© 2007 The Authors *Insect Science* (2007) 14, 133–143
Journal compilation © Institute of Zoology, Chinese Academy of Sciences

Host-herbivore studies of *Stenoscepa* sp. (Orthoptera: Pyrgomorphidae), a high-Ni herbivore of the South African Ni hyperaccumulator *Berkheya coddii* (Asteraceae)

ROBERT S. BOYD[1], MICHEAL A. DAVIS[2], MICHAEL A. WALL[3] and KEVIN BALKWILL[4]

[1]Department of Biological Sciences, Auburn University, Auburn, Alabama, [2]Department of Biological Sciences, University of Southern Mississippi, Hattiesburg, MS, [3]Entomology Department, San Diego Natural History Museum, San Diego, California, USA and [4]C.E. Moss Herbarium, School of Animal and Plant Sciences, University of the Witwatersrand, Johannesburg, South Africa

Abstract Nymphs of *Stenoscepa* sp. feed on leaves of the Ni hyperaccumulator *Berkheya coddii* at serpentine sites in Mpumalanga Province, South Africa. These sites contain Ni hyperaccumulators, Ni accumulators, and plants with Ni concentrations in the normal range. We conducted studies to: (i) determine the whole-body metal concentration of nymphs (including those starved to empty their guts); (ii) compare *Stenoscepa* sp. nymphs against other grasshoppers in the same habitat for whole-body metal concentrations; and (iii) compare the suitability of Ni hyperaccumulator and Ni accumulator plants as food sources for *Stenoscepa* sp. and other grasshoppers. *Stenoscepa* nymphs had extremely high whole-body Ni concentrations (3 500 μg Ni/g). This was partly due to food in the gut, as starved insects contained less Ni (950 μg Ni/g). *Stenoscepa* nymphs survived significantly better than other grasshoppers collected from either a serpentine or a non-serpentine site when offered high-Ni plants as food. In a host preference test among four *Berkheya* species (two Ni hyperaccumulators and two Ni accumulators), *Stenoscepa* sp. preferred leaves of the Ni hyperaccumulator species. A preference experiment using leaves of three *Senecio* species (of which one species, *Senecio coronatus*, was represented by both a Ni hyperaccumulator and a Ni accumulator population) showed that *Stenoscepa* sp. preferred Ni accumulator *Senecio coronatus* leaves to all other choices. We conclude that *Stenoscepa* sp. is extremely Ni-tolerant. *Stenoscepa* sp. nymphs prefer leaves of hyperaccumulator *Berkheya* species, but elevated Ni concentration alone does not determine their food preference. We suggest that the extremely high whole-body Ni concentration of *Stenoscepa* nymphs may affect food web relationships in these serpentine communities.

Key words *Berkheya coddii*, biomagnification, food webs, heavy metals, hyperaccumulation, South Africa
DOI 10.1111/j.1744-7917.2007.00135.x

Introduction

Plants and their herbivores are engaged in an evolutionary struggle in which the survival of each depends on the ability to counter the adaptations of the other. Plant chemical defenses are ubiquitous and can explain many plant/herbivore interactions. Herbivores faced with plant defenses use counterdefenses that, in many cases, enable them to feed on chemically defended plant tissues. Most studies of plant chemical defenses have focused upon organic plant constituents (secondary compounds). A great many secondary compounds have been discovered and the roles of some of these in plant defense have been well

Correspondence: R. S. Boyd, Department of Biological Sciences, Auburn University, Auburn, Alabama 36849-5407, USA. Tel: 334 844 1626; fax: 334 844 1645; email: boydrob@auburn.edu

studied (Harborne, 1988). It has been recently recognized that some plants, called hyperaccumulators by Brooks *et al*. (1977), are chemically unusual: they contain large amounts of elements (often metals) not normally found in abundance in plants. Other plants, called accumulators (Iturralde, 2004), have levels less elevated yet still unusually high. For Ni, hyperaccumulation has been defined as > 1 000 μg Ni/g dry mass and accumulation as 100−1 000 μg Ni/g dry mass (Reeves & Baker, 2000). Reeves and Baker (2000) report that normal plant Ni concentrations are < 10 μg Ni/g. At the time of their review, Reeves and Baker (2000) estimated that 418 hyperaccumulator taxa had been documented, with the majority of these (76%) hyperaccumulating Ni.

Several explanations have been offered for the unusual elemental composition of metal hyperaccumulators (Boyd & Martens, 1992). Of these, the defense hypothesis suggests that hyperaccumulated metals may protect plants from herbivores and other natural enemies (Boyd & Martens, 1998; Boyd, 2004). 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; Davis *et al*., 2001) and pathogens (e.g., Boyd *et al*., 1994; Ghaderian *et al*., 2000) or deter herbivore damage altogether (e.g., Pollard & Baker, 1997). Boyd (1998) pointed out several differences between elemental and organic plant defenses. First, elemental defenses are acquired from the soil and are not synthesized by a plant. Second, elements cannot be chemically degraded and thus this mechanism of detoxification is not a herbivore counterdefense option.

The elevated metal concentration of hyperaccumulator plants creates a chemically unusual food source for herbivores. Although it is clear that metals hyperaccumulated by plants defend them against some herbivores and pathogens (Boyd, 2004), it also is clear that some herbivores and pathogens circumvent metal-based plant defenses (Jhee *et al*., 2005). Information from field studies regarding herbivory and metal hyperaccumulators is almost non-existent, but Boyd and Martens (1998) suggested three ways that circumvention of elemental defenses may occur: (i) by selective feeding on low-metal tissues; (ii) through a generalist diet that dilutes metal-containing food; and (iii) by possessing physiological tolerance of high dietary metal content. Studies of a Californian Ni hyperaccumulator, *Streptanthus polygaloides* Gray (Brassicaceae), have documented two Ni-tolerant insects and a Ni-tolerant plant parasite that attack the Ni hyperaccumulator with no apparent harm. The Ni-tolerant insects, *Melanotrichus boydi* Schwartz & Wall (Heteroptera: Miridae) and *Coquilletia insignis* Uhler (Heteroptera: Miridae), contained whole-body Ni concentrations of 780 μg Ni/g (Schwartz & Wall, 2001) and 500 μg Ni/g (Boyd *et al*., 2004), respectively. The Ni-tolerant plant parasite, *Cuscuta californica* var. *breviflora* (Cuscutaceae), contained 800 μg Ni/g (Boyd *et al*., 1999).

The elevated whole-body metal concentrations of insects feeding on hyperaccumulators may have consequences for the applied uses of metal hyperaccumulating plants. Hyperaccumulators have been studied for potential use in phytoextraction, the removal of metals from soils using plants (Reeves, 2003). Phytoextraction includes cleanup of metal-polluted sites (Pilon-Smits, 2004) in addition to using metal hyperaccumulators to mine metals from high-metal sites, termed phytomining (Anderson *et al*., 1999). *Berkehya coddii* Roessl. (Asteraceae) in particular has attracted attention for its potential uses in these applications, due to its high Ni concentration and relatively high biomass yield (Robinson *et al*., 1997; Brooks *et al*., 2001). However, there is concern that herbivores feeding on hyperaccumulators may mobilize metals into ecosystems (Whiting *et al*., 2004; Angle & Linacre, 2005) and affect local food webs. Studies of food webs involving hyperaccumulators in their native habitats can illustrate the pathways through which metals move between plants and higher trophic levels.

In South Africa, pioneering studies of insects associated with the Ni hyperaccumulator *Berkheya coddii* have documented a number of species feeding on this plant (Mesjasz-Przybylowicz *et al*., 2004). Surveys of *B. coddii* by Mesjasz-Przybylowicz and Przybylowicz (2001) and Boyd *et al*. (2006) have reported a number of insect taxa found in association with this hyperaccumulator species. One of these, *Chrysolina pardalina* Fabricius (Coleoptera: Chrysomelidae), has been investigated in detail (Mesjasz-Przybylowicz *et al*., 2004). Others, including a grasshopper of the genus *Stenoscepa* that may be an undescribed species (P. Naskrecki, personal communication, 2006), have not yet received in-depth study. Boyd *et al*. (2006) reported that whole-body analysis of *Stenoscepa* sp. nymphs collected from *B. coddii* contained a mean of 3500 μg Ni/g, the highest mean Ni concentration yet reported for an arthropod associated with a Ni hyperaccumulator plant.

The extraordinarily elevated whole-body Ni concentration of *Stenoscepa* sp. suggested that it deserved further study. For example, it is unclear if high Ni insects such as *Stenoscepa* sp. have high whole-body Ni concentrations because their tissues contain elevated Ni levels, their guts contain high Ni food, or both. We also do not know the dietary breadth of this species, including whether it preferentially feeds on Ni hyperaccumulator plants. The research we report here was designed to: (i) determine the whole-body metal concentrations of nymphs (including those starved to empty their guts); (ii) compare *Stenoscepa* sp. nymphs against other field-collected grasshopper species in their ability to use *B. coddii* as a food source; (iii) compare *Stenoscepa* sp. nymphs against other grasshopper species in their ability to use hyperaccumulating and accumulating *Senecio coronatus* as a food source; and (iv) examine the food preference of *Stenoscepa* sp. nymphs when offered a choice of four *Berkheya* species (including two Ni hyperaccumulators) and three *Senecio* species (of which one species, *Senecio coronatus*, was represented by both a Ni hyperaccumulator and a Ni accumulator population, to give a total of four *Senecio* food choices).

Materials and methods

Study site

The area east of Badplaas in Mpumalanga Province, South Africa, contains scattered outcrops of serpentine soils (Morrey *et al*., 1992; Smith *et al*., 2001). Several Ni hyperaccumulator Asteraceae species, including *B. coddii*, *B. rehmannii* Thell. var. *rogersiana* Thell. (hereafter referred to simply as *B. rehmannii*), and *Senecio coronatus* (Thunberg) Harvey, grow on serpentine soils in this area, along with other species in those genera that have not been reported as hyperaccumulators (Smith *et al*., 2001). *Senecio coronatus* is unusual among hyperaccumulators in that some populations on serpentine soils hyperaccumulate Ni but others do not. For example, Boyd *et al*. (2002) reported mean leaf Ni concentrations of 12 100 and 680 μg Ni/g from two populations growing on serpentine soils in the Badplaas area. Furthermore, this species also can be found growing on non-serpentine soils (Smith *et al*., 2001).

The main field site for this work was a serpentine site (Groenvaly) that was included in the survey of serpentine sites of Mpumalanga by Smith *et al*. (2001). Vegetation of this site is predominantly grassland and contains extensive stands of *B. coddii*. We collected *Stenoscepa* sp. nymphs from *B. coddii* on this site for both descriptive studies of whole-body metal concentrations and feeding trials. A general collection of grasshoppers from serpentine plants other than *B. coddii* was also made at the Groenvaly site, targeting open areas dominated by grasses and low forbs. We also made a general collection of grasshoppers from a non-serpentine site in the vicinity of Groenvaly. This site (called here the Airstrip site) hosted an airstrip dominated by grasses and low-growing forbs and thus was similar in physiognomy to the Groenvaly serpentine site.

Descriptive studies

We collected samples of grasshoppers from field sites to compare the metal composition of whole bodies of *Stenoscepa* sp. nymphs against those of other species. Four samples of *Stenoscepa* sp. nymphs were collected from *B. coddii* at the Groenvaly site. Samples of two morphotypes of other grasshoppers (called Serpentine 1 and Serpentine 2) were collected from this same site (a morphotype consists of individuals that are similar in appearance in the field and may represent a single species). We also collected two samples of a grasshopper morphotype (called Non-Serpentine 1) from the non-serpentine Airstrip site. Samples were dried at 60°C for 72 h and analyzed for eight metals (Co, Cr, Cu, Mg, Mn, Ni, Pb and Zn) using the elemental analysis techniques described below. Metal concentrations were compared among grasshopper morphotypes using one-way Analysis of Variance (ANOVA). Fisher's Protected Least Significant Difference (PLSD) test was used for post-hoc means comparisons (Abacus Concepts, 1998).

Field-collected *Stenoscepa* sp. nymphs were raised in plastic containers so that we could collect frass and exuviae for analysis of their metal concentrations. *Stenoscepa* sp. nymphs were collected from *B. coddii* plants at the Groenvaly site and kept in plastic containers at room temperature. Fresh stem tips (terminal 5 cm of stem with associated young leaves) were collected from *B. coddii* plants and provided to nymphs every few days so nymphs could feed *ad libitum*. Exuviae and frass were carefully separated from plant material and air-dried. We dried those samples at 60°C for 72 h and analyzed them for elemental concentrations, using the same technique we used for insect bodies. Five samples of the *B. coddii* tips used to feed the nymphs also were dried at 60°C for 72 h and analyzed for elemental concentrations as described below for plant material. Elemental concentrations were compared between whole insects and exuviae using a *t*-test (Abacus Concepts, 1998). Elemental concentrations of *B. coddii* stem tips and frass also were compared with a *t*-test to determine if they changed during gut passage.

To test the influence of gut contents on whole-body metal concentrations, we designed an experiment in which groups of nymphs were either fed *B. coddii* or starved. Eight groups of nymphs that had been fed *B. coddii* stem tips for 4 days were placed into plastic containers (at least 5 nymphs per container). Containers were paired and one member of each pair was randomly selected for the starvation treatment. We removed all food from the selected container and no additional food was provided to generate insects with relatively empty guts. The other container in each pair was provided with fresh *B. coddii* stem tips. After 49 h, insects in each container were killed, dried and analyzed for metal concentration. Metal concentrations of fed versus starved insects were compared using a *t*-test.

Host suitability of Ni hyperaccumulator leaves

Two no-choice studies were conducted to compare the suitability of Ni hyperaccumulator tissue as a food source for different groups of grasshoppers. The first study was a no-choice feeding experiment using leafy stem tips of *B. coddii*. Three groups of 20 grasshoppers each were used. One group was *Stenoscepa* sp. nymphs collected from *B. coddii* at the Groenvaly site. A second group was composed of nymphs of other grasshopper morphotypes (Serpentine 1 and Serpentine 2) collected from other plants at that serpentine site. The third group was a collection of nymphs of grasshoppers collected from a non-serpentine site (Non-Serpentine 1).

Each grasshopper was placed into a 10 cm diameter Petri plate containing the terminal 5 cm of a *B. coddii* stem. Remaining food was removed and a new stem tip provided after 4 days. Survival of each grasshopper was noted at 3, 4, 7, 10 and 11 days. On day 3, we also made observations on whether the plant material in each container showed evidence of feeding. Each stem tip was classified as either "damaged" or "not damaged".

The second study was also a no-choice experiment in which grasshoppers were offered leaves of either Ni-hyperaccumulating or Ni-accumulating *Senecio coronatus*. We used three groups of grasshoppers. One group contained 40 *Stenoscepa* sp. nymphs. The second group was 36 nymphs and four adults of grasshoppers collected from plant species other than *B. coddii* at the serpentine site. Most of these grasshoppers (21) were the morphotypes we referred to above as Serpentine 1 and Serpentine 2; the remainder belonged to four other morphotypes. The third sample was 36 nymphs and four adults of grasshoppers collected from the non-serpentine site. The non-serpentine group contained 16 nymphs and four adults of three morphotypes: 10 of the 20 members of this group were the morphotype Non-Serpentine 1 referred to above. The remaining non-serpentine grasshoppers were members of two other morphotypes.

To the extent possible, grasshoppers within a group were paired by morphotype, stage (nymph *vs*. adult), and size. One member of each pair was randomly assigned either Ni-hyperaccumulating or Ni-accumulating *S. coronatus* as food. Each grasshopper was placed into a 5-cm diameter Petri plate containing a rectangular piece of *S. coronatus* leaf (ca. 2 × 3 cm) cut from the middle portion of a leaf (adjacent to the midrib). Survival of each grasshopper was noted 3, 5 and 9 days after the start of the experiment. On days 5 and 9, we noted whether there was evidence of feeding damage to the leaf sample in each plate. Damage observations were summarized as either "damaged" or "not damaged" for each grasshopper. Two samples of leaf material from each *S. coronatus* population used for this experiment were dried at 60°C for 72 h, ground and analyzed for elemental composition as described below.

Data from both experiments were analyzed similarly. Survival of the grasshopper groups in each experiment was compared with survival analysis using the Kaplan-Meier estimate (Abacus Concepts, 1998). Treatment significance was determined by the Peto-Peto-Wilcoxon test at $\alpha \leqslant 0.05$ (Abacus Concepts, 1998). Observations on the frequency of feeding damage were compared among grasshopper groups in each experiment using contingency table analysis.

Stenoscepa *sp. host preference studies*

Two host preference studies were conducted using *Stenoscepa* sp. nymphs collected from the Groenvaly site. In one, we tested host preference by allowing them to choose between foliage of four *Berkheya* species (*B. coddii*, *B. rehmannii*, *B. echinacea* and *B. insignis*). The terminal 5 cm was collected from stems of each *Berkheya* species. One stem tip (with associated leaves) from each species was placed into each of 20 Petri plates (10 cm diameter). A nymph was placed into each plate and allowed to feed for 3 days (plates were kept at room temperature). At the end of that time, we ranked the stem tips in each plate for degree of feeding damage from "1" to "4", assigning a "1" to the species that had been damaged most and a "4" to that damaged least. These rank data were analyzed by a Kruskal-Wallis test to determine if species varied in damage ranks. We then used paired Mann-Whitney *U*-tests to determine which species varied in damage rank relative to the others. Additional samples of plant material were saved for elemental analysis. Samples were dried at 60°C for 72 h, ground, and analyzed as described below for plant material. Elemental concentrations of these plant materials were compared using one-way ANOVA followed by Fisher's PLSD test for post-hoc means separations at $\alpha < 0.05$ (Abacus Concepts, 1998).

The other *Stenoscepa* sp. host preference experiment also was a choice experiment, using leaves of several *Senecio* species. We selected *Senecio coronatus* because this species has both Ni hyperaccumulator and Ni accumulator populations growing on serpentine soils. We selected two other *Senecio* species, *S. conrathii* N.E. Br. and *S. oxyriifolius* D.C., because they also were found on serpentine soils in the study area and neither hyperaccumulated Ni. *Stenoscepa* sp. nymphs were collected from *B. coddii* plants at Groenvaly and were offered four types of *Senecio* leaves, including Ni-hyperaccumulating *S. coronatus*, Ni-accumulating *S. coronatus*, *S. conrathii* and *S. oxyriifolius*. Leaves were collected from plants in the field, placed into plastic bags and transported on ice to the laboratory. Small (1 cm ×

1 cm) squares were cut from the leaf blades next to the midrib. One square from each leaf type was impaled through its center upon one of four small toothpicks that projected from a square of moistened cellulose sponge. The arrangement of the species on the four toothpicks was randomized for each sponge so that the identity of the adjacent pieces varied. Marks made using a permanent pen on each toothpick identified the species of leaf piece. One sponge was placed in the center of 20 plastic Petri plates (5 cm in diameter) along with two *Stenoscepa* sp. nymphs.

We visually estimated the amount of each leaf square that had been consumed at 23 and 50 h after the experiment was initiated. Estimates were made to the nearest 5%, except that damage less than 2.5% was assigned a numerical value of 1% to differentiate it from cases in which no damage (0%) was observed. Damage data were analyzed by one-way ANOVA after damage values were arcsin-transformed so that they more closely fit the statistical assumptions underlying that procedure (Zar, 1996). Fisher's PLSD test was used to provide post-hoc mean separations at $\alpha < 0.05$ (Abacus Concepts, 1998).

Samples of plant material used in this experiment were dried at 60°C for 72 h and analyzed for elemental concentrations as described below. Three samples were analyzed for each *Senecio* population/species (except for *S. conrathii*, for which insufficient material remained after the experiment). Elemental concentrations of these plant materials were compared using one-way ANOVA followed by Fisher's PLSD test (Abacus Concepts, 1998).

Elemental analyses

Plant material was analyzed by dry-ashing and analysis of the ash dissolved in concentrated acids (Boyd & Davis, 2001). Plant material was not rinsed with water prior to grinding and analysis to avoid leaching metals from the tissues. Plant samples were dry-ashed at 485°C, further oxidized in 1 mol/L HNO_3, and the residues were re-dissolved in 1 mol/L HCl. For all metals but Ni (Co, Cr, Cu, Mg, Mn, Pb, Zn), the solutions were analyzed using an inductively coupled argon plasma spectrometer (Jarrell-Ash, ICAP 9000). Nickel concentrations were determined using an atomic absorption spectrophotometer (Instrumentation Laboratory, IL 251).

Insect samples were analyzed for metal concentrations in a manner similar to the plant samples except that they were acid-digested (Wall & Boyd, 2002). As with plant samples, insect samples were not rinsed with water prior to analysis to avoid leaching metals from the insect bodies. Samples were digested in borosilicate glass test tubes using 3–5 mL of concentrated HNO_3 at 110°C for 6–8 h. Residue was dissolved in 3–5 mL of 1 mol/L HCl at 110°C for 2–4 h. The solutions were then diluted with distilled water to a volume of 25 mL. Solutions were analyzed for Co, Cr, Cu, Mg, Mn, Pb and Zn using an inductively coupled argon plasma spectrometer (Jarrell-Ash, ICAP 9000). Nickel concentrations were determined using an atomic absorption spectrophotometer (Instrumentation Laboratory, IL 251).

Results

Descriptive studies

Grasshopper taxa differed in concentrations of three metals (Cr, Mn, Ni) but did not differ for the remaining five metals analyzed (Table 1). The morphotype Serpentine 1 contained significantly more Cr and Mn than the other three grasshoppers. The greatest difference between taxa was for Ni, which varied 875-fold among the four taxa (Table 1). *Stenoscepa* sp. contained significantly more Ni

Table 1 Whole-body metal concentrations (means with SE in parentheses) of samples of the four grasshopper morphotypes. Means for a metal with differing superscripts differ significantly among morphotypes (Fisher's PLSD test, $\alpha < 0.05$). Metal concentrations are expressed on a dry mass basis.

Metal (μg/g)	Grasshopper taxon or morphotype			
	Stenoscepa ($n = 4$)	Serpentine 1 ($n = 2$)	Serpentine 2 ($n = 3$)	Non-Serpentine 1 ($n = 2$)
Co	4.8 (2.8)	6.9 (2.1)	0 (0)	0 (0)
Cr	0^b (0)	9.3^a (0.3)	0^b (0)	0^b (0)
Cu	44 (5.9)	62 (19)	53 (12)	63 (9.7)
Mg	1 600 (160)	1 900 (330)	1 400 (190)	940 (88)
Mn	18^b (3.9)	59^a (14)	21^b 6.7)	18^b (2.8)
Ni	$3\,500^a$ (230)	50^b (15)	53^b (32)	4^b (4)
Pb	4.8 (2.8)	6.9 (2.1)	3.3 (3.3)	3.8 (3.8)
Zn	230 (32)	260 (26)	330 (42)	380 (140)

Table 2 Metal concentrations of starved *Stenoscepa* sp., *Stenoscepa* sp. fed *B. coddii* stem tips and *Stenoscepa* sp. exuviae. The column labeled comparison *P*-value is the result of a *t*-test ($\alpha < 0.05$) between the metal levels in the materials listed in that column.

				Comparison *P*-value	
Metal (μg/g)	Starved *Stenoscepa* ($n = 5$)	Fed *Stenoscepa* ($n = 5$)	Exuviae ($n = 2$)	Starved *vs.* Fed *Stenoscepa*	Fed *Stenoscepa* *vs.* Exuviae
Co	0 (0)	0 (0)	0 (0)	–	–
Cr	0 (0)	0 (0)	0 (0)	–	–
Cu	44 (5.3)	51 (7.7)	33 (33)	0.48	0.46
Mg	930 (14)	2 000 (260)	630 (33)	0.003 4	0.026
Mn	6.3 (4.0)	16 (1.6)	0 (0)	0.048	0.001 6
Ni	950 (63)	3 000 (670)	1 200 (160)	0.015	0.17
Pb	0 (0)	7.4 (4.6)	50 (7.4)	0.15	0.18
Zn	510 (180)	430 (72)	680 (180)	0.71	0.17

than all other taxa. Values for *Stenoscepa* sp. samples varied from 3 050 to 4 110 μg Ni/g. The next highest Ni value of any sample was 110 μg Ni/g from a sample of one of the taxa (Serpentine 2) collected from the serpentine Groenvaly site.

Whole body metal analysis for *Stenoscepa* sp. nymphs either starved or fed *B. coddii* leaves showed differences in concentrations of three metals. Fed nymphs contained more (2–3 times) Mg, Mn and Ni than those that were starved (Table 2). Comparison of metal concentrations in exuviae versus whole bodies of fed insects showed more Mg and Mn in fed insects, but no significant difference in Ni concentrations (Table 2). Analyses of *B. coddii* plant material and *Stenoscepa* sp. frass revealed significantly more Mn and Zn in frass but no significant difference in the other elements analyzed, including Ni (Table 3).

Table 3 Elemental concentrations (means, SE in parentheses) of material fed to *Stenoscepa* sp. nymphs and the frass collected from those insects. Means for an element with differing superscripts differ significantly (*t*-test, $\alpha < 0.05$). Element concentrations are expressed on a dry mass basis.

Element (μg/g)	*B. coddii* stem tips ($n = 5$)	*Stenoscepa* sp. frass ($n = 3$)
Ca	13 000 (1 000)	17 000 (1 500)
Cu	15 (1.7)	13 (6.6)
Fe	180 (12)	320 (79)
K	14 000 (1 500)	15 000 (600)
Mg	5 100 (510)	6 600 (900)
Mn	77^{b} (11)	200^{a} (23)
Ni	11 000 (1 800)	13 000 (1 600)
P	2 400 (250)	2 800 (390)
Zn	78^{b} (6.2)	200^{a} (25)

No-choice studies: multiple grasshopper taxa and B. coddii *foliage*

Survival of the four groups of grasshoppers offered *B. coddii* leaves differed significantly (survival analysis, Peto-Peto-Wilcoxon test: $P < 0.000 1$). *Stenoscepa* sp. nymphs survived well whereas the other three groups died rapidly (Fig. 1). Pairwise survival analyses showed no significant difference in survival between Serpentine 1 and Non-Serpentine 1 morphotypes ($P = 0.41$) and Serpentine 1 and Serpentine 2 morphotypes ($P = 0.40$), whereas *Stenoscepa* sp. survival differed significantly from that of the next best-surviving group (Non- Serpentine 1: $P < 0.000 1$).

Observations of feeding damage showed that only

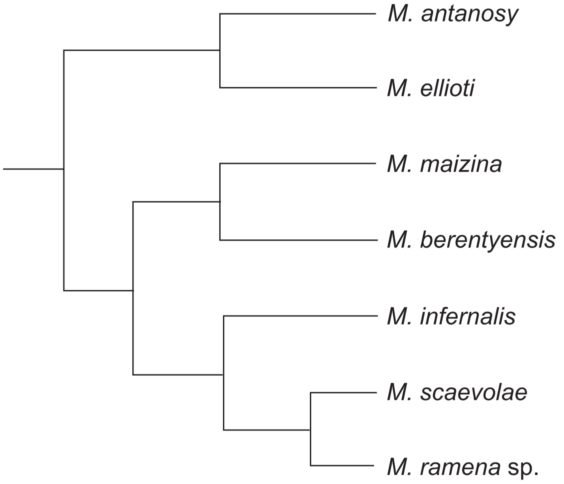

Fig. 1 Survival curves of grasshoppers offered leaves of *B. coddii* in a no-choice feeding experiment. Grasshoppers used included *Stenoscepa* sp. nymphs, nymphs of a grasshopper morphotype (Non-Serpentine 1) collected from the non-serpentine grassland site and two morphotypes (Serpentine 1 and 2) collected from the serpentine site at Groenvaly.

Stenoscepa sp. nymphs readily consumed *B. coddii* leaves. Data from day 3, in which the material in each Petri plate was scored as damaged or undamaged, showed significant variation in the frequency of damage among taxa (contingency table analysis: $\chi^2 = 32$, df = 3, $P < 0.000\,1$). The percentages of insects from each group that damaged the plant material in their Petri plates were: *Stenoscepa* sp., 85%; Serpentine 1, 25%; Serpentine 2, 15%; and Non-Serpentine, 10%. Subdivision of the contingency table showed that *Stenoscepa* differed significantly from Serpentine 1 ($\chi^2 = 15$, df = 1, $P < 0.000\,1$) and that Serpentine 1 did not differ significantly from Non-Serpentine ($\chi^2 = 1.6$, df = 1, $P = 0.21$). Thus, the highly significant result for the complete contingency table stemmed primarily from the high frequency of damage by *Stenoscepa* sp.

No-choice studies: multiple grasshopper taxa and hyperaccumulator vs. accumulator S. coronatus *foliage*

Stenoscepa sp. survived at high rates when fed either Ni hyperaccumulator or Ni accumulator leaves (Fig. 2), whereas other grasshoppers rapidly died when offered either type of leaf (survival analysis: Peto-Peto-Wilcoxon test, $P < 0.000\,1$). As shown by several separate survival analyses, this significant difference was largely due to the high survival of *Stenoscepa* and the low survival of the other grasshopper groups (Fig. 2) rather than due to effects of leaf Ni status. Survival analysis comparing *Stenoscepa* sp. nymphs offered hyperaccumulator or accumulator leaves showed no significant difference (Peto-Peto-Wilcoxon test: $P = 0.97$). Similarly, survival analyses comparing survival of the other groups of grasshoppers showed no influence of leaf Ni status. Survival curves did not differ between Serpentine grasshoppers fed high or low Ni leaves (Peto-Peto-Wilcoxon test: $P = 0.15$), nor did they differ between Non-Serpentine grasshoppers fed high or low Ni leaves (Peto-Peto-Wilcoxon test: $P = 0.78$).

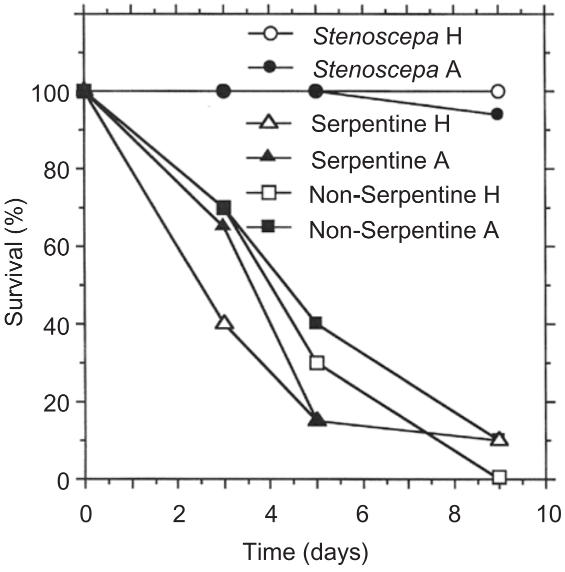

Fig. 2 Survival curves of grasshoppers offered either Ni-hyperaccumulating (H) or Ni-accumulating (A) leaf pieces from *S. coronatus* as food. Grasshopper groups were *Stenoscepa* sp. nymphs, a group of nymphs (plus a few adults) from the serpentine site at Groenvaly (Serpentine), and a group of nymphs (plus a few adults) from the non-serpentine site (Non-Serpentine).

As with the *B. coddii* experiment, observations of feeding damage showed that only *Stenoscepa* sp. readily accepted *S. coronatus* leaves. Percentages of insects that damaged the leaf piece in their Petri plate were: 10% for Serpentine grasshoppers offered hyperaccumulator and 5% for those offered accumulator leaves; 10% for Non-Serpentine grasshoppers offered high-Ni and 0% for those offered accumulator leaves; and 95% for *Stenoscepa* sp. offered hyperaccumulator and 90% for those offered accumulator leaves. Damage frequency varied significantly among grasshopper groups (contingency table analysis: $\chi^2 = 82$, df = 5, $P < 0.000\,1$). Subdividing the contingency table by excluding *Stenoscepa* sp. revealed no significant difference in damage frequency among the other grasshoppers ($\chi^2 = 1.3$, df = 3, $P = 0.72$), indicating that the high damage frequency by *Stenoscepa* sp. nymphs caused the highly significant result for the full contingency table.

Elemental analysis of *S. coronatus* leaf samples showed similar concentrations of seven elements in hyperac-cumulator and accumulator leaves but significant differences for two metals: Ni and Zn (Table 4). Both Ni and Zn concentrations were greater in Ni hyperaccumulator leaves (37-fold and 3.5-fold, respectively) than in accumulator leaves.

Table 4 Elemental analysis means (SE in parentheses) for the *Senecio coronatus* leaves used in the *Stenoscepa* sp. no-choice feeding trial. Means for an element with differing superscripts differ significantly (*t*-test, $\alpha < 0.05$); $n = 2$ for all means.

Element (μg/g)	Hyperaccumulator *S. coronatus*	Accumulator *S. coronatus*
Ca	13 000 (1500)	17 000 (1700)
Cu	8.8 (1.3)	6.5 (0.98)
Fe	390 (31)	480 (11)
K	10 000 (140)	14 000 (1000)
Mg	19 000 (930)	21 000 (610)
Mn	53 (4.5)	37 (7.1)
Ni	15 000^{a} (670)	400^{b} (46)
P	870 (56)	790 (62)
Zn	70^{a} (9.0)	21^{b} (1.2)

Stenoscepa *sp. host preference studies:* Berkheya *species*

Stenoscepa sp. showed distinct food preferences between *Berkheya* species. Damage ranks varied significantly among species (Kruskal-Wallis test: $H = 30$, df = 3, $P < 0.000\,1$). The two Ni hyperaccumulator species (*B. coddii* and *B. rehmannii*) were damaged more greatly than the non-hyperaccumulator species (*B. insignis* and *B. echinata*). Mean damage ranks (SE in parentheses) of each species were (with "1" being most damaged and "4" least damaged): *B. coddii*, 1.1 (0.26); *B. rehmannii*, 2.3 (0.31); *B. insignis*, 3.9 (0.11); and *B. echinata*, 4.0 (0). Mann-Whitney *U*-tests showed no significant difference in damage levels between the two hyperaccumulators (*B. coddii* and *B. rehmannii*: $P = 0.15$) or between the two non-hyperaccumulators (*B. insignis* and *B. echinata*: $P = 0.63$).

Plant material of the four *Berkheya* species varied significantly for four elements (Table 5): Mg, Ni, P and Zn. Both hyperaccumulator species contained more Mg than the two non-hyperaccumulators. Nickel concentration was greatest in *B. coddii*, whereas both of the non-hyperaccumulators barely exceeded the minimum value for Ni accumulation (100 μg Ni/g). Phosphorus was greater in *B. coddii* compared to the other three species, and Zn was also greatest in *B. coddii* (Table 5).

Stenoscepa *sp. host preference studies:* Senecio *populations/ species*

Nymphs of *Stenoscepa* sp. offered a choice between the four types of *Senecio* leaves significantly preferred Ni accumulator *S. coronatus* leaves to all other leaf types. This result was observed at both 23 and 50 h (Table 6). Some feeding damage was observed to samples of each leaf type offered and mean feeding damage for each leaf type increased between 23 and 50 h (Table 6).

Table 6 Results of the feeding choice experiment for *Stenoscepa* sp. nymphs offered leaf pieces from three *Senecio* species. Data are mean percent damage (SE) to leaf pieces of each population/species. Different superscripts denote means for a particular time that differed significantly (Fisher's PLSD test, $\alpha < 0.05$); $n = 20$ for all means.

Time	Hyperaccumulator *S. coronatus*	Accumulator *S. coronatus*	*S. conrathii*	*S. oxyriifolius*
23 h	5.5%^{b} (2.0)	16%^{a} (0.6)	4.8%^{b} (1.3)	1.5%^{b} (0.60)
50 h	18.0%^{b} (5.6)	60%^{a} (6.9)	18.0%^{b} (5.3)	5.8%^{b} (1.40)

Table 7 Elemental analyses of plant material offered to *Stenoscepa* sp. nymphs in the *Senecio* choice experiment. Values are means (SE). Different superscripts denote means for a particular element that differed significantly among *Senecio* leaf types (Fisher's PLSD test, $\alpha < 0.05$); $n = 3$ for all means.

Element (μg/g)	Hyperaccumulator *S. coronatus*	Accumulator *S. coronatus*	*S. oxyriifolius*
Ca	18 000^{b} (1 500)	32 000^{a} (1 400)	8 900^{c} (140)
Cu	9.8 (0)	9.8 (0)	8.8 (0.65)
Fe	320^{c} (3.5)	390^{b} (10)	200^{a} (2.6)
K	18 000^{b} (650)	11 000^{c} (590)	70 000^{a} (2 400)
Mg	12 000^{b} (400)	16 000^{a} (300)	6 800^{c} (270)
Mn	51^{b} (1.9)	77^{b} (2.0)	130^{a} (14)
Ni	12 000^{a} (1 800)	680^{b} (64)	300^{b} (190)
P	680^{b} (9.8)	790^{a} (24)	690^{b} (29)
Zn	80^{a} (1.6)	26^{b} (1.6)	23^{b} (5.0)

Table 5 Elemental analyses of plant material offered *Stenoscepa* sp. nymphs in the *Berkheya* choice experiment. Values are means (SE). Different superscripts for an element denote means that differed significantly among *Berkheya* species (Fisher's PLSD test, $\alpha < 0.05$); $n = 6$ for all means.

Element (μg/g)	*Berkheya* species			
	B. coddii	*B. rehmannii*	*B. insignis*	*B. echinata*
Ca	7 580 (1 150)	10 800 (926)	9 250 (1 260)	9 960 (1360)
Cu	12.2 (0.850)	13.8 (0.748)	13.4 (0.628)	14.2 (0.649)
Fe	201 (4.68)	195 (5.72)	297 (54.7)	227 (37.7)
K	13 800 (1 370)	10 800 (612)	13 000 (1 850)	15 300 (1 370)
Mg	6 060^{a} (235)	5 020^{a} (204)	2 610^{b} (314)	5 050^{a} (1 030)
Mn	39.5 (6.30)	29.5 (2.01)	27.6 (5.00)	29.1 (2.31)
Ni	14 700^{a} (1280)	2 920^{b} (403)	104^{c} (25)	133^{c} (40)
P	1 200^{a} (143)	746^{b} (24.9)	724^{b} (96.8)	825^{b} (114)
Zn	91.6^{a} (2.79)	30.3^{b} (2.29)	21.7^{c} (2.94)	27.5^{b,c} (1.09)

The plant materials used in this experiment differed in elemental concentrations for all elements except Cu (Table 7). The preferred food (Ni accumulator *S. coronatus*) contained more Ca, Mg, and P, and less K, than both other foods offered the grasshoppers. Hyperaccumulator *S. coronatus* leaves contained very large concentrations of Ni (12 000 μg Ni/g), whereas accumulator *S. coronatus* and *S. oxyriifolius* leaves contained levels in the hundreds of μg/g (Table 7).

Discussion

Earlier surveys of *B. coddii* in Mpumalanga Province (Mesjasz-Przybylowicz & Przybylowicz, 2001) reported a number of insects that feed on *B. coddii*, including the beetle *Chrysolina pardalina*. Extensive feeding trials with *Chrysolina pardalina* showed it is a specialist on *B. coddii* (Augustyniak *et al*., 2002) and has physiological mechanisms that allow it to deal with the high Ni concentration of its food (Przybylowicz *et al*., 2003). Our work with *Stenoscepa* sp. shows that it likely is another specialist herbivore of *B. coddii*. Similar investigations into its physiology would form a useful comparison to the results of investigations of *C. pardalina*.

Whole-body Ni concentrations of *Stenoscepa* sp. were extremely high (3 500 μg Ni/g; Table 1). To our knowledge, this is the highest yet reported for an insect species feeding on a hyperaccumulator. Prior studies of other high Ni insects have reported the bioaccumulation index (B) of Laskowski (1991), which compares the level in the insect against that in the tissue it consumes. For *Stenoscepa* sp., our data yield a B-value of 0.24 (3 500 μg Ni/g ÷ 14 700 μg Ni/g) for insects with full guts and 0.065 (950 μg Ni/g ÷ 14 700 μg Ni/g) for those that had been starved. Both values are markedly less than 1 and show that Ni is not bioaccumulated by *Stenoscepa* sp. This result is consistent with other reports of the bioaccumulation index for other insects feeding on hyperaccumulator plants (e.g. Boyd & Wall, 2002), including *Chrysolina pardalina* (Mesjasz-Przybylowicz *et al*., 2004).

The high concentration of Ni in leaves of *B. coddii* suggests that Ni might be a feeding cue for specialist herbivores. However, Ni apparently was not a feeding cue for *Stenoscepa* sp. Leaves of both *B. coddii* and hyperaccumulator *S. coronatus* had similarly high Ni concentrations (14 700 μg Ni/g and 12 000 μg Ni/g, respectively; Tables 3 and 4) and, if Ni alone signified an acceptable host to *Stenoscepa* sp., we would expect both species to be preferred. Among *Berkheya* species *B. coddii* was preferred, but *Stenoscepa* sp. preferred accumulator *Senecio coronatus* leaves to hyperaccumulator *S. coronatus* leaves. We suggest that *Stenoscepa* sp. must use chemical cues other than Ni to discriminate between hosts. It may be that insects are unable to taste Ni: Behmer *et al*. (2005) concluded that a generalist grasshopper (the desert locust, *Schistocerca gregaria* Forskål) was unable to directly detect the Zn concentration of its food. Instead, they concluded that *S. gregaria* relied on organic chemical cues to associate high Zn food with negative aftereffects of ingestion. To our knowledge, Wall and Boyd (2006) have performed the only herbivore choice test using a high Ni insect species offered hyperaccumulator and non-hyperaccumulator leaves of a Ni hyperaccumulator plant species. Using *Melanotrichus boydi* (Heteroptera: Miridae), a specialist on the Ni hyperaccumulator *S. polygaloides* (Brassicaceae) from California, USA, they found no feeding preference, suggesting that Ni is not used by *M. boydi* as a feeding cue.

Boyd and Martens (1998) proposed that hyperaccumulator plants affect food webs in several ways, including by influencing predator-prey interactions. For example, if some insects that consume hyperaccumulator tissues contain elevated whole-body metal levels, they may be toxic to predators in these communities (Boyd & Wall, 2001). Outridge and Scheuhammer (1993) reported toxicity of dietary Ni at 500−2 500 μg Ni/g to mammals and at 300−800 μg Ni/g for young birds. Our samples of *Stenoscepa* sp. contained from 3 050 to 4 110 μg Ni/g (mean of 3 500 μg Ni/g) and thus certainly contain enough Ni to cause toxicity if consumed by vertebrate predators in adequate quantities. However, Boyd (1998) pointed out that dietary dilution of Ni can allow generalist herbivores to circumvent elemental defenses in plants. Dietary dilution also may allow generalist predators to dilute Ni consumed with their prey. Thus, the effect of high-metal insects on food webs will probably depend on many factors, including the prey preferences of predators and the relative abundance of high-metal insects among available prey. The impact of a high-metal insect such as *Stenoscepa* sp. upon the food web of its serpentine community awaits further studies that specifically target those issues.

Acknowledgments

We wish to thank Auburn University and the University of the Witwatersrand for providing travel funds for this research. We are grateful to Sappi Limited for access to study sites and permission to sample insects and collect plant samples. Aid with elemental analyses of plant and insect samples was provided by J. Odom of Auburn University. We thank two anonymous reviewers for helpful comments on the original manuscript.

References

Abacus Concepts (1998) *StatView*. SAS Institute Inc., Cary, USA.

Anderson, C.W.N., Brooks, R.R., Chiarucci, A., LaCoste, C.J., Leblanc, M., Robinson, B.H., Simcock, R. and Stewart, R.B. (1999) Phytomining for nickel, thallium and gold. *Journal of Geochemical Exploration*, 67, 407–415.

Angle, J.S. and Linacre, N.A., (2005) Metal phytoremediation - A survey of potential risks. *International Journal of Phytoremediation*, 7, 241–254.

Augustyniak, M., Mesjasz-Przybylowicz, J., Miroslaw, N., Dybowska, M., Przybylowicz, W. and Migula, P. (2002) Food relations between *Chrysolina pardalina* and *Berkheya coddii*, a nickel hyperaccumulator from South African ultramafic outcrops. *Fresenius Environmental Bulletin*, 11, 85–90.

Behmer, S.T., Lloyd, C.M., Raubenheimer, D., Stewart-Clark, J., Knight, J., Leighton, R.S., Harper, F.A. and Smith, J.A.C. (2005) Metal hyperaccumulation in plants: mechanisms of defence against herbivores. *Functional Ecology*, 19, 55–66.

Boyd, R.S. (2004) Ecology of metal hyperaccumulation. *New Phytologist*, 162, 563–567.

Boyd, R.S. (1998) Hyperaccumulation as a plant defensive strategy. *Plants That Hyperaccumulate Heavy Metals: Their Role in Phytoremediation, Microbiology, Archaeology, Mineral Exploration and Phytomining* (ed. R.R. Brooks), pp. 181–201. CAB International, Wallingford, Oxon, UK.

Boyd, R.S., Davis, M.A., Wall, M.A. and Balkwill, K. (2006) Metal concentrations of insects associated with the South African Ni hyperaccumulator *Berkheya coddii* (Asteraceae). *Insect Science*, 13, 85–102.

Boyd, R.S., Wall, M.A. and Davis, M.A. (2004) The ant-mimetic plant bug, *Coquilletia insignis* (Heteroptera: Miridae) feeds on the Ni hyperaccumulator plant, *Streptanthus polygaloides* (Brassicaceae). *Ultramafic Rocks: Their Soils, Vegetation and Fauna* (eds. R.S. Boyd, A.J.M Baker & J. Proctor), pp. 227–231. Science Reviews 2000 Ltd, St Albans, UK.

Boyd, R.S., Davis, M.A., Wall, M.A. and Balkwill, K. (2002) Nickel defends the South African hyperaccumulator *Senecio coronatus* (Asteraceae) against *Helix aspersa* (Mollusca: Pulmonidae). *Chemoecology*, 12, 91–97.

Boyd, R.S. and Davis, M.A. (2001) Metal tolerance and accumulation ability of the Ni hyperaccumulator *Streptanthus polygaloides* Gray (Brassicaceae). *International Journal of Phytoremediation*, 3, 353–367.

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

Boyd, R.S., Martens, S.N. and Davis, M.A. (1999) The nickel hyperaccumulator *Streptanthus polygaloides* (Brassicaceae) is attacked by the parasitic plant *Cuscuta californica* (Cuscutaceae). *Madroño*, 46, 92–99.

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

Boyd, R.S. and Martens, S.N. (1998) The significance of metal hyperaccumulation for biotic interactions. *Chemoecology*, 8, 1–7.

Boyd, R.S. and Martens, S.N. (1994) Nickel hyperaccumulated by *Thlaspi montanum* var. *montanum* is acutely toxic to an insect herbivore. *Oikos*, 70, 21–25.

Boyd, R.S., Shaw, J. and Martens, S.N. (1994) Nickel hyperaccumulation defends *Streptanthus polygaloides* (Brassicaceae) against pathogens. *American Journal of Botany*, 81, 194–300.

Boyd, R.S. and Martens, S.N. (1992) The raison d'être for metal hyperaccumulation by plants. *The Vegetation of Ultramafic (Serpentine) Soils* (eds. A.J.M. Baker, J. Proctor & R.D. Reeves), pp. 270–289. Intercept, Andover, UK.

Brooks, R.R., Lee, J., Reeves, R.D. and Jaffré, T. (1977) Detection of nickeliferous rocks by analysis of herbarium specimens of indicator plants. *Journal of Geochemical Exploration*, 7, 49–57.

Brooks, R.R., Robinson, B.H., Howes, A.W. and Chiarucci, A. (2001) An evaluation of *Berkheya coddii* Roessler and *Alyssum bertolonii* Desv. for phytoremediation and phytomining of nickel. *South African Journal of Science*, 97, 558–560.

Davis, M.A., Pritchard, S.G., Boyd, R.S. and Prior, S.A. (2001) Developmental and induced responses of nickel-based and organic defences of the nickel-hyperaccumulating shrub, *Psychotria douarrei*. *New Phytologist*, 150, 49–58.

Ghaderian, S.M., Lyon, A.J.E. and Baker, A.J.M. (2000) Seedling mortality of metal hyperaccumulator plants resulting from damping-off by *Pythium* spp. *New Phytologist*, 146, 219–224.

Harborne, J.B. (1988) *Introduction to Ecological Biochemistry*. Academic Press, London, UK.

Iturralde, R.B. (2004) Notes on tropical American nickel hyperaccumulating plants. *Ultramafic Rocks: Their Soils, Vegetation and Fauna* (eds. R.S. Boyd, A.J.M. Baker & J. Proctor), pp. 255–258. Science Reviews Ltd, St Albans, UK.

Jhee, E.M., Boyd, R.S. and Eubanks, M.D. (2005) Nickel hyperaccumulation as an elemental defence of *Streptanthus polygaloides* (Brassicaceae): influence of herbivore feeding mode. *New Phytologist*, 168, 331–344.

Laskowski, R. (1991) Are the top carnivores endangered by heavy metal biomagnification? *Oikos*, 60, 387–390.

Martens, S.N. and Boyd, R.S. (1994) The ecological significance of nickel hyperaccumulation: a plant chemical defense. *Oecologia*, 98, 379–384.

Mesjasz-Przybylowicz, J., Migula, P., Nakonieczny, M., Przybylowicz, W., Augustyniak, M., Tarnawska, M. and Glowacka, E. (2004) Ecophysiology of *Chrysolina pardalina*

Fabricius (Chrysomelidae), a herbivore of the South African Ni hyperaccumulator *Berkheya coddii* (Asteraceae). *Ultramafic Rocks: Their Soils, Vegetation and Fauna* (eds. R.S. Boyd, A.J.M. Baker & J. Proctor), pp. 233–241. Science Reviews 2000 Ltd, St Albans, UK.

Mesjasz-Przybylowicz, J. and Przybylowicz, W.J. (2001) Phytophagous insects associated with the Ni-hyperaccumulating plant *Berkheya coddii* (Asteraceae) in Mpumalanga, South Africa. *South African Journal of Science*, 97, 596–598.

Morrey, D.R., Balkwill, K., Balkwill, M.-J. and Williamson, S. (1992) A review of some studies of the serpentine flora of Southern Africa. *The Vegetation of Ultramafic (Serpentine) Soils* (eds. A.J.M. Baker, J. Proctor & R.D. Reeves), pp. 147–157. Intercept, Andover, UK.

Outridge, P.M. and Scheuhammer, A.M. (1993) Bioaccumulation and toxicology of nickel: implications for wild animals and birds. *Environmental Review*, 1, 172–197.

Pilon-Smits, E. (2004) Phytoremediation. *Annual Review of Plant Biology*, 56, 15–39.

Pollard, A.J. and Baker, A.J.M. (1997) Deterrence of herbivory by zinc hyperaccumulation in *Thlaspi caerulescens* (Brassicaceae). *New Phytologist*, 135, 655–658.

Przybylowicz, W.J., Mesjasz-Przybylowicz, J., Migula, P., Glowacka, E., Nakonieczny, M. and Augustyniak, M. (2003) Functional analysis of metals distribution in organs of the beetle *Chrysolina pardalina* exposed to excess of nickel by Micro-PIXE. *Nuclear Instruments and Methods in Physics Research B*, 210, 343–348.

Reeves, R.D. (2003) Tropical hyperaccumulators of metals and their potential for phytoextraction. *Plant and Soil*, 249, 57–65.

Reeves, R.D. and Baker, A.J.M. (2000) Metal-accumulating plants. *Phytoremediation of Toxic Metals: Using Plants to Clean Up the Environment* (eds. I. Raskin & B.D. Ensley), pp. 193–228. John Wiley & Sons, New York, USA.

Robinson, B.H., Brooks, R.R., Howes, A.W., Kirkman, J.H. and Gregg, P.E.H. (1997) The potential of the high-biomass nickel hyperaccumulator *Berkheya coddii* for phytoremediation and phytomining. *Journal of Geochemical Exploration*, 60, 115–126.

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

Smith, S., Balkwill, K. and Williamson, S. (2001) Compositae on serpentine in the Barberton Greenstone Belt, South Africa. *South African Journal of Science*, 97, 518–520.

Wall, M.A. and Boyd, R.S. (2002) Nickel accumulation in serpentine arthropods from the Red Hills, California. *Pan-Pacific Entomologist*, 78, 168–176.

Wall, M.A. and Boyd, R.S. (2006) *Melanotrichus boydi* (Heteroptera: Miridae) is a specialist on the nickel hyperaccumulator *Streptanthus polygaloides* (Brassicaceae). *Southwestern Naturalist*, 51, 481–489

Whiting, S.N., Reeves, R.D., Richards, D., Johnson, M.S., Cooke, J.A., Malaisse, F., Paton, A., Smith, J.A.C., Angle, J.S., Chaney, R.L., Ginocchio, R., Jaffré, T., Johns, R., McIntyre, T., Purves, G.W., Salt, D.E., Schat, H., Zhao, F.J. and Baker, A.J.M. (2004) Research priorities for conservation of metallophyte biodiversity and their potential for restoration and site remediation. *Restoration Ecology*, 12, 106–116.

Zar, J.H. (1996) *Biostatistical Analysis*. Prentice-Hall, Englewood Cliffs, USA.

Accepted August 22, 2006