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Nickel defends the South African hyperaccumulator *Senecio coronatus* (Asteraceae) against *Helix aspersa* (Mollusca: Pulmonidae)

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Summary. The elevated Ni concentration of Ni hyperaccumulator plants has been proposed to be an effective chemical defence against herbivores. To test this hypothesis, we fed leaves from hyperaccumulator and non-hyperaccumulator populations of South African *Senecio coronatus* to a generalist herbivore species, the brown garden snail (*Helix aspersa*). Snails fed hyperaccumulator leaves experienced significantly greater mortality than those fed non-hyperaccumulator leaves and also contained 10-fold greater concentrations of Ni. A choice experiment showed snails preferred non-hyperaccumulator leaves in two of three trials. Snails fed cornmeal diet amended with Ni had significantly reduced mass for diets containing as little as 140 μg Ni g^{-1}, and significantly greater mortality occurred for snails consuming diets containing 830 μg Ni g^{-1} and greater. Because hyperaccumulator *S. coronatus* leaves contained far more Ni (12,100 μg Ni g^{-1}) than the toxic threshold shown in the diet experiment, we concluded that the Ni concentration of hyperaccumulator leaves was sufficient to cause the elevated mortality of snails fed those leaves. This research adds another example to the growing literature showing the toxicity of hyperaccumulated Ni to generalist folivores.

Key words. Elemental defences – heavy metals – herbivory – hyperaccumulation – nickel

Introduction

Metal hyperaccumulator plants have unusually elevated concentrations of one or more metals in their tissues. In a recent review, Reeves and Baker (2000) estimated that 418 metal hyperaccumulator taxa have been discovered. Most of these taxa (318, or 76%) hyperaccumulate Ni, with Ni hyperaccumulation defined as at least 1,000 μg Ni g^{-1} dry mass in the aboveground parts of a field-collected plant specimen (Reeves & Baker 2000).

Several ecologic or physiologic explanations have been offered to explain the unusual elemental composition of metal hyperaccumulators (Boyd & Martens 1992). Of these, the defence hypothesis has received the most supporting evidence (Boyd & Martens 1998). A number of laboratory studies have shown that high-metal plant tissues are toxic to generalist insect herbivores (*e.g.*, Boyd & Martens 1994; Martens & Boyd 1994; Boyd & Moar 1999) and pathogens (*e.g.*, Boyd *et al.* 1994; Ghaderian *et al.* 2000) or deter herbivore damage altogether (*e.g.*, Pollard & Baker 1997). However, some herbivores or plant parasites are able to attack metal hyperaccumulators despite their elemental defence. These include an aphid (Boyd & Martens 1999), a mirid bug (Schwartz & Wall 2001), a parasitic plant (Boyd *et al.* 1999), and a virus (Davis *et al.* 2001). Thus, it is clear that elemental defences, like other modes of plant defence, can be circumvented.

The defence hypothesis holds that hyperaccumulated metals are a means of chemical defence. However, this type of chemical defence, termed an ‘‘elemental’’ defence by Martens & Boyd (1994), differs from the more widespread organic chemical defences of plants because metals are taken up by plants from the soil instead of manufactured from photosynthate. Elemental defences also offer an avenue to the study of plant defence biology not available for most organic plant defences because plant metal content can be manipulated by manipulating soil metal content. Thus, defended and undefended plants can be created in a glasshouse setting and used to directly study the effect of metal on herbivores. Boyd (1998) and Pollard (2000) agreed that this unique feature of metal hyperaccumulator plants made them a model system for the study of plant/herbivore coevolution.

The study presented here uses another comparative approach not usually available for Ni hyperaccumulator species. In other studies of hyperaccumulator plant defence, plants are grown on high- and low-metal substrates (*e.g.*, Boyd & Martens 1994; Martens & Boyd 1994; Pollard & Baker 1997; Ghaderian *et al.* 2000; Davis *et al.* 2001) and/or a closely-related species is used for comparative purposes (*e.g.*, Davis *et al.* 2001). *Senecio coronatus* (Thunberg) Harvey (Asteraceae) is a grassland herb of southern Africa (Hilliard 1977) found on both ultramafic and non-ultramafic soils. However, populations growing on ultramafic soils and that hyperaccumulate Ni are morphologically indistinguishable

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from non-hyperaccumulator populations (Balkwill pers. obs.). This situation allows an experimenter to collect plant material from a single species in the field that varies greatly in Ni concentration and thus offers a heretofore unexplored experimental approach to questions regarding elemental plant defences.

Mollusks are important herbivores in some ecological settings, as indicated by the notoriety of some species in agricultural and horticultural systems (Henderson 1996). The effectiveness of elemental defences against molluscan herbivores has received little attention. Pollard & Baker (1997) used the slug *Deroceras caruanae* (Pollonera) to investigate the effectiveness of hyperaccumulated Zn by *Thlaspi caerulescens* J. & C. Presl (Brassicaceae). We know of no other published information dealing with herbivorous mollusks and metal hyperaccumulator plants.

In this study, we fed leaves from hyperaccumulator and non-hyperaccumulator *Senecio coronatus* populations to generalist herbivore snails (*Helix aspersa* Müller). The objectives of this research were to determine if:

1. a diet of leaves from hyperaccumulating *S. coronatus* populations negatively affects *H. aspersa* relative to a diet of leaves from non-hyperaccumulating populations;
2. *H. aspersa* avoided hyperaccumulator leaves when offered a choice of hyperaccumulator and non-hyperaccumulator *S. coronatus* leaves; and
3. the Ni concentration of hyperaccumulator *S. coronatus* was capable of producing a toxic effect upon *H. aspersa* by significantly elevating body Ni concentrations.

Materials and methods

Study organisms: *Senecio coronatus* is a perennial herb with elliptic, rather fleshy leaves ranging in length to about 40 cm (Hilliard 1977). Morrey *et al.* (1992) reported that *S. coronatus* specimens collected from two ultramafic sites in northeastern South Africa contained up to 24,000 μg Ni g^{-1}, but noted that the species also occurred on non-ultramafic soils. Our preliminary field exploration on ultramafic areas east of Badplaas in South Africa's Mpumalanga Province (Morrey *et al.* 1992) revealed a number of *S. coronatus* localities. Plants at some of these sites hyperaccumulated Ni, whereas others did not. Identification in the field of plants as hyperaccumulator or non-hyperaccumulator was accomplished using filter paper impregnated with dimethylglyoxime (DMG). In this technique, leaves of hyperaccumulator plants pressed upon DMG paper turn the paper a pink color, whereas leaves of non-hyperaccumulators result in a greenish color (Reeves 1992).

Helix aspersa Müller is native to Mediterranean Europe but has been introduced into many parts of the globe, including South Africa. It is a generalist herbivore and thus can cause problems by damaging a wide range of crop and garden plants (*e.g.*, Sakovich 1996, Young 1996). *Helix aspersa* is also easy to rear and this feature, coupled with its wide host range, has led to its use in studies of herbivory (*e.g.*, Diaz *et al.* 1998; Fraser & Grime 1999) and of plant chemical defences (*e.g.*, Linhart & Thompson 1999).

Leaf experiments (Force feeding experiment): *Helix aspersa* individuals were collected from gardens of two resorts (Deo Volente and Aventura) in Badplaas, Mpumalanga Province, South Africa. Snails were fed lettuce (*Lactuca sativa* L., Asteraceae) for several days before force feeding began. Each of 46 snails was weighed to the nearest 0.01 g and placed into a lidded 150 ml plastic container. The snails were then ranked from lowest to highest mass and divided into pairs with similar mass. Members of each pair were randomly assigned to receive either high- or low-Ni *S. coronatus* leaves. Each snail was given a cut piece of leaf (generally > 8 mm^2) and allowed to feed *ad libitum*. Snails were maintained at room temperature and weighed every 3–4 days. After weighing, each snail was dipped in water to ensure adequate hydration and given a fresh leaf piece. The experiment was conducted for a total of 37 days.

Survival data were compared between hyperaccumulator and non-hyperaccumulator leaf diets to determine if survival was significantly affected by leaf type. Data were analyzed by survival analysis using the Kaplan-Meier estimate (Abacus Concepts 1998). Treatment significance was determined by the Peto-Peto-Wilcoxon test at $\alpha \leq 0.05$.

Three replicates of leaf pieces taken from the hyperaccumulator and non-hyperaccumulator leaves used at the beginning of the experiment were dried at *ca.* 60°C and used for elemental analysis. Dried samples were ground and elemental analysis was performed for nine elements. Plant samples were dry-ashed at 485°C, further oxidized with boiling 1 *M* HNO_3, dissolved in 1 *M* HCl, and analyzed for Ca, K, Mg, P, Cu, Fe, Mn and Zn using an inductively-coupled argon plasma spectrometer (Jarrell-Ash ICAP 9000, Franklin, MA, USA). Nickel was determined by analyzing the same extract with an atomic absorption spectrophotometer (Instrumentation Laboratory IL 251, Wilmington, MA, USA). Concentrations of each element in leaves from hyperaccumulator and non-hyperaccumulator *S. coronatus* populations were compared by one-way Analysis of Variance (ANOVA).

Snails that died during the course of the experiment were dried at *ca.* 60°C. At the end of the experiment, all remaining live snails were also dried. The dried remains of snails were analyzed for concentrations of Ni, Ca, K, Mg, P, Cu, Fe, Mn, Cr, Pb, Co and Zn following the technique of Wall (1999) and Schwartz & Wall (2001). Two or three snails that had received the same diet (high- or low-Ni) from consecutively numbered pairs of snails were combined to provide adequate biomass for the analysis. This procedure resulted in eight samples of snails from each leaf diet. Samples were dried for at least 48 h at 67°C, weighed, and placed in borosilicate glass test tubes. We digested samples using 3–5 ml of concentrated nitric acid, maintaining samples at 110°C for 6–8 h (until most of the liquid had evaporated). Residue was re-dissolved in 3–5 ml of 1 *M* HCl at 110°C for 2–4 h, and diluted with distilled water to a final volume of 10 ml. Elemental determinations for snail samples were performed as described above. Element concentrations of snails were compared by one-way ANOVA.

Leaf experiments (Choice experiment): A choice experiment was conducted to determine if snails differentially damaged one leaf type when presented with both hyperaccumulator and non-hyperaccumulator leaves. Twenty large *H. aspersa* were collected from the gardens at Deo Volente. Each snail was placed into a lidded 150 ml volume plastic cup. Rectangular (1.5 × 2.5 cm) pieces were cut from leaves taken from hyperaccumulator and non-hyperaccumulator *S. coronatus*. Each piece was cut from a leaf blade on one side of the leaf midrib and all pieces were examined to ensure that each showed no signs of herbivory prior to the experiment. One hyperaccumulator and one non-hyperaccumulator leaf piece were placed leaning against opposite sides of each cup and the containers were stored at room temperature. After 4, 6, and 7 d, the cups were opened and the leaf pieces were removed. The percentage of each leaf piece eaten was visually estimated and each piece was tested with DMG paper to identify hyperaccumulator and non-hyperaccumulator leaf pieces. Fresh leaf pieces were added to each container at 4 and 6 d.

Choice experiment data were analyzed with the Wilcoxon signed rank test using StatView 5.0 (Abacus Concepts 1998). This analysis determined if hyperaccumulator or non-hyperaccumulator leaf pieces were more greatly damaged. Separate analyses were performed for the data collected for 4, 6, and 7 d. Snails that did not feed on either leaf piece during a portion of the experiment were not included in the analysis of that experimental period.

Diet experiment: Yellow cornmeal was used as artificial snail diet. Diets containing 140, 240, 430, 830, 1600 and 3200 μg Ni g^{-1} were made by amending cornmeal with powdered anhydrous $NiCl_2$ (Fisher Scientific, Suwanee, GA, USA). An additional treatment of unamended diet was used as a control.

Helix aspersa individuals used for this experiment were obtained from a commercial supplier (Pennsylvania Snail Farms, Meyersdale, PA, USA). Snail fresh weights ranged from 3.66 g to 8.09 g and snails were fed lettuce for 4 d before the experiment began. Each snail was placed into a 150 ml plastic cup containing 1 cm of wet sand to elevate humidity within the cup. Diet treatments were randomly assigned to the seven snails that comprised each of 20 replicates. Snails were fed *ad libitum*: moistened diet was placed into a plastic weigh boat and a snail was added to each container. Each snail was misted with water after weighing to maintain hydration. Snails were weighed at the start of the experiment and every 3–4 d thereafter. Fresh diet was provided at the time of each weighing.

Survival curves were compared to determine if mortality was significantly affected by diet treatments. Data were analyzed by survival analysis using the Kaplan-Meier estimate (Abacus Concepts 1998). Treatment significance was determined by the Peto-Peto-Wilcoxon test at $\alpha \leq 0.05$. Once an overall significant treatment effect was determined, the dataset was subdivided to make comparisons between specific treatments. The survival curve for snails fed unamended diet was compared to that of snails fed 440 and 830 µg Ni g^{-1} diets to determine the threshold diet Ni concentration for snail mortality.

Mass data were also analyzed to determine dietary effects. Data from each snail and each weighing interval were expressed as a decimal fraction of initial mass and analyzed by repeated measures ANOVA. In an effort to detect sublethal metal effects, we only analyzed data from those Ni-amended diet treatments that did not result in statistically increased mortality relative to control diet. These diets were those amended to contain 140, 240, and 430 µg Ni g^{-1}. Because high mortality during the course of the experiment created missing data points that interfered with the analysis, mass data were analyzed only through Day 22 of the experiment.

Results

Leaf experiments (Force feeding experiment): Snails fed hyperaccumulator leaves experienced high mortality during the course of the experiment (Fig. 1). Relative to snails fed non-hyperaccumulator leaves, survival of those fed hyperaccumulator leaves began to decline at 7 d and rapidly declined by 27 d so that only 30% were alive at 37 d. In contrast, snails fed non-hyperaccumulator leaves suffered very low mortality (Fig. 1). None died until 34 d, and at 37 d 91% remained alive. These survival curves differed significantly (Peto-Peto-Wilcoxon test, Chi-square = 19.8, $P < 0.0001$).

Leaves from hyperaccumulator and non-hyperaccumulator plant populations varied significantly in composition for all elements measured excepting Cu (Table 1). Three elements (Ni, K, and Zn) were at greater concentrations in hyperaccumulator leaves. The greatest difference was for Ni concentration, which was 18-fold greater in hyperaccumulator leaves (Table 1). Values for Zn and K were 3-fold and 1.7-fold greater in hyperaccumulator leaves, respectively (Table 1). Non-hyperaccumulator leaves had greater concentrations than hyperaccumulator leaves for five elements (Ca, Mg, P, Fe, and Mn). Differences for these elements were less extreme, ranging from 1.2-fold greater concentrations in non-hyperaccumulator leaves for P and Fe to 1.8-fold greater for Ca (Table 1).

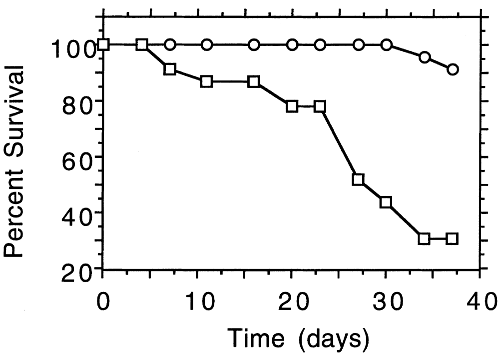

Fig. 1 Survival of *H. aspersa* fed leaves of *S. coronatus* from hyperaccumulator (squares) and non-hyperaccumulator (circles) populations

Analyses of snails showed only a few significant differences in elemental concentrations. The greatest difference was for Ni concentration, which was 10.3-fold greater in the bodies of snails fed hyperaccumulator leaves (Table 2). Another significant difference was observed for Pb, which was also greater (2.1-fold) in snails fed hyperaccumulator leaves. Finally, Mg concentrations were significantly greater (2.1-fold) in snails fed non-hyperaccumulator leaves (Table 2). Other elements did not differ significantly between snails fed hyperaccumulator and non-hyperaccumulator leaves (Table 2).

Leaf experiments (Choice experiment): Snails significantly preferred non-hyperaccumulator leaves, but only during the later portions of the choice experiment. At 4 d, 12 of 20 snails (60%) had fed on the leaves in their containers. Mean damage (% removed, SE in parentheses) was 9.5% (4.3%) for hyperaccumulator leaf pieces and 7.8% (2.0%) for non-hyperaccumulator leaf pieces. The Wilcoxon signed rank test revealed no significant difference in damage levels (Tied Z-value = -0.157, $P = 0.88$). When new leaf pieces were added and snails were allowed to feed for two more days, they significantly preferred non-hyperaccumulator leaves (Wilcoxon paired rank test, Tied Z-value = -2.4, $P = 0.017$). All seven feeding snails consumed more non-hyperaccumulator leaf. Mean damage levels (SE in parentheses) were 1.3% (1.3%) for hyperaccumulator leaves and 7.4% (2.1%) for non-hyperaccumulator leaves. The final trial also showed a significant preference for non-hyper-accumulator leaves. All eight feeding snails consumed more non-hyperaccumulator leaf (Wilcoxon paired rank test, Tied Z-value = -2.5, $P = 0.012$). Mean damage was 1.9% (1.1%) for hyperaccumulator leaves and 20% (8.5%) for non-hyperaccumulator leaves.

Snails consumed both types of leaves, but there was a significant tendency for some individuals to avoid hyperaccumulator leaves. Of snails that consumed leaves during the first trial, five snails did not consume any hyperaccumulator leaf whereas only one snail did not consume any non-hyperaccumulator leaf. In the second trial, seven snails did not consume hyperaccumulator leaves and, in the third trial, four snails did not consume hyperaccumulator leaves. In both these later trials, all feeding snails consumed some non-hyperaccumulator leaf material. If the activities of individual snails are examined over the course of the entire experiment, 11 individual snails showed at least one case in

Table 1 Elemental concentrations (in µg g^{-1}, dry mass) of leaf samples from hyperaccumulator and non-hyperaccumulator populations of *S. coronatus*. Data are means (SE), $N = 3$

Element	Plant population type		ANOVA *P*-value
	Hyperaccumulator	Non-hyperaccumulator	
Ni	12,100 (1790)	684 (64.3)	0.0030
K	18,400 (653)	11,000 (589)	0.0011
Zn	80.0 (1.59)	26.0 (1.63)	<0.0001
Ca	18,000 (1470)	32,500 (1410)	0.0021
Mg	12,500 (395)	16,100 (297)	0.0019
P	681 (9.76)	786 (23.7)	0.015
Fe	318 (3.47)	391 (10.5)	0.026
Mn	51.0 (1.92)	76.9 (1.99)	0.0007
Cu	10 (0)	10 (0)	1.0

which they consumed part of the non-hyperaccumulator leaf but did not damage the hyperaccumulator leaf, whereas only one did the opposite. This pattern of avoidance of feeding on the hyperaccumulator leaf is statistically significant from random expectations (Chi-square = 8.33, df = 1, $P < 0.005$).

Diet experiment: Diet treatments significantly affected snail survival (Chi-square = 26.3, df = 6, $P = 0.0002$). In general, survival was greatest for snails fed unamended diet and decreased as diet Ni concentration increased (Fig. 2). Comparison of survival of snails fed unamended diet against that of snails fed 440 µg Ni g^{-1} diet showed no significant difference (Chi-square = 1.08, df = 1, $P = 0.299$). In contrast, comparison of survival of snails fed unamended diet against survival of those fed 830 µg Ni g^{-1} diet was significant (Chi-square = 5.32, df = 1, $P = 0.0211$), indicating that the toxic threshold was between 440 and 830 µg Ni g^{-1}.

Snail mass data were investigated for those Ni-amended diets that did not produce significant survival differences relative to the unamended diet (140, 240, and 440 µg Ni g^{-1}). Repeated measures ANOVA showed a highly significant treatment × mass change interaction ($F_{18,162} = 3.67$, $P < 0.0001$), indicating that over time changes in snail mass differed due to treatments. Snails fed unamended diet gained mass, whereas those fed Ni-amended diets lost mass (Fig. 3). To show that the significant interaction effect was due to the contrast between unamended and Ni-amended diets, we excluded data from the unamended diet and re-analyzed the dataset. The re-analysis showed no significant treatment × mass change interaction ($F_{12,108} = 0.59$, $P = 0.849$), indicating that mass change patterns were similar for all Ni-amended diets.

Table 2 Elemental concentrations of *H. aspersa* fed leaves from hyperaccumulator and non-hyperaccumulator populations of *S. coronatus*. Data are means (SE), $N = 8$

Element	Diet type		ANOVA *P*-value
	Hyperaccumulator	Non-hyperaccumulator	
Ni (µg g^{-1})	380 (98)	37 (6.3)	0.0035
K (µg g^{-1})	6,300 (640)	6,400 (640)	0.90
Zn (µg g^{-1})	220 (32)	210 (25)	0.82
Ca (%)	19 (3.2)	16 (1.1)	0.44
Mg (µg g^{-1})	3,100 (280)	6,400 (740)	0.0010
P (µg g^{-1})	8,500 (820)	7,400 (690)	0.31
Fe (µg g^{-1})	330 (57)	330 (33)	0.92
Mn (µg g^{-1})	85 (7.6)	90 (13)	0.73
Pb (µg g^{-1})	31 (7.2)	15 (2.1)	0.047
Cu (µg g^{-1})	92 (17)	54 (5.5)	0.057
Cr (µg g^{-1})	35 (6.6)	34 (3.1)	0.92
Co (µg g^{-1})	0.97 (0.51)	0.87 (0.38)	0.88

Discussion

We concluded that toxicity to snails occurred due to the elevated Ni concentration of hyperaccumulator *S. coronatus* leaves. Our force-feeding experiment showed that hyperaccumulator leaves of *S. coronatus* are toxic to *H. aspersa*. This toxicity likely stemmed from the Ni in those leaves: the greatest difference in element concentration between hyperaccumulator and non-hyperaccumulator leaves was that for Ni (18-fold; Table 1). Furthermore, the artificial diet study showed that concentrations of Ni much less than that in hyperaccumulator leaves were toxic to snails. Significantly decreased survival was observed for snails fed diet containing 840 µg Ni g^{-1}, whereas hyperaccumulator leaves contained 12,100 µg Ni g^{-1}.

We also observed a sublethal effect of Ni on snails. In the artificial diet experiment, weight gain of snails consuming diet containing only 140 µg Ni g^{-1} was significantly reduced relative to unamended diet (Fig. 3). The effectiveness of such a small Ni concentration was surprising, as Boyd & Moar (1999) documented significantly reduced mass of larval *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) at a dietary Ni concentration of 535 µg g^{-1}. This sublethal effect of Ni upon *H. aspersa* indicates that plants with Ni concentrations less than the 1,000 µg g^{-1} threshold used for defining Ni hyperaccumulation (Reeves & Baker 2000) may receive a small defensive benefit. Incremental increases in Ni concentration could magnify this initial benefit and thus provide the selective advantage underlying the evolution of Ni hyperaccumulation.

Toxicity is one consequence of a chemical herbivore defence tactic, but deterrence is yet another that may be even more valuable to a plant. Deterrence is the prevention of herbivore damage, and is valuable because no damage cost is incurred by the defended plant (Pollard & Baker 1997). Pollard & Baker (1997) demonstrated deterrence by high-Zn plants for a lepidopteran folivore, and Sägner *et al.* (1998) reported that the high-Ni sap of a Ni hyperaccumulator repelled fruit flies. Our choice experiment showed some evidence for deterrence of snails by hyperaccumulator *S. coronatus* leaves. Whereas in the initial trial leaves of both types were damaged, in the later two trials damage was primarily concentrated on non-hyperaccumulator leaves. Furthermore, over the course of the experiment, significantly more cases of zero damage to the hyperaccumulator leaf occurred than would be expected due to chance

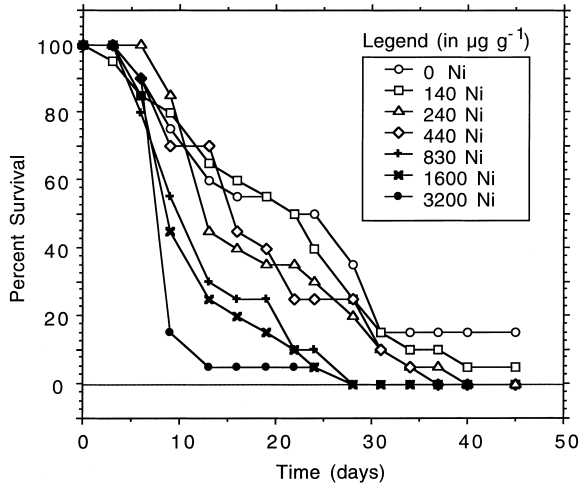

Fig. 2 Survival of *H. aspersa* when fed experimental cornmeal diets. Survival curves drawn with open symbols did not differ significantly from the unamended diet curve, whereas those with solid symbols differed significantly (see text)

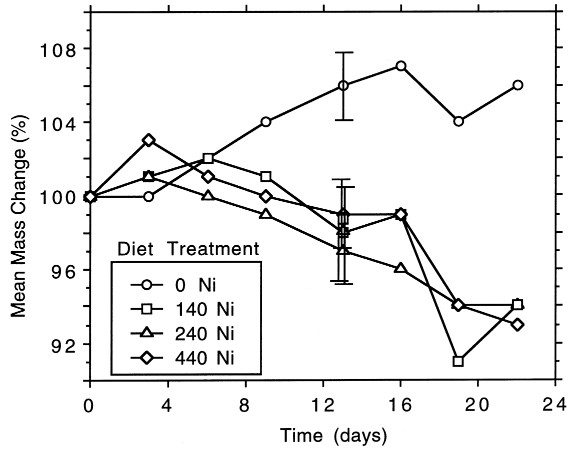

Fig. 3 Mean mass changes of *H. aspersa* fed three experimental diets during the first 22 d of the artificial diet experiment. All Ni-amended diets shown did not cause elevated mortality relative to the unamended diet. Error bars for means at 13 d are SE and are presented as an example of error bars for time periods in the later portion of the experiment. Error bars for other data points are not shown as considerable overlap occurs among error bars

alone. This latter fact is evidence of deterrence and suggests that hyperaccumulator *S. coronatus* plants are protected against herbivory by two mechanisms: toxicity and partial deterrence. The lack of damage to hyperaccumulator leaves (in some cases) indicates that snails can detect leaf suitability without inflicting noticeable levels of damage. Recent reports that Ni in hyperaccumulator leaves is concentrated in epidermal cells (*e.g.*, Mesjasz-Przybylowicz *et al.* 1996; Heath *et al.* 1997; Küpper *et al.* 2000; Psaras *et al.* 2000) suggest that snails may be able to directly assess the Ni concentration of leaves.

Much previous research has focussed on snails (such as *H. aspersa*) as indicators of industrial metal pollution (*e.g.*, Coughtrey & Martin 1977; Laskowski & Hopkin 1996; Gomot-de Vaufleury & Pihan 2000). Several studies have shown *H. aspersa* to be relatively tolerant of some metals. For example, Gomot-de Vaufleury (2000) showed no negative growth or survival effect for *H. aspersa* consuming a diet of 30,000 μg Pb g^{-1}. Other metals impacted snails at lower concentrations: Zn decreased snail growth at 6,000 μg g^{-1} and Cu at 1,000 μg g^{-1}. These other studies did not examine Ni, the metal most often hyperaccumulated by plants. Reeves & Baker (2000) suggested that Ni is often hyperaccumulated because it is widely available: Ni-enriched ultramafic rocks are globally widespread and form large exposures. We add that Ni may also be hyperaccumulated because of its relative toxicity to generalist herbivores such as *H. aspersa*. Nickel hyperaccumulation is defined as at least 1,000 μg Ni g^{-1} dry mass (Reeves & Baker 2000). In our study, snail growth was reduced at 140 μg Ni g^{-1} and survival was decreased at 830 μg Ni g^{-1}. From reports of other metals tested on *H. aspersa* in the literature (Pb, Cu, Zn, and Cd), only Cd has been reported to show negative effects at lesser concentrations (Laskowski & Hopkin 1996; Gomot-de Vaufleury & Kerhoas 2000; Gomot-de Vaufleury 2000).

Our results show that hyperaccumulator populations of *S. coronatus* are defended, via both toxicity and partial deterrence, against the generalist folivore *H. aspersa*. This study reinforces prior investigations of hyperaccumulated metals as elemental defences, such as Martens & Boyd (1994), Boyd & Martens (1994), Pollard & Baker (1997), and Boyd & Moar (1999). However, these prior studies have been limited to use of hyperaccumulator species from the Brassicaceae which are native to Europe or North America. Our research extends the defence hypothesis of Ni hyperaccumulation both taxonomically (to the Asteraceae) and geographically (to Africa). We also provide evidence of the effectiveness of Ni hyperaccumulation against a herbivorous mollusk. Most prior research regarding Ni hyperaccumulators and the defence hypothesis has used insects as example herbivores (*e.g.*, Martens & Boyd 1994; Boyd & Martens 1994; Boyd & Moar 1999), although Pollard & Baker (1997) used an herbivorous mollusk, the slug *Deroceras caruanae* (Pollonera), in their investigation of the defence hypothesis using a hyperaccumulator of Zn.

Our experiments were also unique in that the hyperaccumulator species used shows an unusual combination of edaphic substrates and hyperaccumulator behavior. The majority of hyperaccumulator taxa are only found on ultramafic soils and thus all samples collected have high levels of metal (Reeves & Baker 2000). This is especially true for Ni hyperaccumulator taxa that have been reported to have maximum leaf Ni concentrations $> 10,000$ μg Ni g^{-1} (Reeves & Baker 2000). In contrast, *Senecio coronatus* is not endemic to ultramafic soils (Hilliard 1977). Furthermore, when it does occur on ultramafics, it may or may not hyperaccumulate Ni (Balkwill pers. obs.). Given that Ni hyperaccumulation can poison generalist herbivores, the question then arises as to why some ultramafic populations are not protected by hyperaccumulated Ni. We hypothesize that non-hyperaccumulator *S. coronatus* populations are chemically defended, but by organic-based secondary chemicals rather than by Ni. Davis & Boyd (2000) showed that, in the Ni hyperaccumulator *Streptanthus polygaloides* Gray (Brassicaceae), concentrations of organic defence chemicals (in this case, glucosinolates) were less than in a congeneric non-hyperaccumulator species. This suggested that an evolutionary trade-off may occur between elemental and organic plant defences. A similar trade-off may occur in *S. coronatus*, which is likely chemically defended by pyrrolizidine alkaloids, as has been reported for other members of the genus (Stegelmeier *et al.* 1999), and perhaps by other organic chemicals as well. In this case, we suggest that non-hyperaccumulator *S. coronatus* are defended by elevated secondary chemical (alkaloid) levels whereas hyperaccumulator populations are able to reduce secondary chemical production because they are defended by Ni. Generalist herbivores, such as *H. aspersa* in our study, are capable of withstanding the secondary chemicals but are susceptible to the novel defence posed by hyperaccumulated Ni.

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