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High-nickel insects and nickel hyperaccumulator plants: A review

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Abstract Insects can vary greatly in whole-body elemental concentrations. Recent investigations of insects associated with Ni hyperaccumulator plants have identified insects with relatively elevated whole-body Ni levels. Evaluation of the limited data available indicates that a whole-body Ni concentration of 500 μg Ni/g is exceptional: I propose that an insect species with a mean value of 500 μg Ni/g or greater, in either larval/nymphal or adult stages, be considered a "high-Ni insect". Using the 500 μg Ni/g criterion, 15 species of high-Ni insects have been identified to date from studies in Mpumalanga (South Africa), New Caledonia and California (USA). The highest mean Ni concentration reported is 3 500 μg Ni/g for nymphs of a South African *Stenoscepa* species (Orthoptera: Pyrgomorphidae). The majority of high-Ni insects (66%) are heteropteran herbivores. Studies of high-Ni insect host preference indicate they are monophagous (or nearly so) on a particular Ni hyperaccumulator plant species. Much of the Ni in bodies of these insects is in their guts (up to 66% – 75%), but elevated levels have also been found in Malpighian tubules, suggesting efficient elimination as one strategy for dealing with a high-Ni diet. Tissue levels of Ni are generally much lower than gut concentrations, but up to 1 200 μg Ni/g has been reported from exuviae, suggesting that molting may be another pathway of Ni elimination. One ecological function of the high Ni concentration of these insects may be to defend them against natural enemies, but to date only one experimental test has supported this "elemental defense" hypothesis. Community-level studies indicate that high-Ni insects mobilize Ni into food webs but that bioaccumulation of Ni does not occur at either plant-herbivore or herbivore-predator steps. Unsurprisingly, Ni bioaccumulation indices are greater for high-Ni insects compared to other insect species that feed on Ni hyperaccumulator plants. There is some evidence of Ni mobilization into food webs by insect visitors to flowers of Ni hyperaccumulator plants, but no high-Ni insect floral visitors have been reported.

Key words bioaccumulation, elemental defense, heavy metals, herbivore defense, hyperaccumulation, metal tolerance, nickel

Introduction

Organisms can vary widely in their elemental makeup. Since the late 1970s, there has been considerable interest among botanists in plants that take up large amounts of particular soil elements into their tissues. The most extreme cases are hyperaccumulator plants (Brooks *et al.*, 1977) and an extensive literature has developed around the identification (e.g., Reeves & Baker, 2000; Reeves *et al.*, 2007), physiology and genetics (e.g., Hanikenne *et al.*, 2008), and applied uses of these unusual plants. Applied uses include phytomining, in which harvest of these plants allows recovery of the element accumulated in their tissues (e.g., Anderson *et al.*, 1999), and phytoremediation, in

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which harvest of the plants allows removal of an element from an element-contaminated site (Pilon-Smits, 2004; Pilon-Smits & Freeman, 2006). The threshold values set for hyperaccumulation vary by element: Mn and Zn hyperaccumulators contain > 10 000 μg/g dry mass (Reeves & Baker, 2000), hyperaccumulators of As, Co, Cu, Ni, Se and Pb contain > 1 000 μg/g (Brooks, 1998; Reeves & Baker, 2000; Ma *et al.*, 2001), and hyperaccumulators of Cd contain > 100 μg/g (Reeves & Baker, 2000). The hyperaccumulator plant concept also has been applied to a few other elements, including Al (Jansen *et al.*, 2002), B (Babaoglu *et al.*, 2004) and Fe (Rodríguez *et al.*, 2005).

Most hyperaccumulator plants hyperaccumulate Ni: at the time of their review, Reeves & Baker (2000) estimated that 418 hyperaccumulator taxa had been documented, with the majority of these (76%) hyperaccumulating Ni. Nickel hyperaccumulators usually grow on serpentine soils (Brooks, 1987; Reeves & Baker, 2000), which have relatively high levels of Cr, Mg, and Ni, and low levels of Ca, forming a challenging medium for plant growth (Kruckeberg, 1984; Proctor, 1999; Alexander *et al.*, 2007).

Plant communities that contain hyperaccumulator plants are inhabited by insect species that interact with these plants (especially via herbivory). Through these interactions, hyperaccumulated elements may be transferred to members of the insect community and some insects contain relatively elevated concentrations (Boyd, 1998a). Concentrations of elements in insects often have been studied in the context of pollution effects upon communities (e.g. Posthuma & van Straalen, 1993). Human activities have resulted in large releases of elements (including metals) into the environment, sometimes with severe ecological consequences. Understanding how these pollutants move through food webs, and the effects of these pollutants on organisms, is the topic of a large body of scientific literature.

Relative to other heavy metals, such as Cd or Pb, Ni concentrations in terrestrial insects are not often documented (e.g., Posthuma & van Straalen, 1993; Heikens *et al.*, 2001). However, since Ni hyperaccumulators are the most numerous of all hyperaccumulator species and are found in many serpentine areas worldwide (Reeves & Baker, 2000), they probably interact with a wide variety of insect species. Recent studies have begun to explore these insect-Ni hyperaccumulator plant interactions, with a first step being to identify insects that contain unusually high whole-body levels of Ni. These high-Ni insects are likely to: (i) directly interact with Ni hyperaccumulator plants; and (ii) have adaptations that allow the insects to tolerate large Ni burdens. The objective of this review is to explore what we know to date about high-Ni insects and to outline some of the future research directions that they provide. The focus here is on high-Ni insects because: (i) the many Ni hyperaccumulator plant species identified to date have probably evolved in association with a wide variety of insects; and (ii) these insects have been the target of recent research efforts that can serve as model studies for these types of investigations. Plants that hyperaccumulate other elements (especially Se) are also being explored in terms of their interactions with insects (e.g., Quinn *et al.*, 2007; Galeas *et al.*, 2008), but these studies will not be the major focus here.

Defining high-Ni insects

We can use the approach that botanists used to define Ni hyperaccumulator plants as a model for creating a definition of high-Ni insects. In the botanical work, hyperaccumulator plants were defined by surveying the element concentrations of a large number of plant taxa. The distribution of the resulting data was then examined for discontinuities and hyperaccumulation defined by apparent "breaks" in the data distribution (Brooks, 1987; Pollard *et al.*, 2002). The resulting definition has been criticized as being arbitrary (Macnair, 2003) but has served a useful purpose by attracting scientific attention to these plants. Once hyperaccumulators were defined and identified as being biologically unusual, the cellular and physiological processes that underlie this trait began to be explored and we have learned much about these phenomena (Salt, 2004; Hanikenne *et al.*, 2008). A similar benefit may be realized from defining and characterizing high-Ni insects.

To define high-Ni insects, we need to examine data on whole-body Ni concentrations of large numbers of insect species. In particular, surveys of the Ni concentrations of the insect community of a site that contains hyperaccumulator plants would be particularly useful for this purpose. Unfortunately, such survey data are rare for any element hyperaccumulated by plants, including Ni [but see recent work by Galeas *et al.* (2008) on insects associated with the Se hyperaccumulator *Stanleya pinnata* (Pursh.) Britt. (Brassicaceae) in Colorado, USA]. To my knowledge, the only study to attempt a general faunal survey of the Ni concentration of arthropods (mainly insects) at a site hosting an Ni hyperaccumulator species is that of Wall and Boyd (2002). That study measured Ni in 134 samples of insect morphospecies (morphospecies are visually distinct specimens, likely representing biological species, based on their appearance in the field) collected by pitfall traps, night-lighting, and sweep-netting. The distribution of those Ni concentration data (Fig. 1) shows that most of the values (119, or 89%) were < 100 μg/g dry mass and that there was an absence of values between 250 μg/g and 550 μg/g. Values > 550 μg/g included only 6% of the

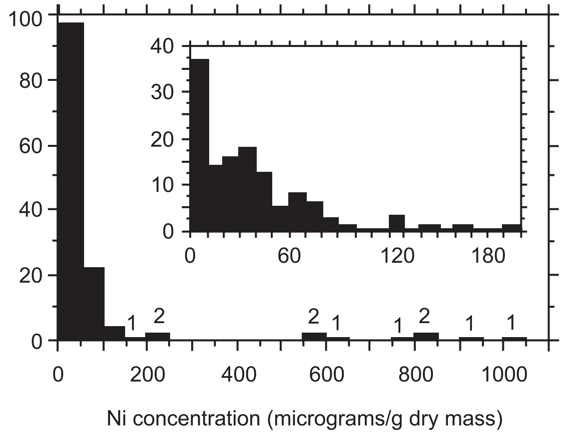

Fig. 1 Histogram showing distribution of Ni concentration data from 134 samples of arthropods (mainly insects) collected from a serpentine site in California, USA, hosting the Ni hyperaccumulator *Streptanthus polygaloides* (Wall & Boyd, 2002). Data are individual sample values. For concentrations of > 150 μg Ni/g (on the *x*-axis), sample sizes for small bars in the main graph are denoted by numbers on top of each bar. The inset shows the distribution of data points in the range of 0 - 180 μg Ni/g, using an expanded scale on the *x*-axis, to provide more detail on the distribution of relatively low Ni values.

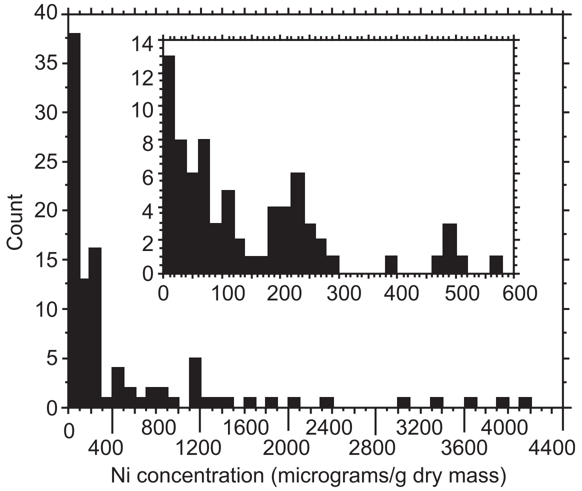

Fig. 2 Histogram showing distribution of Ni concentration data from 99 samples of insects collected from the Ni hyperaccumulator *Berkheya coddii* in Mpumalanga, South Africa (Boyd *et al.*, 2006a). Data are individual sample values. The inset shows the distribution of data points in the range of 0 - 600 μg Ni/g, using an expanded scale on the *x*-axis, to provide more detail on the distribution of relatively low Ni values.

dataset and all were samples of one species, *Melanotrichus boydi* Schwartz & Wall (Heteroptera: Miridae). This insect is monophagous on the Ni hyperaccumulator *Streptanthus polygaloides* Gray: Brassicaceae, a plant species endemic to serpentine soils and found on that field site (Wall & Boyd, 2006).

There is a second faunal survey, smaller in scope, which provides a more limited dataset. Boyd *et al.* (2006a) collected insects from the South African Ni hyperaccumulator *Berkheya coddii* Roessler (Asteraceae), restricting collection only to insects captured directly from these plants. A total of 99 samples were analyzed for Ni. The distribution of those data (Fig. 2) shows a similar clustering of values at low Ni concentrations, but considerably more spread of the data among Ni concentrations > 100 μg/g. Values > 400 μg/g represent 31% of the data values and 18% are > 1 000 μg/g.

Statistical comparison of the datasets of Wall and Boyd (2002) in Figure 1 (mean = 80 μg/g), and Boyd *et al.* (2006a) in Figure 2 (mean = 540 μg/g), shows these means differ significantly (Mann-Whitney *U*-test, tied Z-value = 7.7, $P < 0.000\,1$). There are several potential reasons for this difference. First, the samples were not collected in a similar way. The California sample included insects captured directly from the hyperaccumulator plant, but also included insects collected during more general habitat sampling. In South Africa, only insects collected directly from the hyperaccumulator were included in the study. This difference in approach may be important, as the California study (Wall & Boyd, 2002) reported that a comparison of insect Ni concentrations between taxa collected directly from the hyperaccumulator (*S. polygaloides*) with the Ni concentrations of taxa collected from general habitat sampling showed that those collected from the hyperaccumulator had significantly greater Ni concentrations. Thus we might expect the South African data to be greater in Ni because only insects collected directly from the hyperaccumulator plant were included. Second, the plant species in these two studies differ greatly in biomass and degree of Ni accumulation. *Streptanthus polygaloides* is a relatively small (up to 100 cm) winter annual (Hickman, 1993) for which maximum Ni concentrations reported by Reeves *et al.* (1981) were 16 400 μg/g. *Berkheya coddii* is a much larger herbaceous perennial (growing to 150 cm: Anderson *et al.*, 1997) for which a maximum of 77 000 μg Ni/g has been reported from leaves (Mesjasz-Przybylowicz & Przybylowicz, 2001). The two species thus differ greatly in the amount of Ni mobilized by their biomass into their communities, since *B. coddii* contributes more biomass to its community and that biomass also has a greater Ni concentration than *S. polygaloides*. From this standpoint, it is unsurprising that Ni concentrations of the South African insect samples were generally greater than the California ones. Finally, the differences may reflect other biological factors, such as differing degrees of adaptation to Ni of the insect herbivores in the different locations. Detailed studies will be needed to assess the relative importance of each of these factors to data collected from different field sites.

It is clear that we need more surveys of insects from all types of habitats (including those containing Ni hyperaccumulator plants) in order to more effectively judge what concentration of Ni is exceptionally elevated. The few data available to date, and summarized above, indicate that 500 μg Ni/g is a remarkably high Ni concentration. Thus I suggest that 500 μg Ni/g be used as the minimum level to define a high-Ni insect. I further suggest that this threshold be set for the mean concentration of sample values for a particular insect species, in either its larval or adult stages. Reeves (1992) defined Ni hyperaccumulation in plants as a report of at least one sample value of 1 000 μg Ni/g or greater from a field-collected sample. By defining high-Ni insects based on a mean, rather than a single sample value, the standard is more conservative and also will be less influenced by outliers. Other issues involved in setting this standard, such as the importance of gut contents in measures of whole-body Ni concentrations, are discussed below.

Table 1 lists the high-Ni insect species identified to date that have been collected from Ni hyperaccumulator plants, using the 500 μg Ni/g criterion. Note that, as mentioned previously, an insect species may meet the criterion described above for samples of larvae or nymphs but not for samples of adults (or vice versa), from the same insect species. This is illustrated by *Anoplocnemis dallasiana* Lethierry & Serville (Heteroptera: Coreidae) in Table 1: Migula *et al.* (2007) reported 1 300 μg Ni/g in nymphs but only 280 μg Ni/g in adults. In the reverse situation, they reported less from nymphs (43 μg Ni/g) but more from adults (520 μg Ni/g) of *Rhinocoris neavei* Bergroth (Heteroptera: Reduviidae).

Inspection of Table 1 shows the majority of high-Ni insects identified to date are heteropterans (73%), implying that this group is unusually well represented among high-Ni insects. Whether heteropterans are, in fact, disproportionately represented relative to other insect orders cannot be judged at this time. This can only be determined by comparing the proportions of insect orders in the insect community against the proportions of orders in the high-Ni insect fauna. Information on the insect communities of hyperaccumulator plant habitats is not available and so a rigorous test of this

Table 1 Mean Ni concentrations of high-Ni insects (defined by a whole-body Ni concentration 500 μg/g) discovered to date from surveys of Ni hyperaccumulators.

Insect taxa	Mean Ni (μg/g)	Ni hyperaccumulator host species	Locality	References
Heteroptera				
Agonoscelis erosa Westwood	1300	*Berkheya coddii*	Mpumalanga, South Africa	4
Anoplocnemis curvipes Fabricius	510	*Berkheya coddii*	Mpumalanga, South Africa	4
A. dallasiana Lethierry & Serville (nymphs)	1300	*Berkheya coddii*	Mpumalanga, South Africa	6
Boerias ventralis Dallas	580	*Berkheya coddii*	Mpumalanga, South Africa	4
Coquilletia insignis Uhler	500	*Streptanthus polygaloides*	California, USA	2
Melanotrichus boydi Schwartz & Wall	750	*Streptanthus polygaloides*	California, USA	1
Rhinocoris neavei Bergroth	520	*Berkheya coddii*	Mpumalanga, South Africa	6
Sphaerocoris testudogriseus DeGeer	530	*Berkheya coddii*	Mpumalanga, South Africa	4
Spilostethus rivularis Germar	630	*Berkheya coddii*	Mpumalanga, South Africa	4
Utana viridipuncta Bergroth (adults)	780	*Hybanthus austrocaledonicus*	New Caledonia	3
Utana viridipuncta (nymphs)	2600	*Hybanthus austrocaledonicus*	New Caledonia	3
Veterna sanguineirostris Thunberg	1800	*Berkheya coddii*	Mpumalanga, South Africa	4
Coleoptera				
Chrysolina pardalina Fabricius	2700	*Berkheya coddii*	Mpumalanga, South Africa	5
C. pardalina	1300	*Berkheya coddii*	Mpumalanga, South Africa	6
Unidentified Curculionidae	1100	*Berkheya coddii*	Mpumalanga, South Africa	4
Orthoptera				
Stenoscepa sp. nov.	3500	*Berkheya coddii*	Mpumalanga, South Africa	4

References listed for the data presented are: 1 = Schwartz and Wall (2001); 2 = Boyd *et al.* (2004); 3 = Boyd *et al.* (2006a); 4 = Boyd *et al.* (2006b); 5 = Mesjasz-Przybylowicz and Przybylowicz (2001) and Augustyniak *et al.* (2002); 6 = Migula *et al.* (2007).

idea is not feasible at this time. However, it is suggestive that the three widely separated localities that have been investigated to date for high-Ni insects (New Caledonia, California, and South Africa: Table 1) have each yielded at least one high-Ni heteropteran. In addition, Peterson *et al.* (2003) reported a very high level of Ni (1 300 μg Ni/g) for composite (multispecies) samples of insects collected from areas with high densities of a Portuguese Ni hyperaccumulator (*Alyssum pintodasilvae* Dudley: Brassicaceae). These samples included several species of "large-bodied Heteroptera": Peterson *et al.* (2003) suspected those heteropterans may have contained high Ni concentrations and thus been responsible for the overall high values of those composite samples. It seems likely that one or more high-Ni heteropteran species were present in those Portuguese serpentine sites: this would be yet another example of high-Ni heteropterans, and from a site on yet another continent (Europe).

We would expect that herbivores, because they occupy the trophic level which directly ingests Ni hyperaccumulator tissues, to be well represented in Table 1. This is the case: almost all of the insects in Table 1 are herbivores, with the lone exception being the predacious assassin bug *Rhinocoris neavei*. Herbivores may feed on Ni hyperaccumulator plants in many ways (Jhee *et al.*, 2005), and we can evaluate the insects in Table 1 with regard to their feeding mode. Because many of the herbivores are heteropterans, the piercing-sucking feeding mode typical of this group is also well represented in Table 1. Leaf-chewing insects are represented by the genera *Stenoscepa* (Orthoptera: Pyrgomorphidae) and *Chrysolina* (Coleoptera: Chrysomelidae) and perhaps the unidentified Curculionid (Coleoptera).

In light of the prevalence of heteropterans in Table 1, it is interesting that the sole predator high-Ni insect found to date (*R. neavei*) is also a heteropteran. But it is difficult to ascribe much significance to this fact because we have so few data from other carnivorous insects. Boyd *et al.* (2006a) reported relatively low values of Ni (36 μg Ni/g) from mantids collected from the same Ni hyperaccumulator species (*Berkheya coddii*) as the *R. neavei* collected by Migula *et al.* (2007). Nickel values have also been reported from *Polyrhachis* ants (Hymenoptera: Formicidae) associated with *B. coddii*, but those ants were also tending aphids that were feeding on the hyperaccumulator and so Ni in the ants may not solely have come from the ants' predacious activities. Even so, Ni concentrations of these ants were relatively low: 97 μg Ni/g reported by Boyd *et al.* (2006a) and 85 μg Ni/g reported by Migula *et al.* (2007).

Feeding specialization and Ni tolerance

Insect herbivores range widely in host specificity and we can examine this question for the high-Ni insect herbivores in Table 1. Host specificity has been addressed for only a few species. Host choice tests have been conducted for three of the species in Table 1: *Chrysolina pardalina* (Coleoptera: Chrysomelidae), *Melanotrichus boydi*, and *Stenoscepa* sp. No-choice tests, that compare growth or survival of a high-Ni insect when individuals are restricted to feeding on one of a small collection of potential hosts, have also been conducted in these cases. For *Chrysolina pardalina*, choice tests showed a preference for the host Ni hyperaccumulator *B. coddii*, even when another Ni hyperaccumulator species, *B. zehyeri* (Sond. & Harv.) Oliv. & Hiern subsp. *rehmannii* (Thell.) Roessler (Asteraceae), also known as *B. rehmannii* Thell. var. *rogersiana* Thell., was offered. Mesjasz-Przybylowicz and Przybylowicz (2001) showed that *C. pardalina* could be raised on *B. coddii* for its entire life cycle in a no-choice test. Studies of *M. boydi* showed a preference for the host Ni hyperaccumulator *S. polygaloides* in several choice tests (Wall & Boyd, 2006): these used other dominant plant species in the habitat of *M. boydi* as well as other species in the same host family (Brassicaceae) and host genus (*Streptanthus*). Wall and Boyd (2006) also tested for population-level host preference, using insects and *S. polygaloides* plants collected from several *S. polygaloides* populations. They found no preference of *M. boydi* for plants from their "home" population. Host preference studies of *Stenoscepa* sp. (Boyd *et al.*, 2007) included no-choice tests that contrasted *Stenoscepa* sp. against other grasshopper species: these showed *Stenoscepa* sp. survived better than the other grasshopper species when fed *Stenoscepa*'s putative host (*B. coddii*). Follow-up choice experiments with *Stenoscepa* sp., comparing preference for hosts within the genus *Berkheya*, showed that both *B. coddii* and the co-occurring Ni hyperaccumulator *B. rehmannii* were preferred over two relatively low-Ni *Berkheya* species. Other choice experiments, using *Stenoscepa* and several *Senecio* species as hosts, showed a preference of *Stenoscepa* for Ni hyperaccumulator *Senecio coronatus* (Thunberg) Harvey leaves, although leaves from a low-Ni population *S. coronatus* also were available (but not preferred as a choice) in the experiment.

Results of the above studies, although restricted to just a handful of high-Ni insects, suggest that their host range is relatively narrow: *C. pardalina* and *M. boydi* are monophagous while *Stenoscepa* sp. has a slightly more broad host range. While the tests conducted have revealed monophagy or near-monophagy in the cases examined, a further question is whether high-Ni insects are more likely to be host specialists than other insect herbivores that feed on Ni hyperaccumulator plants. An affirmative answer would suggest that host specialization by high-Ni insects is achieved at the cost of host choice breadth. In turn, limited host breadth of high-Ni insects would imply that adaptations of high-Ni insects that allow them to tolerate their high-Ni diet exclude them from consuming other potential host species. Identifying those adaptations and understanding their evolutionary history is an exciting area ripe for exploration.

One likely adaptation of high-Ni insects is physiological tolerance of Ni. It seems reasonable that high-Ni insects are relatively Ni-tolerant, because they consume high-Ni food without apparent harm. But rigorous tests of this tolerance assumption, using controlled experiments that compare the toxicity of Ni to high-Ni herbivores against its toxicity to other insect herbivores, are lacking. That such experimental confirmation is necessary stems from studies (e.g., Boyd, 2007a; Sorenson *et al.*, 2009) in which relatively high levels of metal tolerance are reported from insects that are unlikely to be exposed to high metal levels in their natural environments. Thus it seems unwise to assume Ni tolerance is greater in high-Ni insects, especially since there are herbivores of Ni hyperaccumulator plants that do not have high whole-body Ni concentrations yet also feed upon high-Ni plant tissues (Wall & Boyd, 2002; Boyd *et al.*, 2006a, b; Migula *et al.*, 2007).

If some high-Ni insects are monophagous on a Ni hyperaccumulator species, then it follows to ask how the insect recognizes its host plant and what feeding cues may be involved. Because the high Ni concentration in Ni hyperaccumulator tissues is a distinctive chemical signature, it is tempting to suggest that it may serve as a host recognition or feeding cue. I know of no direct tests of this hypothesis using a high-Ni insect species. Important to this question is whether Ni levels in plant tissues can be detected directly by insects (most likely using taste). Although I know of no tests using Ni, Behmer *et al.* (2005) performed experiments regarding this question for Zn, using the grasshopper *Schistocerca gregaria* (Forskål) (Orthoptera: Acrididae) and an artificial diet that could be amended with various chemicals (including Zn). Their results suggested that Zn levels in diets were not directly detectable, but that grasshoppers could associate the negative physiological effects of a high-Zn diet with other components (also added to that diet by experimenters) that they could taste. Similar experiments with Ni have not yet been conducted, but the Zn work suggests that plant secondary chemical differences between Ni hyperaccumulator and other plants may be the cues used by high-Ni insects in detecting suitable hosts.

An indirect experimental approach to the role of Ni in host choice is to offer a high-Ni insect species an array of plant species that differ greatly in level of hyperaccumulation and then document their feeding choice. The host-choice experiments summarized above provide equivocal evidence. For example, Augustyniak *et al.* (2002) included a second Ni hyperaccumulator *Berkheya* species as a host choice and found it was consumed by the *B. coddii* specialist, *C. pardalina*. On the other hand, Boyd *et al.* (2007) reported a choice experiment in which *Stenoscepa* sp. did not discriminate between the hyperaccumulators *B. coddii* and *B. rehmannii*. In another choice experiment with *Stenoscepa*, insects preferred *Senecio coronatus* leaves from a hyperaccumulator population relative to leaves from a non-hyperaccumulator population, a result that suggests high Ni levels might be a host cue (Boyd *et al.*, 2007). A slightly different approach is to exploit natural variation in Ni levels of a hyperaccumulator species to construct a within-species choice test. Augustyniak *et al.* (2002) conducted this type of experiment using the high-Ni beetle *Chrysolina pardalina* and the Ni hyperaccumulator *B. coddii*. They reported that plant Ni concentration did not affect insect host choice but leaf age did: beetles preferred relatively young leaves despite large differences in leaf Ni concentration among those samples of young leaves.

Another indirect approach to the host choice cue question, and one that avoids confounding experimental factors associated with species identity, is to raise a Ni hyperaccumulator species upon either high- or low-Ni soil. This yields plants that differ greatly in Ni concentration but otherwise have species-specific traits in common (Boyd, 1998b; Pollard, 2000). To my knowledge, the only test of host choice using a Ni hyperaccumulator species raised on either high-Ni or low-Ni soil is that of Wall and Boyd (2006). In their experiment, they allowed *M. boydi* individuals to choose *Streptanthus polygaloides* plants either harvested from the field (and thus hyperaccumulating Ni) or raised in a greenhouse on low-Ni soil (plants raised on low-Ni soil typically contain only 20 - 70 μg Ni/g: Jhee *et al.*, 2005). They reported no evidence of a feeding preference by the insects. It should be noted that this last approach does not completely avoid confounding factors. For example, Martens and Boyd (1994) showed that *S. polygaloides* plants raised on high- or low-Ni soil differ in traits other than Ni concentration, so that Ni is not the only variable manipulated by such an experiment. As exemplified by the experiments of Behmer *et al.* (2005) on Zn mentioned above, the influence of Ni on host choice may be more precisely isolated by use of an artificial diet and manipulating its Ni concentration. This has yet to be done with Ni.

Where is Ni in insect bodies?

High-Ni insects must, by definition, have large amounts of

Ni in them, but the exact location of the Ni is important to our understanding of the physiology of these insects and to our ability to detect adaptations they may possess for dealing with large metal burdens. Because of the small size of most insects, special research techniques usually are needed to investigate these questions (Mesjasz-Przybylowicz & Przybylowicz, 2002; Przybylowicz *et al.*, 2004). These techniques include cryofixation of specimens to immobilize Ni in tissues after death, and elemental analysis using sensitive microscopic techniques (such as microscopic proton-induced X-ray emission, or Micro-PIXE). In a series of pioneering studies using this technique (Mesjasz-Przybylowicz & Przybylowicz, 2002; Przybylowicz *et al.*, 2003; Przybylowicz *et al.*, 2004; Augustyniak *et al.*, 2008), five insect species (including the high-Ni insects *Chrysolina pardalina* and *Stenoscepa* sp.) that feed on the South African Ni hyperaccumulator *Berkheya coddii* have been analyzed. Two generalizations have emerged from this initial work. First, and unsurprisingly given the hyperaccumulator level of Ni in host plant tissues, relatively high levels of Ni have been found in the guts of these insects. Second, in the case of the high-Ni insects that have been examined (*C. pardalina* and *Stenoscepa* sp.), relatively large amounts of Ni have been reported from Malpighian tubules (Przybylowicz *et al.*, 2003; Augustyniak *et al.*, 2008). The conclusion suggested by this latter finding is that efficient excretion of Ni from high-Ni insect tissues is one adaptation whereby internal Ni concentrations are controlled (Przybylowicz *et al.*, 2003; Augustyniak *et al.*, 2008). Levels of Ni in the Malpighian tubules of other (non-high-Ni) insects feeding on this hyperaccumulator plant have not yet been investigated, so it is not known if this finding is specific to high-Ni insects or if it is found in other insects that feed on Ni hyperaccumulators.

Micro-PIXE studies have generally found that levels of Ni measured in tissues outside the gut of high-Ni insects are relatively low. The greatest Ni concentration reported, 1 012 μg/g, was found in exuviae of larvae of *Chrysolina pardalina* (Przybylowicz *et al.*, 2003). Przybylowicz *et al.* (2003) suggested that molting may be a mechanism whereby Ni is eliminated from the bodies of insects feeding on hyperaccumulators, a suggestion supported by the report of Boyd *et al.* (2007) of a relatively large amount of Ni (1 200 μg/g) in exuviae of *Stenoscepa* sp. The second highest Ni concentration reported by micro-PIXE work is that of 760 μg/g in Malpighian tubules of *Stenoscepa* sp. (Augustyniak *et al.*, 2008). Other Ni concentrations within *C. pardalina* tissues ranged from 0.8 μg/g (in fat bodies) to the 1 012 μg/g reported from the exuvium, but values for tissues other than exuvium were < 60 μg/g (Przybylowicz *et al.*, 2003). Studies of Ni concentrations in organs of insects other than high-Ni insects show similarly low values, less than 110 μg/g (Migula *et al.*, 2007).

The finding of large amounts of Ni in the guts of insects (including high-Ni insects) that feed on Ni hyperaccumulators suggests that gut contents may greatly contribute to whole-body Ni measurements. For example, Migula *et al.* (2007) suggested that how collected specimens are handled after capture could affect whole-body Ni measurements. Insects killed immediately upon capture would retain their gut contents, whereas those captured but not immediately killed would have the opportunity to at least partially empty their digestive tracts. This is certainly an important factor, and probably contributes to the variation in whole-body Ni concentrations of the high-Ni insects reported in Table 2. For example, gut contents are likely one reason why the same heteropteran (*Spilostethus rivularis* Germar: Lygaeidae), collected from *Berkheya coddii* by both Boyd *et al.* (2006a) and Migula *et al.* (2007), was reported by the latter as having only 24% of the Ni concentration found by Boyd *et al.* (2006a). Migula *et al.* (2007) allowed captured insects to reduce their gut contents by delaying their deaths for 1 - 2 days, whereas Boyd *et al.* (2006a) killed captured insects immediately after collection. The contribution of gut contents to whole-body Ni levels was tested by Boyd *et al.* (2007) with the high-Ni grasshopper *Stenoscepa* sp., by analyzing insects killed immediately after removal from containers supplied with food and comparing their Ni concentrations with those starved for 49 hours. They indeed found that starved insects contained significantly less Ni: starved insects contained 950 μg/g versus 3 000 μg/g for those not starved, showing that Ni levels in starved insects declined to about 32% of that in those with relatively full guts.

The influence of gut contents on whole-body Ni measures suggests that investigators interested in assessing tissue Ni concentrations should starve insects before killing them. How long a starvation period is necessary, and how much residual food may remain in a starved insect gut, are potentially important variables that cannot be known in most situations. It is important to realize that investigators who desire to determine the content of Ni in an insect that is eaten whole by a predator, and thus targeting ecological rather than physiological questions, may prefer to measure whole-body concentrations of insects that are not starved. This topic is addressed more fully in the following section.

Ecological consequences of high-Ni insects: effects on predators

Because hyperaccumulation in plants has been suggested

Table 2 Mean whole-body Ni, host Ni, and bioaccumulation index (B) values for herbivorous insects collected from Ni hyperaccumulator plants.

Taxon	Insect Ni (μg/g)	Host Ni (μg/g)	Index (B)	Reference
Heteroptera				
Achaemenes pseudocostalis Stål[†]	160	12500	0.013	Migula *et al.* (2007)
Agonoscelis erosa[†]	**1310**	**4700**	**0.28**	**Boyd *et al.* (2006a)**
Anoplocnemis curvipes[†]	**510**	**4700**	**0.11**	**Boyd *et al.* (2006a)**
***Anoplocnemis dallasiana* Lethierry & Serville**	**1260**	**12500**	**0.10**	**Migula *et al.* (2007)**
Boerias ventralis[†]	580	4700	0.12	Boyd *et al.* (2006a)
Coquilletia insignis	**500**	**3800**	**0.13**	**Boyd *et al.* (2004)**
Liorhyssus hyalinus Fabricius	48	2900	0.017	Wall and Boyd (2002)
Lygus hesperus Knight	130	2900	0.045	Wall and Boyd (2002)
Melanotrichus boydi[†]	**750**	**2900**	**0.26**	**Wall and Boyd (2002)**
Norialsus berkheyae	151	9000	0.017	Migula *et al.* (2007)
Orthesia sp. Bosc[†]	260	9000	0.029	Migula *et al.* (2007)
Protaphis pseudocardui Theobald	92	12500	0.0074	Migula *et al.* (2007)
Sphaerocoris testudogrisea	**530**	**9600**	**0.055**	**Boyd *et al.* (2006a)**
***Spilostethus rivularis* 1**	**630**	**9600**	**0.066**	**Boyd *et al.* (2006a)**
Spilostethus rivularis 2	154	10660	0.014	Migula *et al.* (2007)
Thyanta pallidovirens Stål	40	2900	0.014	Wall and Boyd (2002)
Veterna sanguineirostris[†]	**1800**	**4700**	**0.38**	**Boyd *et al.* (2006a)**
Coleoptera				
Chrysolina clathrata Clark	260	16000	0.016	Boyd *et al.* (2006a)
Chrysolina pardalina 1	301	23000	0.013	Mesjasz-Przybylowicz *et al.* (2002)
***Chrysolina pardalina* 2**	**1260**	**12500**	**0.10**	**Migula *et al.* (2007)**
***Chrysolina pardalina* 3**	**2650**	**7750**	**0.34**	**Mesjasz-Przybylowicz *et al.* (2001)**
Hycleus lunatus Pallas	170	9600	0.018	Boyd *et al.* (2006a)
Unidentified Curculionidae	**1100**	**4700**	**0.23**	**Boyd *et al.* (2006a)**
Orthoptera				
***Stenoscepa* sp. nov.**	**3500**	**14700**	**0.24**	**Boyd *et al.* (2007)**
Diptera				
Bactrocera psidii Froggatt	420	6900	0.061	Boyd *et al.* (2006b)

Bold lines are data from high-Ni insects (defined by a whole-body Ni concentration   500 μg/g). [†]Taxa for which more than one host tissue was reported. In these cases, the host Ni value reported in the table is the least value, so that the value for B is the maximum in these cases.

to constitute an elemental defense against herbivores (Martens & Boyd, 1994; Boyd, 2007b), it is natural to ask if elemental defense extends to organisms occupying other trophic levels (Boyd, 1998a). Host quality is an important feature of the prey of parasitoids and predators, and the elemental composition of hosts can affect quality (Ye *et al.*, 2009). For example, Vickerman and Trumble (2003) showed that consumption of high-Se herbivore prey negatively affected the predacious bug *Podisus maculiventris* (Heteroptera: Pentatomidae). I know of only two tests of the elemental defense hypothesis focusing on predator-prey relations using a high-Ni insect: one used a suite of predators and one used several pathogens. Boyd and Wall (2001) fed predators either high-Ni *Melanotrichus boydi* or various low-Ni prey and monitored predator survival: predators included two spider species, *Misumena vatia* Clerk (Araneae: Thomisidae) and *Pholcus phalangioides* Fuesslin (Araneae: Pholcidae), larvae of a lacewing (*Chrysoperla carnea* Stephens: Neuroptera, Chrysopidae), and nymphs of a mantid (*Stagmomantis californica* Rehn & Hebard: Dictyoptera, Mantidae). In one case, that of *Misumena vatia*, predator survival was significantly reduced when fed *M. boydi*, suggesting toxicity of the high-Ni insect prey. In the pathogen experiment, Boyd (2002) used three entomopathogens, the nematodes *Steinernema carpocapsae* Weiser (Nematoda: Rhabditidae) and

Heterorhabditis bacteriophora Poinar (Nematoda: Rhabditidae) and the fungus *Beauveria bassiana* (Balsamo) Vuillemin (Ascomycota: Clavicipitaceae), and compared their effectiveness against both nymphs and adults of high-Ni *M. boydi* and a low-Ni mirid bug, *Lygus hesperus* Knight (Heteroptera: Miridae). He reported that *M. boydi* survived no better than *L. hesperus* when challenged by any of the three pathogens. Although these initial tests of Ni-based defense against natural enemies of high-Ni herbivores have some value, additional experiments should be conducted using a single prey/host insect species to control for possible species-specific host differences. This would require experimental prey/host insects that are either raised on an artificial diet (which can be either amended with an element or unamended) or upon high- or low-element host plants (that themselves are raised on element-amended or unamended growth media). To my knowledge, this has yet to be attempted for any high-Ni insect species.

In general, the effectiveness of body element concentration as a defense against predators/pathogens will depend upon several factors (Boyd, 1998b). First, the feeding mode of a predator/pathogen likely will affect the dose of the element received. For example, a bird predator that ingests a high-Ni insect whole (and thus ingests the prey's gut contents) may experience a much different dose than a pathogen that attacks the hemolymph. Second, variation in element concentration among different parts of a high-Ni insect's body may be important to predators/pathogens that target particular tissues in their attack. Finally, feeding specificity of a predator will affect the total dose of an element received. For example, a polyphagous predator will likely be able to include a small number of high-Ni insect prey without suffering ill effects because the Ni dose will be diluted among other prey in the diet. However, how many high-Ni prey can be included in the diet without ill effect depends on how high-Ni prey might affect digestive processes. If there is a threshold concentration of Ni in a predator gut at which negative impacts occur, then a certain proportion of high-Ni prey might act as a "poison pill" that interferes with digestion of low-element food that is ingested at about the same time as the high-Ni prey. This "poison pill" hypothesis has yet to be investigated.

The above discussion of elemental defense against predators/pathogens assumed that an element acts as a passive defense, by which I mean that its effectiveness is not dependent upon the behavior of a prey insect. However, there is the potential for elemental defenses to play a role in active defense against predators, in which the elemental defense is part of a behavioral defensive action. For example, grasshoppers may regurgitate gut contents as a defensive behavior and, if those gut contents contain host plant chemicals, those chemicals may negatively affect an attacking predator (Sword, 2001). Sword (2001) showed that plant secondary compounds from ingested leaves of *Ptelea trifoliata* (Rutaceae) could result in rejection of grasshoppers by an insectivorous lizard. I hypothesize that the very high Ni concentration of *B. coddii* leaves may create a similar effect for *Stenoscepa* sp. This grasshopper species feeds on *B. coddii* and thus its regurgitate contains a relatively high Ni concentration. It would be interesting to conduct experiments similar to those of Sword (2001), involving *Stenoscepa* sp. allowed to feed on *B. coddii* plants that were growing on high- or low-Ni soils, so that the Ni concentrations of leaves (and hence concentrations in grasshopper regurgitate) would vary greatly. Experiments that evaluate the success of predator attack, as affected by regurgitate Ni concentration, would be a fascinating test of active elemental defense of herbivores against their predators.

Ecological consequences of heavy metal insects: community effects

Because hyperaccumulator plants mobilize large amounts of an element from the soil into their tissues, they represent a gateway whereby those elements can enter food webs (Boyd, 1998a; Pilon-Smits & Freeman, 2006). In this section, I will expand the focus beyond heavy metal insects in order to explore more generally how elements can be spread into communities by herbivorous insects feeding upon hyperaccumulator plants, emphasizing high-Ni insects where possible. High-Ni insects would be more effective than those with lower concentrations in generating community-level effects.

A few studies have reported evidence consistent with elemental mobilization into food webs via hyperaccumulator plants. For example, Peterson *et al.* (2003) collected arthropods from areas in Portugal on which a Ni hyperaccumulator species (*Alyssum pintodasilvae* Dudley: Brassicaceae) was either present or absent, and compared Ni concentrations in those arthropod samples. They reported significantly more Ni in samples collected from habitats containing the Ni hyperaccumulator, in some cases as much as 100-fold more. Similar but more recent investigations have been done regarding the spread of Se in communities containing Se hyperaccumulators in Colorado, USA (Quinn *et al.*, 2007; Galeas *et al.*, 2008). However, in general the extent of elemental mobilization and its effects on the structure and function of communities are unexplored.

Spread in food webs: herbivores as gatekeepers

If hyperaccumulator plants represent gateways by which elements may enter food webs, herbivores (and detritivores) are probably the most important gatekeepers. Feeding by herbivores will determine how much of an element becomes available to predators, and herbivore movement patterns will influence how the element they consume (and eventually eliminate from their bodies) will be transported around a habitat. Consumption of hyperaccumulator tissue may spread an element laterally in the environment (due to movement of a herbivore) and into the next trophic level if the herbivore is consumed by a predator.

One phenomenon that can magnify the effect of an element on a food web is bioaccumulation, the increase in concentration of an element in successive trophic levels (Heikens *et al.*, 2001). Laskowski (1991) presented a simple bioaccumulation index (B), calculated as the concentration of an element in an organism divided by the concentration of that element in the organism's food, that can document degree of bioaccumulation (B values > 1 indicate bioaccumulation is occurring). Table 2 presents values of B calculated for herbivorous insects reported from Ni hyperaccumulators, including most of the high-Ni insects listed in Table 1. Table 2 also includes other herbivorous insects for which whole-body Ni concentration is elevated (yet < 500 μg/g), but for which data were available in the literature to allow calculation of B. Values of B in Table 2 are all < 1, indicating no bioaccumulation of Ni by any herbivore yet studied. In fact, all the B values for herbivores in Table 2 are less than 0.4. One might expect that high-Ni insect herbivores, those with 500 μg/g, would have greater values of B than those not classified as high-Ni insects. This is indeed the case: using the data in Table 2, comparison of data from high-Ni herbivores (mean = 0.18, SD = 0.11, $n = 13$) to those from other herbivores (mean = 0.022, SD = 0.016, $n = 12$) shows B values are statistically greater for the high-Ni herbivores (Mann-Whitney *U*-test, $Z = -4.2$, $P < 0.000\,1$).

Spread in food webs: predators

Although high-Ni insect herbivores do not bioaccumulate Ni, they do contain high whole-body Ni concentrations and thus may transmit Ni to organisms that prey upon them. While in principle all insects must have predator natural enemies, documentation of predation upon high-Ni insects is sparse. Boyd and Wall (2001) observed crab spiders (*Misumena vatia*) capturing the high-Ni insect *Melanotrichus boydi* in the field, and Migula *et al.* (2007) likewise reported predation of the high-Ni insect *Chrysolina pardalina* by the assassin bug *Rhinocoris neavei*. High-Ni insects must be prey for vertebrate predators of insects but to my knowledge no cases have been reported.

As with herbivores, we can ask whether bioaccumulation occurs between herbivore and predator trophic levels. Surveys of Ni hyperaccumulator plants that present data for both a predator and its herbivore prey can provide an initial exploration of this question. To my knowledge, there is only one predator species for which these data are available from field studies. Migula *et al.* (2007) analyzed adults of the predaceous assassin bug *Rhinocoris neavei* (520 μg Ni/g) and its reported prey, *Chrysolina pardalina* (1 260 μg Ni/g), both collected from the Ni hyperccumulator *Berkheya coddii*. They reported a B value of 0.41, much less than unity, indicating that bioaccumulation did not occur, yet showing transfer of Ni between trophic levels.

Bioaccumulation of elements by predators also can be tested directly by feeding prey to predators and comparing body element concentrations. To my knowledge, only Boyd and Wall (2001) have performed this type of experiment with Ni. They fed the high-Ni bug *Melanotrichus boydi* to three predators: two spiders, *Misumena vatia* and *Pholcus phalangioides*, and a praying mantid, *Stagmomantis californica*. These predators differ in feeding mode: the spiders suck fluids from their prey whereas the mantid is a chewing predator. They analyzed bodies of the predators for Ni concentrations at the end of the experiment. The predators all contained relatively large mean levels of Ni, despite the difference in feeding mode between the spiders and the mantid: 420 μg Ni/g for *M. vatia*, 470 μg Ni/g for *P. phalangioides* and 460 μg Ni/g for *S. californica*. Using 780 μg Ni/g as a mean value for the *M. boydi* prey (as reported by Wall, 1999), values of B calculated by Boyd and Wall (2001) were all less than unity: 0.54, 0.60 and 0.59, respectively. As with the report of Migula *et al.* (2007) for *Rhinocoris neavei* (also a piercing/sucking predator) these B values are generally greater than those reported for herbivores from hyperaccumulator plants (Table 2).

These studies of predator bioaccumulation indicate that predators may bioaccumulate Ni to a greater extent than herbivores. Comparison of the mean value of B for predators, combining results from the Migula *et al.* (2007) and Boyd and Wall (2001) studies (mean = 0.54, SD = 0.087, $n = 4$), to values for the herbivores in Table 2, suggests that trophic level may influence the degree of bioaccumulation. Statistical analysis (Mann-Whitney *U*-test) shows B for predators is significantly greater than B for the herbivores in Table 2, regardless of whether the predators are compared to all herbivores (*Z*-value $= -3.2$, $P = 0.001\,6$: all herbivores mean = 0.11, SD = 0.11, $n = 25$), the high-Ni herbivores in Table 2 (*Z*-value $= -2.9$, $P = 0.0016$: high-Ni herbivores mean = 0.18, SD = 0.11, $n = 13$), or the non-high-Ni herbivores in Table 2 (*Z*-value $= -2.9$, $P = 0.001\,6$: non-high-Ni herbivores mean = 0.022, SD = 0.016, $n = 12$). This may reflect a greater availability of Ni in the bodies of prey compared to the availability of Ni in plant tissues, but more data are needed before firm conclusions can be reached.

Spread in food webs: floral visitors

Pollination mutualisms are another plant-animal interaction through which hyperaccumulated elements may be mobilized into food webs (Boyd, 1998a). The typical rewards for floral visitors, pollen and nectar, have not often been assessed for their element concentrations in hyperaccumulator plants because of the small quantities of material that are usually available (Boyd, 1998a). However, some studies have compared whole-body Ni values of floral visitors collected from flowers of a hyperaccumulator species with those of visitors collected from flowers of non-hyperaccumulators. None of these floral visitors are high-Ni insects, but more Ni has been reported from visitors collected from hyperaccumulator flowers. Boyd *et al.* (2006b) measured Ni in the bodies of honeybees (*Apis mellifera* L.) collected from flowers of the Ni hyperaccumulator *Hybanthus austrocaledonicus* (Vieill.) Schinz & Guillaumin ex Melchior (Violaceae) and from flowers of the non-hyperaccumulator *Myodocarpus fraxinifolius* Brongn. & Gris (Myodocarpaceae), both growing on a serpentine soil site in New Caledonia. They reported significantly more Ni (8-fold more) in honeybees from the Ni hyperaccumulator (mean = 40 μg Ni/g). A study in California, USA, compared Ni concentrations in bodies of two bee species (*Apis mellifera* and *Bombus vandykei* Frisson: Hymenoptera, Apidae) collected from flowers of the Ni hyperaccumulator *Streptanthus polygaloides* on a serpentine site (Wall & Boyd, 2006). Wall and Boyd (2006) also collected both bee species from flowers of a non-hyperaccumulator plant, *Heteromeles arbutifolia* (Lindley) Roemer (Rosaceae), growing on a non-serpentine soil site. Both bee species contained more Ni when collected from the hyperaccumulator flowers: honeybees had 2.9-fold more Ni (46 μg Ni/g vs. 16 μg Ni/g) and *B. vandykei* 3.2-fold more (38 μg Ni/g vs. 12 μg Ni/g).

Thus the limited evidence available indicates that floral visitors can mobilize Ni from flowers of hyperaccumulators, although the levels measured are relatively low compared to whole-body Ni levels of some herbivores (Table 2). It would be interesting to know if the elevated metal levels of the floral resources used to provision bee offspring might affect development of the young. This might be yet another way in which Ni hyperaccumulators impact associated species through food web connections with insects.

Conclusion

It is clear that our knowledge of high-Ni insects is rudimentary and in need of much scientific investigation. Insect faunal surveys of serpentine sites that contain Ni hyperaccumulator plants are vital to seek out and identify more of these insects. Studies of the distribution of Ni within bodies of high-Ni insects, and the physiological adaptations that allow them to consume high-Ni food, may illustrate new mechanisms of metal tolerance. These insects may have impacts on food webs and species interactions that can help us better understand the serpentine ecosystems in which they live. By bringing attention to these insects, it is hoped that they will receive more research effort from the scientific community.

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