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Lygus hesperus (Heteroptera: Miridae) tolerates high concentrations of dietary nickel

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Abstract Nickel hyperaccumulator plants contain unusually elevated levels of Ni (> 1 000 μg Ni/g). Some insect herbivores, including *Lygus hesperus* (Western tarnished plant bug), have been observed feeding on the California Ni hyperaccumulator *Streptanthus polygaloides*. This bug may be able to utilize *S. polygaloides* as a host either through its feeding behavior or by physiological tolerance of Ni. This experiment determined the Ni tolerance of *L. hesperus* by offering insects artificial diet amended with 0, 0.4, 1, 2, 4.5, 10, 20 and 40 mmol Ni/L and recording survival. Survival varied due to Ni concentration, with diets containing 10 mmol Ni/L and greater resulting in significantly lower survival compared to the control (0 mmol Ni/L) treatment. Insects tolerated diet containing as much as 4.5 mmol Ni/L, a relatively elevated Ni concentration. I conclude that *L. hesperus* can feed on *S. polygaloides* because it is Ni-tolerant, probably due to physiological mechanisms that provide it with resistance to plant chemical defenses including elemental defenses such as hyperaccumulated Ni.

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

Hyperaccumulators are chemically unusual plants that contain large amounts of elements (often metals) not normally found in abundance in plants (Brooks, 1998). Hyperaccumulation of Ni is defined as concentrations in plant tissues > 1 000 μg Ni/g on a dry mass basis, in contrast to normal plant Ni concentrations of < 10 μg Ni/g (Reeves & Baker, 2000). Other metals and metalloids, such as Cd, Co, Cu, Mn, Se, Tl and Zn, are also hyperaccumulated by plants (Brooks, 1998). However, hyperaccumulation of Ni is predominant: Reeves and Baker (2000) estimated that > 400 hyperaccumulator taxa have been documented and most of these (76%) hyperaccumulate Ni. Nickel hyperaccumulation is restricted to plants that grow on serpentine (ultramafic) soils, substrates having low Ca: Mg ratios, low amounts of N, P and K, and often containing high amounts of metals such as Cr and Ni (Brooks, 1987; Kruckeberg, 1984).

Little is known about insect herbivores of Ni hyperaccumulator plants because few surveys have targeted these plant species (Boyd, 2004). Limited surveys in California, USA (Wall & Boyd, 2002), Mpumalanga, South Africa (Mesjasz-Przybylowicz & Przybylowicz, 2001; Boyd *et al*., 2006a; Boyd *et al*., 2007) and New Caledonia (Boyd *et al*., 2006b) have identified some herbivores of Ni hyperaccumulators. Still less is known of the physiological mechanisms that allow these herbivores to consume tissues unusually elevated in Ni. With the exception of studies by Przybylowicz *et al*. (2003) on *Chrysolina pardalina* Fabricius (Coleoptera: Chrysomelidae), a specialist herbivore of the Ni hyperaccumulator *Berkheya coddii* Roessl. (Asteraceae) from Mpumalanga, South Africa, this area of research remains unexplored.

The California Ni hyperaccumulator *Streptanthus polygaloides* Gray (Brassicaceae) is one of a handful of Ni hyperaccumulators known from continental North America (Kruckeberg & Reeves, 1995) and contains up to 17 000 μg Ni/g in its leaves (Reeves *et al*., 1981). All parts of the plant

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hyperaccumulate Ni (Reeves *et al*., 1981) and the Ni may provide *S. polygaloides* with an "elemental defense" (Martens & Boyd, 1994) against some herbivores and pathogens (Boyd *et al*., 1994; Martens & Boyd, 1994; Boyd & Moar, 1999; Davis *et al*., 2001a). However, hyperaccumulated Ni is ineffective as a defense against some herbivores (Boyd & Martens, 1999; Boyd *et al*., 2004; Wall & Boyd, 2006), plant parasites (Boyd *et al*., 1999) and plant pathogens (Davis *et al*., 2001b). One such herbivore is a specialist plant bug, *Melanotrichus boydi* Schwartz & Wall (Heteroptera: Miridae), which feeds on *S. polygaloides* and has whole body Ni concentrations of almost 800 μg/g (Schwartz & Wall, 2001). Besides this specialist plant bug, a survey of *S. polygaloides* (Wall & Boyd, 2002) also encountered the generalist plant bug *Lygus hesperus* Knight (Heteroptera: Miridae). This bug is a broad generalist that has been reported from more than 100 plant species in 24 families (Scott, 1977). The specimens collected from *S. polygaloides* contained elevated Ni (130 μg Ni/g), but less than the specialist *M. boydi* (Wall & Boyd, 2002). The elevated Ni concentration of these *L. hesperus* specimens, as well as field observations, showed they were feeding on *S. polygaloides* (Wall & Boyd, 2002). A laboratory study of *L. lineolaris* Palisot de Beauvois, another *Lygus* species with a broad host range (Snodgrass, 1986; Young, 1986), showed no significant effect of plant Ni concentration on insect survival (Jhee *et al*., 2005).

Nickel hyperaccumulator plants are an unusual food resource for herbivores due to their extraordinary elemental makeup. Successful herbivores may be able to circumvent high Ni concentrations of hyperaccumulator plants by three mechanisms (Boyd, 1998): (i) by feeding on relatively low Ni tissues or cells (avoidance); (ii) by dietary dilution (mixing low and high Ni foods to lower total dietary Ni concentration); or (iii) through physiological mechanisms that allow tolerance of a high Ni diet. Observations of feeding by *L. hesperus* on *S. polygaloides* in the field (Wall & Boyd, 2002), and the ineffectiveness of hyperaccumulated Ni in protecting *S. polygaloides* plants from *L. lineolaris* in laboratory experiments (Jhee *et al*., 2005), raise the question of how *Lygus* bugs circumvent the Ni-based defense of *S. polygaloides*.Two basic possibilities explain the success of *Lygus* bugs: (i) they use behavioral traits, such as selective feeding on low-Ni tissues within hyperaccumulator plants or through a mixed diet; or (ii) they are tolerant of the elevated Ni concentrations of tissues of the hyperaccumulator. This experiment was designed to test the latter possibility by determining the tolerance threshold of *L. hesperus* to dietary Ni. I did this by creating artificial *L. hesperus* diets (Cohen, 2004) containing a range of Ni concentrations and comparing the survival of *L. hesperus* allowed to feed on those diets.

Materials and methods

Lygus hesperus individuals were obtained from a commercial supplier (BioTactics, Riverside, CA, USA) as a mix of late instar and adult stages. These insects had been reared on artificial diet (Debolt, 1982). Artificial *Lygus* diet used for amendment with Ni was obtained from Bio-Serv® (Frenchtown, NJ, USA). Because this is a liquid diet, Ni concentrations are reported as mmol Ni/L rather than the μg Ni/g used to document the Ni concentrations of dried hyperaccumulator plants (see Discussion). The diet was amended with Ni in the form of the chloride salt (obtained from Sigma-Aldrich, St. Louis, MO, USA) to create the following Ni concentrations (mmol Ni/L): 0, 0.4, 1, 2, 4.5, 10, 20 and 40. Approximately 25 mL of diet was vacuum-sealed into small plastic bags in which one side consisted of Parafilm M (American National Can Company, Neenah, WI, USA).

Ten *L. hesperus* were placed into each of 80 150-mL plastic containers. Approximately 20% of insects used were adults, with the number of adults ranging from 0–4 per container. The top of each container was covered with fine mesh plastic screen to prevent insects from escaping. I divided the containers into 10 blocks of eight containers each and randomly assigned one of the eight diet treatments to each container in a block. A bag containing the appropriate diet was placed on top of the screen on each container with the Parafilm side facing down. Bugs were able to feed by piercing the Parafilm with their stylets to access the diet in the bags. Containers were kept at room temperature (22°C) during the experiment.

I counted surviving insects in each container daily (but skipping day 2) for 13 days. Survival among all treatment groups was compared with survival analysis using the Kaplan-Meier estimate and the Peto-Peto-Wilcoxon test (Abacus Concepts, 1998). I then conducted several pairwise survival analyses, contrasting survival of insects fed the control (0 mmol Ni/L) diet with those fed certain Ni-amended diets to determine the least concentration of Ni that resulted in a statistically significant treatment effect.

Results

Survival of *L. hesperus* was significantly affected by dietary Ni concentration (Fig. 1). Survival analysis of the full dataset showed that survival varied significantly among Ni treatments (Peto-Peto-Wilcoxon test: $P < 0.000\,1$). The control (0 mmol Ni/L) and Ni concentrations of 4.5 mmol Ni/L or less yielded similar survival curves (Fig. 1). Survival analysis comparing the control treatment (0 mmol Ni/L) with the greatest concentration of Ni in this group (the 4.5 mmol Ni/L diet) showed no significant difference (Peto-Peto-Wilcoxon test: $P = 0.91$). In contrast, survival analyses comparing the control with the 10 mmol Ni/L and 20 mmol Ni/L treatments both showed highly significant treatment effects (Peto-Peto-Wilcoxon tests: $P < 0.000\ 1$ in both cases). Thus, the lethal threshold in this experiment for Ni in artificial diet was between 4.5 and 10 mmol Ni/L . Although it is clear that diet containing 10 mmol Ni/L resulted in lowered *L. hesperus* survival, it is unknown if that Ni concentration was toxic to *L. hesperus* or if insects refused to feed because I was unable to observe insect feeding behavior. The results do show that *L. hesperus* was tolerant of diet containing as much as 4.5 mmol Ni/L.

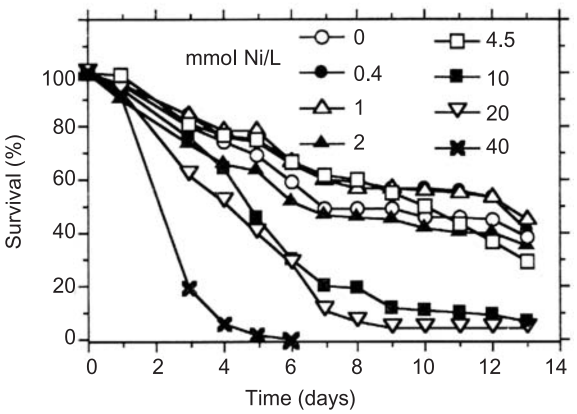

Fig. 1 Survival of *L. hesperus* offered artificial diet containing from 0–40 mmol Ni/L . Each curve summarizes the survival of 80 *L. hesperus* individuals.

Discussion

Observations of feeding by *L. hesperus* upon *S. polygaloides* in the field (Wall & Boyd, 2002), plus my result showing decreased *L. hesperus* survival on diet containing 10 mmol Ni/L, suggest that *L. hesperus* feeding on *S. polygaloides* is consuming material containing at most a Ni concentration between 4.5 and 10 mmol/L. The Ni concentration of *S. polygaloides* tissues on a molar basis is unknown, but Boyd and Martens (1994) reported that plants grown on Ni-amended soil in a greenhouse contained 7 400 μg Ni/g dry mass and 91% water. If all the Ni is dissolved in that water, the Ni concentration would be 12.5 mmol/L. This figure is a maximum, because studies of other Ni hyperaccumulator species have shown that not all hyperaccumulated Ni is water-soluble. Brooks (1987) reported that water extracts of Ni hyperaccumulator tissues may contain 23%–75% of the total Ni, depending on the species involved. Thus, it is likely that the mmol Ni/L concentration of *S. polygaloides* leaf tissues is in the range of 3.0–9.4 mmol Ni/L, close to or within the tolerance limit of *L. hesperus*.

The effects of Ni on insects are, in general, little known. Although other heavy metals have been more extensively investigated [e.g., the study by Behmer *et al.* (2005) on the response of *Schistocerca gregaria* (Orthoptera: Acrididae) to Zn], the review by Heliovaara and Vaisanen (1993) stated that "effects of Ni on insects are poorly documented." To my knowledge, the only information on elemental composition of *L. hesperus* is the study of Cohen *et al.* (1985), which documented whole-body levels of some metals (but not Ni) in laboratory and field-collected insects. I conclude that the ability of *L. hesperus* to tolerate 4.5 mmol Ni/L diet demonstrates Ni tolerance because larvae of the diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), fed artificial diet amended with Ni showed significantly reduced survival at just 0.22 mmol Ni/L (Coleman *et al.*, 2005). I know of only one published study of another heteropteran fed Ni-amended artificial diet. Boyd and Martens (1999) reported that the pea aphid, *Acyrthosiphon pisum* (Harris) (Heteroptera: Aphididae), could tolerate diet containing up to 2 mmol Ni/L. They concluded that the tolerance of this aphid for Ni was one reason it could feed on *S. polygaloides* without ill effect.

Boyd (1998) suggested that herbivores can feed on hyperaccumulator plants through three mechanisms: (i) by selecting to feed on relatively low metal tissues or cells (avoidance); (ii) by dietary dilution through feeding on multiple hosts; or (iii) through physiological mechanisms that allow tolerance of a high metal diet. This artificial diet experiment eliminated the first two possibilities so that I can conclude that *L. hesperus* is relatively Ni-tolerant. Tolerance can be achieved in several ways, including by limiting metal uptake from the gut, by efficiently excreting metal that is taken up from the gut, or by being able to tolerate high tissue concentrations of Ni. Although my experiment does not identify the mechanism of Ni tolerance by *L. hesperus*, as broad generalists *Lygus* bugs probably possess features that allow them to tolerate a variety of plant chemical defenses. These features are not well explored for mirid bugs in general (Wheeler, 2001) but my experiment suggests they may confer tolerance to metal-based defenses such as Ni. To compare results for *L. hesperus* with those from a plant bug that is a specialist herbivore of a Ni hyperaccumulator plant, I attempted an experiment similar to that reported here using *Melanotrichus boydi*, the high-Ni plant bug found only on *Streptanthus polygaloides* (Wall & Boyd, 2006). Unfortunately, that experiment failed because insects in all treatment groups died rapidly, probably because they did not feed on the artificial diet.

If heteropterans as a group are relatively Ni-tolerant, then we would expect them to be well represented among insect herbivores that have been reported to feed on Ni hyperaccumulator species. Only a few surveys of herbivores from Ni hyperaccumulators have been conducted to date. The results from those studies are tantalizing in that all have discovered at least one heteropteran that feeds without apparent harm on a Ni hyperaccumulator species. In Mpumalanga, South Africa, Boyd *et al*. (2006a) found seven heteropterans (including one aphid) feeding on *Berkheya coddii*. In California, USA, Wall and Boyd (2002) and Boyd *et al*. (2004) reported a total of five heteropteran species feeding on *Streptanthus polygaloides*. In New Caledonia, Boyd *et al*. (2006b) reported one heteropteran feeding on developing fruits of *Hybanthus austrocaledonicus*. Finally, insect samples collected by Peterson *et al*. (2003) from habitats containing the Portuguese Ni hyperaccumulator *Alyssum pintodasilvae* included "several unidentified species of relatively large-bodied Hemiptera," one or more of which contained large concentrations of Ni and thus probably fed on *A. pintodasilvae*. These field survey results suggest that heteropterans may be particularly successful herbivores of Ni hyperaccumulator plants. This success may be due to the ability of heteropterans to tolerate elevated levels of dietary Ni, as shown in this experiment using *L. hesperus*.

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