9
Cu (mg kg^{-1})	11	0.66	9
Mn (mg kg^{-1})	9.1	0.50	9
Ni (mg kg^{-1})	7	–	1
Co (mg kg^{-1})	4.7	1.8	9
Cr (mg kg^{-1})	0.72	0.27	9

nants, and thus adding Ca or Ni may have increased the concentrations of elements other than Ni, Ca, or Cl in the diets. If this had occurred, we would expect that the levels of these contaminants would be positively correlated with the amount of Ni or Ca salt added. We checked for this effect via regression of calculated Ni and Ca levels against measured levels of each of the other elements. In every case, there was no significant linear relationship ($P > 0.05$ in all cases). Therefore, values for these elements were pooled from all samples of Ni- and Ca-amended diets to calculate the means for other elements that are documented in Table 2.

Aphid elemental analysis: Aphids collected from high- and low-Ni plants differed in their elemental make-up, but results cannot be statistically analyzed due to the lack of replication. However, aphids from high-Ni plants apparently had elevated elemental concentrations in general (Table 3). It is notable that Ni, which varied markedly in concentration in the tissues of high- and low-Ni plants, was not dramatically increased in aphids feeding on high-Ni plants. Table 3 shows a 1.6-fold greater Ni content of aphids from high-Ni plants, but almost every element analyzed was more abundant in aphids from high-Ni plants. Thus, the results for Ni do not demonstrate unusual elevation of Ni levels in aphids feeding on hyperaccumulating plants.

Discussion

Our experiments clearly showed that Ni-hyperaccumulating *S. polygaloides* plants were not protected from attack by *Acyrthosiphon pisum*. This was a significant departure from results of prior tests of the defence hypothesis of Ni hyperaccumulation. Previous experiments with both pathogens and lepidopteran folivores resulted in, at one extreme, acute toxicity (for folivores and the cabbage black rot bacterium pathogen) or, at the other extreme, reduced growth (for powdery mildew and necrotrophic fungus pathogens), when faced with high-Ni tissues (Boyd & Martens 1994; Boyd *et al.* 1994; Martens & Boyd 1994). The results reported here showed that *A. pisum* was not significantly affected by plant Ni content.

Table 3 Elemental concentrations (dry mass basis) and moisture content of aphids feeding upon high-Ni and low-Ni glasshouse-grown *Streptanthus polygaloides*

Element	Aphid elemental content	
	Low-Ni plant	High-Ni plant
K (%)	3.81	5.92
P (%)	3.06	5.02
Mg (mg kg^{-1})	4560	7490
Ca (mg kg^{-1})	2350	3380
Zn (mg kg^{-1})	796	1520
Fe (mg kg^{-1})	362	583
Mn (mg kg^{-1})	119	124
Cu (mg kg^{-1})	40	60
Ni (mg kg^{-1})	36	57
Pb (mg kg^{-1})	7	0
Co (mg kg^{-1})	0	0
Cr (mg kg^{-1})	0	0
Moisture (%)	74.5	76.2

The difference in aphid survival between Ni- and Ca-amended diets may have been due either to the toxicity of Ni ions or to their deterring aphid feeding. There are two alternative explanations, however. One is that aphid mortality resulted from the increased solute concentration of diets amended with large amounts of either $NiCl_2$ or $CaCl_2$. The second is that the anion used (Cl ion) may have had toxic effects. Neither of these explanations is likely. Calculation of total molarity showed that, for any given concentration of Ni or Ca, the Ca solution had 1.2-fold higher molarity. For example, the 1000 mg Ni kg^{-1} diet contained 6.02 m*M* solute, whereas the 1000 mg Ca kg^{-1} diet contained 8.60 m*M* solute. Similarly, Cl ion concentrations were higher for each Ca-amended diet compared to the corresponding Ni-amended diet. As an example, the 1000 mg Ni kg^{-1} diet contained 4.01 m*M* of Cl ion, whereas the 1000 mg Ca kg^{-1} diet contained 5.73 m*M*. We therefore conclude that the differences in aphid survival between metal-amended diets observed in Figure 1 were due to the greater toxicity of Ni relative to Ca.

The ineffectiveness of plant Ni as a defence against aphids was somewhat surprising, as aphids may be quite sensitive to host plant chemistry in general (Sandstrom & Pettersson 1994; Singh *et al.* 1994; Memmott, Day & Godfray 1995). Furthermore, Culliney & Pimentel (1986) showed that reproduction of the aphid *Myzus persicae* (Sulzer) was negatively affected when feeding on plants fertilized with chemically contaminated sewage sludge. The chemical contaminants included various heavy metals, as well as organic compounds such as PCBs. Unfortunately, they could not determine the relative importance of any one contaminant in producing the negative effect documented by their study, so that the role of metals in producing their results is unknown. More recent work by Crawford *et al.* (1995) showed that the aphid, *Aphis fabae* Scopoli, was unaffected by elevated levels of metals. Crawford *et al.* (1995) reported no effect of relatively high Cu (three-fold increase maximum relative to low-Cu plants) and high Cd (34-fold increase maximum relative to low-Cd plants) levels in host plants, implying that aphids may be relatively insensitive to host plant metal content. Our results with Ni were even more remarkable, as we showed that plants with tremendously elevated levels of Ni (88-fold difference, Table 1, untreated vs. high-Ni soil treatments) had no detectable negative effect on the pea aphid.

Boyd and Martens (1998) suggested that elemental defences could be circumvented by diet dilution (mixing defended and undefended food), tolerance (physiologic ability to withstand or detoxify defended food), or avoidance (selectively feeding on relatively undefended plant tissues). In the case reported here, diet dilution was unlikely to have been a significant factor in the preference experiment, as the aphids would have had to travel constantly between pots. Furthermore, diet dilu-

tion by the aphids was prevented by the experimental design of our no-choice test. Therefore, we must reject diet dilution as an explanation for *A. pisum*'s lack of response to high-Ni plants. The remaining two explanations could be resolved if *A. pisum* were relatively intolerant of Ni added to artificial diet. If this were the case, then we could conclude that phloem Ni levels were simply too low to result in toxicity and, thus, conclude that *A. pisum* was tapping into a relatively undefended resource in otherwise metal-defended plant tissues. However, *A. pisum* was relatively tolerant of dietary Ni in artificial diet. Diet amended with Ni at 1000 mg kg^{-1} dry mass (2.01 m*M* Ni) did not significantly depress aphid survival relative to unamended diet, and a 50% decline in aphid survival relative to unamended diet occurred only at a high Ni level: 10,000 mg Ni kg^{-1} (20.1 m*M* Ni). We should point out that the toxicity of Ni in artificial diets may be affected by chelation or complexation of Ni with other diet ingredients, and this factor may account for the apparent greater tolerance of *A. pisum* to Ni. We conclude that our results may be explained, at least in part, by a relatively high tolerance of the pea aphid to Ni.

Acyrthosiphon pisum may also be avoiding the Ni-based defence of *S. polygaloides* by tapping into relatively low-Ni phloem fluid. Our analysis of the bodies of aphids feeding on high-Ni plants showed that they contained relatively little Ni. Studies using crop plants (Mishra & Kar 1974) show that Ni moves from roots to leaves with the transpiration stream (Cataldo *et al.* 1978), and can then be redistributed by the phloem (Neumann & Chamel 1986). Studies of the tissue-level location of hyperaccumulated metals find them to be concentrated in the epidermis (Vazquez *et al.* 1992; Mesjasz-Przybyowicz *et al.* 1996). We know of no experimental determination of Ni levels in the phloem fluid of a Ni hyperaccumulator plant species. However, our data allow us to make a prediction based upon our experiments with Ni-amended artificial diet. We found a significant decrease in aphid survival at a concentration of 2500 mg Ni kg^{-1} dry mass (5.03 m*M* Ni). Therefore, because we could detect no effect of hyperaccumulated Ni *in planta* on aphids, we conclude that phloem fluid contains less Ni than that (relatively high) level. We hope that direct determination of metal concentrations in phloem fluid of metal hyperaccumulators will be attempted to provide definitive evidence. Until then, we conclude that aphids may be avoiding the elemental defence of *S. polygaloides* by feeding upon a relatively undefended plant resource: phloem fluid.

Complexation of Ni in the phloem fluid may also affect its toxicity to aphids. Unfortunately, there is a lack of information regarding complexation of metals in the phloem of hyperaccumulator plants. Kramer *et al.* (1996) reported that the amino acid histidine plays a pivotal role in hyperaccumulation of metals by an *Alyssum* species, including transport of metals in the xylem sap. Aphids are relatively sensitive to the quantitative and qualitative composition of amino acids in their diet (Mittler & Kleinjan 1970; Sandstrom 1994; Simpson *et al.* 1995). In our preference experiment, we found that aphid population size per unit of plant biomass was highest on high-Ni plants, suggesting that Ni-stimulated changes in host plant quality favoured aphid population growth on those plants. We suggest that high-Ni plants in the choice experiment may have supported greater aphid numbers not because they were nutritionally superior hosts, but because they were more attractive hosts (relative to the other plants in the experimental arrays). We also speculate that differences between high- and low-Ni plants, perhaps in amino acid quantity or quality, may have been responsible for this attraction.

We should note that there is a report of an aphid species attacking a metal-tolerant plant species in the wild. Ernst *et al.* (1990) reported the aphid *Brachycaudus lychnidis* L. feeding on Zn-accumulating *Silene vulgaris* (Moench) Garcke (Caryophyllaceae). This plant species has up to 1400 mg Zn kg^{-1} in its leaves (Ernst *et al.* 1990), an amount falling short of the 10,000 mg kg^{-1} needed to consider it a Zn hyperaccumulator (Baker & Brooks 1989), but nonetheless an elevated concentration. Ernst *et al.* (1990) analyzed the Zn contents of the aphids, which were very high (9000 mg Zn kg^{-1} dry mass). This example shows that aphids can accumulate metals from these plants and tolerate elevated metal levels in their bodies.

The results of this experiment have implications for applied uses of metal hyperaccumulating plants. These plants are the subject of recent attention because they may be useful in extracting metals from polluted soils (Brown 1995). This "green remediation" (Baker *et al.* 1994) may become a major industry. However, metal-hyperaccumulating crop plants may be subject to attack by herbivores that could reduce crop yield and thereby reduce the cost-effectiveness of this technique. Aphids, for example, can decrease plant yield (*e.g.*, Snow & Stanton 1988; Brown *et al.* 1995). Our results show that aphids are able to feed without apparent harm on Ni-hyperaccumulating *S. polygaloides*. Successful phytoextraction therefore may require control of aphid populations that may attack the hyperaccumulator crop.

A second implication of our results deals with the possibility of metal transfer to other trophic levels (van Straalen & Ernst 1991). Aphids can be an important prey base for a wide array of invertebrate and vertebrate predators (Dixon 1985) and, if aphids feeding on hyperaccumulating plants have large amounts of metal in their tissues, the metal may work its way into higher trophic levels. We found relatively low Ni contents of aphids feeding on Ni-hyperaccumulating plants, which implies that aphids will not be an important pathway by which Ni can escape from a remediation site into local food chains. However, the case of high-Zn aphids documented by Ernst *et al.* (1990) indicates that, in other situations, metals may be more ecologically mobile.

Finally, our results predict that *S. polygaloides* growing in the wild will be vulnerable to attack by aphids. On a recent (June 1997) visit to a *S. polygaloides* population growing upon serpentine soil in Ne-

vada Co., California (along Washington Rd., 1.5 miles N of State Route 20), we encountered aphids attacking flowering individuals. The as-yet unidentified aphid species was scarce (noticeably present on $<1\%$ of the plants examined), but its presence demonstrated that the phenomenon documented in our glasshouse experiments is paralleled under natural conditions. The Ni-based defence of *S. polygaloides* does not prevent parasitism of these plants by aphids in the wild.

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