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Responses of Generalist Predators Fed High-Ni Melanotrichus boydi (Heteroptera: Miridae): Elemental Defense Against the Third Trophic Level

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ABSTRACT.—The recent discovery of herbivores that feed on Ni-hyperaccumulating plants and contain elevated Ni concentrations in their bodies suggests that Ni may be transferred to their predators. We tested this hypothesis using the high-Ni herbivore *Melanotrichus boydi* and four predator species: the spiders *Pholcus phalangioides* and *Misumena vatia,* the mantid *Stagmomantis californica* and the lacewing *Chrysoperla carnea*. Survival of each predator species was compared when individuals were fed either *M. boydi* or low-Ni prey. No significant survival difference between diets was observed for *P. phalangioides* or *S. californica,* although individuals fed *M. boydi* contained significantly elevated Ni concentrations (470 μg Ni g^{-1} for *P. phalangioides* and 460 μg Ni g^{-1} for *S. californica*). No significant difference in survival to adulthood was observed for *C. carnea*: adult production was 45% for high-Ni and 55% for low-Ni diets. In contrast, survival of *M. vatia* was significantly decreased when fed high-Ni prey: only 32% of those fed *M. boydi* survived after 20 d, in contrast to 89% of those fed low-Ni prey. *Misumena vatia* collected from Ni-hyperaccumulating *Streptanthus polygaloides* plants in the wild contained some Ni (a maximum of 110 μg Ni g^{-1}), but significantly less than the 420 μg Ni g^{-1} measured in those fed *M. boydi.* This indicated that *M. vatia,* which is native to *S. polygaloides* sites, consumes some *M. boydi* under natural conditions. We concluded that: (1) Ni can be transferred from a high-Ni herbivore to its predators; (2) predators varied in their sensitivity to the Ni contained in *M. boydi,* and; (3) Ni can poison predators which specialize upon high-Ni herbivores and thus may act as an elemental herbivore defense.

INTRODUCTION

Most plant chemical defenses are organic molecules (secondary compounds) synthesized from photosynthate by a plant's biochemical machinery (Harborne, 1988). However, some chemical defenses, termed elemental defenses by Martens and Boyd (1994), are taken up from the soil and sequestered in plant tissues. Plants that take up large amounts of heavy metals from soils are termed hyperaccumulators (Brooks *et al.*, 1977). Baker and Brooks (1989) defined hyperaccumulators of Ni, Cu, Co, Cr and Pb as those species that sequester >1000 μg g^{-1} (dry wt) of one of these metals in their tissues, whereas hyperaccumulators of Zn and Mn contain >10,000 μg g^{-1} (dry wt). Metals in plants may have multiple functions (Boyd and Martens, 1992), but a defensive effect against herbivores has been demonstrated (*e.g.,* Ernst *et al.*, 1990; Boyd *et al.*, 1994; Boyd and Martens, 1994; Martens and Boyd, 1994; Pollard and Baker, 1997; Boyd and Moar, 1999; Jhee *et al.*, 1999). Boyd (1998) reviewed available evidence regarding the defensive nature of hyperaccumulated metals (mainly Ni) and concluded that Ni can defend plant tissues from generalist folivores and pathogens.

Pollard (2000) recently suggested that metal hyperaccumulator plants and their insect

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associates have promise as a model system for coevolutionary studies. Elemental defenses, as with other plant defenses, can be circumvented by some herbivores (Ernst *et al.*, 1990; Boyd and Martens, 1999) or parasitic plants (Boyd *et al.*, 1999). Boyd and Martens (1999) reported that greenhouse populations of the aphid *Acyrthosiphon pisum* [Harris] (Homoptera: Aphididae) tapped hyperaccumulating plants of the California Ni hyperaccumulator species *Streptanthus polygaloides* Gray (Brassicaceae) without harm. In that case, metal contents of aphids feeding upon high-Ni *S. polygaloides* plants were only slightly elevated. Recent studies focused on serpentine sites have detected elevated metal concentrations in some insect species native to these habitats (Davison *et al.*, 1999; Wall, 1999). Wall (1999) detected elevated levels of Ni in some insect herbivores naturally associated with *S. polygaloides* (which is the only Ni hyperaccumulator plant species known from serpentine sites on the western slope of the Sierra Nevada), thus showing transfer of Ni from plants to insects by herbivory. One herbivore, a previously undescribed species of *Melanotrichus* (Heteroptera: Miridae), had a Ni concentration of 770 $\mu g\ g^{-1}$. This level is remarkable, as Outridge and Scheuhammer (1993) reported Ni concentrations in mammals and birds at $<80\ \mu g\ g^{-1}$, even in Ni-polluted environments. Additional study of this species, named *Melanotrichus boydi* by Schwartz and Wall (2001), revealed it to be monophagous on *S. polygaloides* (Wall, 1999). Further investigation showed *M. boydi* was present at all populations of *S. polygaloides* surveyed (Wall, 1999). These samples of *M. boydi* contained from 590–1020 μg Ni g^{-1} (Wall, 1999).

The elevated Ni content of *Melanotrichus boydi* is reminiscent of other cases in which plant chemical defenses are sequestered in herbivores. In some instances these organic chemicals defend the herbivore against its predators, thus providing defense against organisms at the third trophic level in these food chains. Examples of herbivores protected in this manner include some aphids (Harborne, 1988), monarch butterflies (Harborne, 1988) and buckeye butterflies (Strohmeyer *et al.*, 1998). A review of this topic is provided by Price *et al.* (1980). Boyd and Martens (1998) predicted that metals ingested by some herbivores able to feed on hyperaccumulator plants are sequestered in herbivore bodies to defend them against their predators. Metal-based defense of animals has been suggested in several instances (*e.g.,* Gibbs *et al.*, 1981; Hopkin and Martin, 1985; Capon *et al.*, 1993), but to our knowledge has not been investigated for herbivores of hyperaccumulator plants. Heliovaara and Vaisanen (1993) concluded that the effects of Ni on insects were poorly documented, and we know of little research on this topic since their review.

The elevated Ni content of *Melanotrichus boydi* also suggests a pathway whereby Ni may be mobilized into higher levels of food chains, as the Ni present in *M. boydi* may be transferred to predators that consume this herbivore. This question is of interest because heavy metals are important pollutants and their effects on ecosystems deserve exploration. In addition, hyperaccumulator plants are being examined for use as phytoextractors of metals from polluted areas or mining sites (Brooks and Robinson, 1998; McGrath, 1998; Raskin and Ensley, 2000; Terry and Bañuelos, 2000). Movement of metals into consumers in food chains based upon hyperaccumulator plants suggests the possibility of metal transfer offsite or metal transfer into other organisms at a phytoextraction location (Boyd, 1998).

This research addressed two aspects of the relationship between *Melanotrichus boydi* and generalist arthropod predators. First, we explored if *M. boydi* was toxic as prey for several generalist predators. The ideal approach for this question would use both high- and low-Ni *M. boydi* in order to isolate the effect of Ni. However, no naturally occurring low-Ni *M. boydi* populations have yet been discovered (Wall, 1999) and attempts to culture *M. boydi* in the laboratory (and manipulate diet Ni concentration there) have failed. Thus, the only way currently available to test this idea is to contrast the suitability of *M. boydi* as prey against the suitability of low-Ni prey of other species, making the likely assumption that a strong toxic effect found for *M. boydi*, would be due to its extraordinarily elevated Ni concentration. Second, we measured the Ni concentrations of predators fed *M. boydi* to determine if Ni from *M. boydi* was transferred to predators in food chains under laboratory conditions.

MATERIALS AND METHODS

We created high- and low-Ni diets for predators by offering them prey that naturally varied in Ni concentration. High-Ni diets consisted of *Melanotrichus boydi* individuals (ranging from instars of various sizes to adults) captured in the field. These insects were maintained alive in refrigerated containers containing field-collected *Streptanthus polygaloides* plants until offered to predators. *Melanotrichus boydi* individuals used during these experiments were collected from three field sites. One is the Red Hills Management Area located near Chinese Camp, Tuolumne County, CA. Another is a serpentine area along California Highway 49 south of Chinese Camp near Mocassin in Tuolumne County, CA. The third, and most important site because most of the *M. boydi* individuals used in these experiments were collected there, was a serpentine site located south of Sugar Pine Reservoir in El Dorado County, Calif. These sites are further described by Wall (1999).

Low-Ni diets consisted of several types of live food items. One food item of relatively small size was the adult fruit fly, *Drosophila melanogaster* Meig. (Diptera: Drosophilidae), raised on artificial diet. We used a vestigial-wing phenotype, both for its ease of experimental manipulation and its ease of capture by predators. Another low-Ni food item was the nymph or adult of *Lygus hesperus* (Knight) (Heteroptera: Miridae). These were collected by sweep-netting fields of alfalfa (*Medicago sativa* L.: Fabaceae) in the vicinity of Manteca, San Joaquin County, CA. The third low-Ni food category was an adult fly of various species, ca. 4–7 mm in length, collected by sweep-netting in the alfalfa fields mentioned above. Collected *L. hesperus* and sweep-netted flies were maintained until used for feeding experiments in refrigerated containers to which cut *Medicago sativa* plants were added.

We used four predator species for these experiments. The internal location of Ni in *Melanotrichus boydi* is not yet known and this could affect our findings depending upon what tissues a predator consumes (Hopkin, 1989). Thus, we used predators having two feeding modes. Spiders generally feed by injecting digestive juices into their prey, sucking out the digested contents, and then repeating the process (Foelix, 1996). Two spider species were used in our experiments. One was obtained from a commercial source, whereas the other was collected from plants of *Streptanthus polygaloides*. The lacewing species was used as a representative insect that feeds in a similar piercing-sucking fashion (Cohen and Smith, 1998). The fourth predator species was a mantid, a predator with chewing mouthparts (Arnett, 2000). Mantids consume their prey by chewing them: a mode of ingestion different from that of the spider and lacewing species.

All predators were maintained indoors during feeding trials, but temperature and light regimes varied. Specific information about each predator species and its feeding trial is provided below.

PHOLCUS PHALANGIOIDES (FUESSLIN) (ARANEAE: PHOLCIDAE)

Pholcus phalangioides is a web-building spider that occurs in houses almost everywhere in the world (Gertsch, 1949). Nentwig (1983) reported that this species captures a wide variety of insect prey, including chemically-defended Heteroptera. Individuals of *P. phalangioides* were obtained from a commercial source (Carolina Biological Supply Company, Inc.). Each spider was put into a lidded translucent 500 ml plastic cup, along with a few twigs to provide climbing routes and web-building surfaces. Half of the 24 spiders were randomly assigned a high-Ni diet and half assigned a low-Ni diet. Starting 3 June 1999, spiders were checked for survival and fed every other day for 33 d, until the experiment was terminated on 6 July. High-Ni food consisted of an adult *Melanotrichus boydi,* whereas low-Ni food usually was an adult *Lygus hesperus.* In a few cases, the food offered low-Ni treatment spiders was an adult sweep-netted fly. When the experiment was terminated, bodies of the spiders were dried and combined into two samples from each treatment (high-Ni or low-Ni diet) for Ni concentration analysis. The few individuals that died during the study were not analyzed for Ni because their low combined biomass was insufficient for that procedure (*see* analysis description below).

MISUMENA VATIA (CLERK) (ARANEAE: THOMISIDAE)

Misumena vatia has a Holarctic distribution and occurs throughout the U.S. (Fitch, 1963). Like other thomisid spiders, it does not spin a web but instead ambushes prey, seizing them with its forelegs while injecting a quick-acting poison. This species commonly frequents flowers or inflorescences and captures floral visitors (Gertsch, 1949). Because of its preference for flowers as a hunting location, Nentwig (1986) concluded that *Misumena vatia* had a relatively limited prey range for a non-webbuilding spider. However, field studies show that *M. vatia* captures a wide range of prey (Lovell, 1915; Morse, 1981), including a report by Fitch (1963) of *M. vatia* capturing flower-visiting hemipteran insects.

We had noticed this crab spider species using inflorescences of *Streptanthus polygaloides* as hunting sites during several field seasons at the Red Hills study site. Spiders were captured from *S. polygaloides* inflorescences on 11, 12 and 15 June 1999 and placed in capped 150 ml translucent plastic containers. We added one or two small twigs to each container to provide some habitat structure, as well as a section (ca. 5 cm long) of *S. polygaloides* inflorescence. On each day that we captured spiders, we paired captured spiders according to size and randomly assigned each member of a pair to a feeding treatment (high-Ni or low-Ni diet). We began treatments using eight pairs of spiders on 11 June (plus an extra spider fed the low-Ni diet), two pairs were started on 12 June and eight pairs were initiated beginning 16 June.

Feeding treatments began the same day that spiders were collected, and spiders were fed every other day until 6 July, when the experiment was terminated. We fed spiders receiving the low-Ni diet fruit flies, *Lygus hesperus,* or sweep-netted flies. Usually small spiders were given 3–7 fruit flies or a *L. hesperus* nymph, whereas large spiders received an adult *L. hesperus* or a sweep-netted fly. High-Ni diet spiders were fed *Melanotrichus boydi* adults or nymphs. Small spiders were usually fed 1 or 2 nymphs and large spiders were fed an adult each time. Spiders receiving the high-Ni diet that died during the experiment were dried and separated into two samples. A third sample, consisting of spiders fed high-Ni diet that survived to the end of the experiment, was also created when the experiment was terminated. At the end of the experiment, low-Ni diet spiders were dried and divided into three samples for Ni concentration analysis. We did not analyze the bodies of low-Ni spiders that died during the experiment because they were too few to provide sufficient biomass for the Ni analysis procedure.

We also collected *Misumena vatia* from the field, so that their Ni concentrations could be compared to the spiders used in our feeding experiment. On 5 and 6 July 1999 we collected this species from inflorescences of *Streptanthus polygaloides* at the Red Hills Management Area. The spiders were dried and divided into five samples for Ni concentration analysis. We also collected crab spiders more widely, over a 2 wk period (19 June–4 July), from several *S. polygaloides* populations in California. These were combined into two additional samples. Some of these spiders were collected in the Red Hills, some from the Mocassin area and most from the Sugar Pine Reservoir site.

STAGMOMANTIS CALIFORNICA REHN AND HEBARD (ORTHOPTERA: MANTIDAE)

This mantid species is native to California and several other southwestern U.S. states (Powell and Hogue, 1979). Like other mantids, it has raptorial front legs which it uses to seize and hold prey while consuming it with chewing mouthparts (Arnett, 2000). Mantids are highly visual hunters and are reported to consume a wide range of prey items, including flies, bees, wasps, butterflies and other appropriately-sized prey that is not heavily chitinized (Essig, 1926).

Small (5–10 mm length) individuals of *Stagmomantis californica* were captured in the Red Hills on 17 June 1999. Each was placed into a separate capped 250 ml container made of translucent plastic. A small twig was added to each container to provide some habitat structure. Containers were sorted into pairs of similar-sized mantids and members of each pair were randomly assigned to high- or low-Ni diets. Mantids were fed daily until the experiment was terminated on 6 July. The low-Ni diet consisted of fruit flies or a sweep-netted fly, although occasionally a *Lygus hesperus* nymph or adult was fed to an appropriate-sized mantid. Mantids fed the high-Ni diet were given *Melanotrichus boydi* nymphs or adults. The number and type of food item was adjusted depending on the size of the mantid being fed, in an effort to supply similar food quantity per unit of mantid body size throughout the experiment.

After 18 d, bodies of the mantids were dried and separated into three samples for each treatment (high-Ni or low-Ni diet) for Ni concentration analysis. Individuals that died during the course of the study could not be analyzed for Ni concentration due to their low biomass, so we added these individuals to the samples containing mantids that survived to the end of the experiment.

CHRYSOPERLA CARNEA (STEPHENS) (NEUROPTERA: CHRYSOPIDAE)

This lacewing is used as a biocontrol agent (O'Neil *et al.*, 1998) and, thus, larvae were available commercially from Rincon-Vitova Insectaries, Inc. Forty larvae, each 3–4 mm long, were selected and each was placed into a 1.5 μl microcentrifuge tube with five small holes in its lid. Tubes were paired based upon similarity of size of the larvae, and diet treatment was randomly assigned to each tube of the 20 pairs.

The high-Ni diet was three adult *Melanotrichus boydi,* whereas the low-Ni diet consisted of three adult *Lygus hesperus* and 5–7 adult fruit flies. Larvae were fed every other day for the first 4 d, and then daily until the end of the experiment. Survival was noted at each feeding time until larvae either died or spun cocoons as they prepared to eclose into adults. Containers with cocoons were maintained at room temperature until adults emerged. In addition to survival analysis (described below), data for percent of larvae that survived to the cocoon stage and percent of larvae that finally emerged as adults were analyzed for diet effects using contingency table analysis. Too few adults of either diet treatment were obtained to allow for Ni analysis of this predator.

NICKEL CONCENTRATION ANALYSIS

Nickel concentrations in predator samples were determined following the technique of Wall (1999). Samples were dried for at least 48 h at 67 C, weighed and placed in borosilicate glass test tubes. We digested samples using 3–5 ml of concentrated nitric acid, maintaining samples at 110 C for 6–8 h (until most of the liquid had evaporated). Residue was redissolved in 3–5 ml of 1 M HCl at 110 C for 2–4 h, and diluted with distilled water to a final

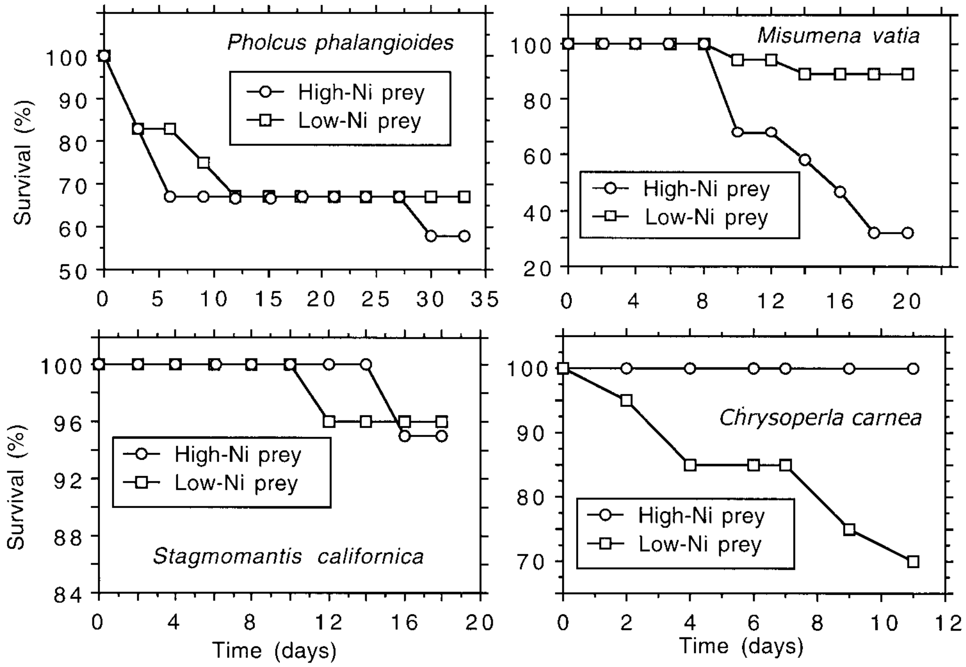

FIG. 1.—Survival curves for each predator species fed high-Ni or low-Ni prey. Note that both axes are scaled differently in the graph for each predator species

volume of 10 ml. A reagent blank was included to detect contamination stemming from the technique. Nickel concentrations of samples were determined with an atomic absorption spectrophotometer (Instrumentation Laboratory, IL.251).

STATISTICAL ANALYSIS

Survival data were analyzed by survival analysis (Abacus Concepts, 1994) using the Kaplan-Meier estimate, with treatment significance determined by the Peto-Peto-Wilcoxon test at alpha ≤0.05. Nickel concentration data were analyzed by Analysis of Variance (ANOVA), with post-hoc mean separations provided by the Fisher's Protected Least Significant Difference (PLSD) Test at alpha ≤0.05.

RESULTS

Pholcus phalangioides.—Survival of *P. phalangioides* declined for spiders from both treatment groups during the first 2 wk of the experiment, but more rapidly for spiders fed high-Ni prey. Survival of spiders receiving either treatment stabilized at 67% for 2 wk more, and then declined by the end of the experiment to 58% for spiders fed high-Ni prey (Fig. 1). The slightly decreased survival for spiders fed high-Ni prey was not statistically significant (Peto-Peto-Wilcoxon test, P = 0.692). However, Ni concentrations of spiders fed high-Ni prey were significantly elevated (ANOVA: $F_{1,2} = 582$, P = 0.0017). The mean Ni concentration of *P. phalangioides* fed high-Ni prey was 470 ± 20 (SE, n = 2) $\mu g\ g^{-1}$, whereas Ni levels of those fed low-Ni prey were below detectable limits (and thus were considered to be zero for the ANOVA) for both samples analyzed.

Misumena vatia.—Crab spiders fed low-Ni prey suffered little mortality over the 20 d duration of the experiment: 89% were still alive when the experiment was terminated (Fig. 1). In contrast, survival of *M. vatia* fed high-Ni prey declined sharply at 10 d and continued to decline, leaving only 32% alive at the experiment's end (Fig. 1). This survival difference was statistically significant (Peto-Peto-Wilcoxon test, $P = 0.0003$).

Nickel concentrations of crab spiders were compared for high-Ni diet spiders, low-Ni diet spiders, wild-collected spiders from the Red Hills Area and wild-collected spiders combined from several areas. These samples varied significantly in Ni concentration (ANOVA: $F_{3,9} = 70$, $P < 0.01$). Crab spiders fed high-Ni prey contained significantly elevated Ni concentrations (420 ± 35, mean $\pm$ SE, $n = 3$) relative to all other samples (Fisher's PLSD test, $P < 0.0001$ for all pairwise comparisons). The two samples of *M. vatia* that died during the feeding experiment contained 360 and 420 μg Ni g^{-1}, values which were slightly less than the sample composed of *M. vatia* individuals still alive at the end of the experiment (480 μg Ni g^{-1}).

Samples of *Misumena vatia* that had been fed low-Ni prey had no detectable Ni in them ($n = 3$), whereas the samples collected in the field from the Red Hills had a mean value of 13 ± 13 (SE, $n = 5$) μg Ni g^{-1} and those collected from several serpentine areas had a Ni concentration of 61 ± 51 (SE, $n = 2$). Maximum Ni concentration for wild-collected samples was 110 μg Ni g^{-1}. Wild-collected and low-Ni diet *M. vatia* samples did not differ significantly from each other in Ni concentration (Fisher's PLSD test, $P > 0.15$ for all pairwise comparisons).

Stagmomantis californica.—Mantids suffered no mortality for the first 10 d, after which survival declined slightly (to 96%) for those fed high-Ni prey (Fig. 1). By the end of the experiment, survival was similar for *S. californica* fed each diet: 96% for those given high-Ni prey and 95% for those fed low-Ni prey (Fig. 1). Because survival rates for mantids receiving either treatment were high, survival curves for mantids receiving the two treatments were not significantly different (Peto-Peto-Wilcoxon test, $P = 1$).

Nickel concentrations of *Stagmomantis californica* bodies were significantly influenced by diet (ANOVA: $F_{1,4} = 22$, $P = 0.009$). Mantids fed high-Ni prey contained 460 ± 67 μg Ni g^{-1} (SE, $n = 3$), whereas the mean Ni concentration of mantids fed low-Ni prey was only 52 ± 52 μg Ni g^{-1} (SE, $n = 3$).

Chrysoperla carnea.—No lacewings fed high-Ni diet died before cocoon formation, whereas after 11 d 30% of those fed low-Ni diet had died before forming cocoons (Fig. 1). These two survival curves were statistically different (Peto-Peto-Wilcoxon test, $P = 0.031$).

Emergence of lacewings from cocoons differed between diet treatments. Only 45% ($n = 20$) of adults emerged from high-Ni treatment cocoons, compared to 79% ($n = 14$) for those formed by larvae fed low-Ni diet. Contingency table analysis showed these results were statistically significant (G-squared = 4.0, df = 1, $P = 0.046$).

In terms of overall success in producing adults from larvae, the different survival responses of larvae and eclosing cocoons compensated for one another so that diet did not significantly influence production of adults. Forty-five percent of larvae fed high-Ni diet became adults, compared to 55% for larvae fed low-Ni diet. Contingency table analysis showed these results were not significantly different (G-squared = 0.401, df = 1, $P = 0.527$).

DISCUSSION

Predators used in these experiments responded differently to the high-Ni diet of *Melanotrichus boydi,* despite the fact that three of them had comparable Ni concentrations at the end of the feeding experiments (470 μg Ni g^{-1} for *Pholcus phalangioides,* 420 μg Ni g^{-1} for *Misumena vatia* and 460 μg Ni g^{-1} for *Stagmomantis californica*). The crab spider (*M. vatia*) demonstrated lower survival when fed *M. boydi,* but this was not observed for either the second spider (*P. phalangioides*) or the mantid. Although it is possible that the lack of toxicity of *M. boydi* for two of the predators used may be due to the short timeframe of the experiments, it is also possible that feeding mode (and the different body materials ingested by predators as a result of feeding mode) may have affected these results. However, the fact that both spider species have similar feeding modes and contained similar concentrations of Ni argues against the hypothesis that feeding mode was responsible for the differential result.

We suggest that *Misumena vatia* was more susceptible to dietary Ni toxicity. Differing responses of invertebrate species to metals have been demonstrated by several studies. For example, Hopkin and Martin (1984) found that a millipede species was unable to control Cd assimilation from high-metal prey and suffered higher mortality due to Cd poisoning, whereas a spider species fed the same high-metal prey did not assimilate Cd and survived (Hopkin and Martin, 1985). Kramarz (1999a, b) demonstrated different metal-processing abilities by two predators: a centipede and a ground beetle. Van Straalen and van Wensem (1986) concluded that the physiological makeup of species inhabiting high-metal forest litter was more important in explaining tissue metal concentrations than either trophic level or body size. Laskowski and Maryanski (1993) also concluded that physiological abilities were more important than trophic level in determining metal concentrations in the bodies of litter-dwelling animals from metal-contaminated forests in Poland. Finally, Heliovaara and Vaisanen (1987) showed that differing life history characteristics of two insect species, feeding on the same host plant species in a metal-polluted area in Finland, resulted in different degrees of metal accumulation. Here, we suggest that *M. vatia* was more physiologically susceptible to Ni toxicity than the other predators used in our experiments.

Our experiments also showed that *Melanotrichus boydi* was defended against the spider predator *Misumena vatia.* We suggest that Ni was the toxic dietary component for *M. vatia.* However, we recognize that many factors contribute to the suitability of prey for a predator species (*e.g.*, Evans *et al.*, 1999) and that *Stagmomantis polygaloides* also possesses organic chemical defenses (Davis and Boyd, 2000) that also might be sequestered by *M. boydi.* Although Heliovaara and Vaisanen (1993) state that, relative to some other metals (*e.g.*, Pb), the effects of Ni on insects are poorly documented, some reports are available. In artificial diet experiments, Martens and Boyd (1994) found that 1000 μg Ni g^{-1} was acutely toxic to larvae of *Pieris rapae* and resulted in 100% mortality in 17 d. Boyd and Moar (1999) concluded from similar experiments that mortality of larvae of *Spodoptera exigua* was significantly elevated at 963 μg Ni g^{-1} and that sublethal effects on growth were detectable at 540 μg Ni g^{-1}. Thus, the concentration of Ni in *M. boydi* is in the range for which harmful effects have been reported for some terrestrial arthropods. If Ni is responsible for the high mortality of *M. vatia,* as seems probable, then our research is the first case reported where: (1) an elemental defense is transferred from a hyperaccumulator plant to an herbivore; and (2) the elemental defense is toxic to a predator of that herbivore. Nentwig (1987) stated that spiders in general may have difficulty in overcoming some prey defensive chemicals, and pointed out that some spiders will accept prey items containing toxic levels of cyanide. In our experiment, *M. vatia* accepted *M. boydi* as prey. This prey item proved acutely toxic to these spiders, and hence we conclude that *M. boydi* possesses a defense against this spider species. This example can be viewed as a tritrophic interaction mediated by a metal (Ni), instead of the secondary (organic) chemicals that are usually involved in such cases (*e.g.,* Sipura, 1999).

Although we conclude that Ni was toxic in one case, it is also clear from our experiments that Ni was not an effective defense against other predators used in our experiments. We observed no reluctance of any of the predator species to attack *Melanotrichus boydi* when they were offered as prey. Also, Ni in *M. boydi* had no demonstrable negative effect against three of the four predators used. Boyd (1998) pointed out that herbivores may circumvent the elemental defense of hyperaccumulating plants in three ways: (1) circumvention of defense by specialized feeding upon low-metal tissues within hyperaccumulator plants; (2) diluting metal content of the diet by means of a generalist diet; and (3) metal tolerance that physiologically enables an herbivore to ingest large amounts of high-metal food. These also pertain to predators attacking high-Ni herbivores. We conclude that the predators unaffected by Ni in our experiment demonstrate circumvention through greater Ni tolerance. Nickel concentrations in all predators were approximately equal, but only *Misumena vatia* suffered mortality. We do not, however, conclude that this greater Ni tolerance is a direct result of selection by life in a serpentine habitat because, of the two tolerant predators, only the *Stagmomantis californica* individuals were collected from a serpentine habitat.

In addition to physiological tolerance, we also conclude that predators may circumvent a Ni-based defense through dietary dilution of Ni. All four of the predator species used here have generalist (mixed) diets under field conditions (Essig, 1926; Gertsch, 1949; Nentwig, 1983; Arnett, 2000). These generalist diets dilute overall Ni intake to relatively low levels. For example, *Misumena vatia,* for which a diet exclusively composed of high-Ni prey proved toxic, is a generalist predator. Fitch (1963) reported that *M. vatia* captures a wide variety of insects, including hemipterans, that visit flowers. We observed *M. vatia* individuals waiting on flowers of *Streptanthus polygaloides* to ambush floral visitors. Because *Melanotrichus boydi* usually feeds on flowers or flower buds of *S. polygaloides* (Schwartz and Wall, 2001; Wall, 1999), it comes into contact with *M. vatia* and our field observations confirm that some become prey. However, we also observed *M. vatia* in the field feeding upon captured floral visitors: bee species that Wall (1999) reported were relatively low in Ni concentration. The Ni concentrations of field-collected *M. vatia* support the conclusion that *M. vatia* has a generalist diet in habitats containing *S. polygaloides.* Some of our *M. vatia* samples had detectable levels of Ni, but none were nearly as elevated as *M. vatia* fed exclusively *M. boydi* for several weeks. We conclude that *M. vatia* do capture and consume some *M. boydi* (or other insects with high Ni concentrations) in the field, but not enough so that a toxic dose of Ni is received by the spiders under natural conditions. Thus, we conclude that *M. vatia,* although susceptible to dietary Ni toxicity, circumvents the defensive effect of Ni in *M. boydi* by diluting it through a generalist diet.

Although the Ni concentration of *Melanotrichus boydi* is not an effective defense against the generalist predators used here, we suspect that Ni may be a valuable defense against specialist predators or parasitoids/pathogens that cannot dilute the metal content of their diet. For example, Ortel *et al.*, (1993) showed a defensive effect of metals using the moth *Lymantria dispar* L. (Lepidoptera: Lymantriidae). Moth larvae fed elevated Zn and Cu diets were less favorable hosts for the parasitoid wasp *Glyptapanteles liparidis* Bouché (Hymenoptera: Braconidae), and parasitoids feeding on high-metal *L. dispar* also contained high levels of metals. We suggest that the high Ni concentration of *M. boydi* may aid resistance against attack by corresponding specialist enemies of *M. boydi.*

Our results also bear upon the question of metal mobility in food chains and whether biomagnification can occur. Movement of metals up food chains has been shown for several metals (*e.g.,* Pb: Price *et al.*, 1974; Cd: Cheng *et al.*, 1984; Hg: Haney and Lipsey, 1973), but the question of metal biomagnification has been controversial (Laskowski, 1991; van Straalen and Ernst, 1991). Laskowski (1991) suggested a biomagnification index, $B = C_n/C_{n-1}$, where C_n is the metal concentration in organisms of a trophic level and C_{n-1} is the concentration in organisms of the previous trophic level. Although the Ni concentrations of the predators used for our experiments may not have reached steady-state levels at the time we had to terminate the experiments, we can calculate values of B for the food chain, *Streptanthus polygaloides–Melanotrichus boydi*–predators, used in this study. The biomagnification index for the herbivore *M. boydi* is B = 780 μg Ni g^{-1} (mean value reported by Wall, 1999)/2900 μg Ni g^{-1} (minimum value for *S. polygaloides* flowers reported by Reeves *et al.*, 1981) or 0.27. For the predators we used, B = ca. 470 μg Ni g^{-1}/780 μg Ni g^{-1} or 0.60. Thus, our results show that Ni is moving up this food chain as predators consume *M. boydi,* but Ni is not biomagnified since $B < 1$ for both herbivore and predator trophic levels. This confirms the conclusion of Outridge and Sheuhammer (1992) and Barceloux (1999), that there is little evidence for the accumulation of Ni in food chains.

Finally, our results have implications for the nascent phytoextraction industry. Metal hyperaccumulators have received recent attention because they may be useful in cleaning metal-contaminated soils (McGrath, 1998) or for mining metals from soils (Brooks and Robinson, 1998). One concern regarding these efforts is their environmental impacts, including effects on local animal species (U.S. Department of Energy, 1994). Schwartz and Wall (2001) and Wall (1999) showed that Ni can be mobilized up food chains through herbivores that feed on a hyperaccumulator species. The results of this article, using *Melanotrichus boydi,* demonstrate that this mobilization continues between the second (herbivores) and third (predators of herbivores) trophic levels. But *M. boydi* is an unusual herbivore because of its very elevated Ni concentration and apparently is a geographically restricted specialist on *Streptanthus polygaloides* (Schwartz and Wall, 2001; Wall, 1999), so that it might be argued that this case has little relevance to phytoremediation locations because they lack such specialist herbivores. However, Ni hyperaccumulator plants also host generalist herbivores. For example, Wall (1999) found that *S. polygaloides* was host to generalist plant-feeding insects such as the mirid, *Lygus hesperus.* Wall (1999) reported that samples of *L. hesperus* collected from *S. polygaloides* contained a mean of 130 μg Ni g^{-1}, much less than *M. boydi* but also much more than the Ni concentration (0.8 μg Ni g^{-1}) typically reported for animal tissues from non-metalliferous sites (Pais and Jones, 1997). Predators feeding on these generalist insects could receive a dose of Ni that might prove either sublethal or toxic over long periods of time, and they would likely move some of the ingested metals off of a phytoremediation or phytomining site. Whether either of these phenomena would prove a significant environmental risk remains an open question at this time.

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