Insect Science (2006) 13, 271–277

Nickel levels in arthropods associated with Ni hyperaccumulator plants from an ultramafic site in New Caledonia

ROBERT S. BOYD[1], MICHAEL A. WALL[2], and TANGUY JAFFRÉ[3]

[1]Department of Biological Sciences, Auburn University, Auburn, Alabama, USA, [2]Entomology Department, San Diego Natural History Museum, San Diego, California, USA, [3]Laboratoire de Botanique et d' Ecologie Végétale Appliquées, IRD - Institut de recherche pour le développement, Centre de Nouméa, Nouméa, New Caledonia

Abstract Arthropods (mainly insects) were collected from a forest site that contained at least six species of Ni hyperaccumulators. Whole body Ni analysis was performed for 12 arthropod taxa, two of which were studied at different life cycle stages. We found two Ni-tolerant insects. The pentatomid heteropteran *Utana viridipuncta,* feeding on fruits of the Ni hyperaccumulator *Hybanthus austrocaledonicus,* contained a mean of 2 600 μg Ni/g in nymphs and 750 μg Ni/g in adults. The tephritid fly *Bactrocera psidii*, feeding on pulp of *Sebertia acuminata* fruits that contained 6 900 μg Ni/g, contained 420 μg Ni/g as larvae that had evacuated their guts and significantly less (65 μg Ni/g) as adults. European honeybees (*Apis mellifera*) visiting flowers of the Ni hyperaccumulator *H. austrocaledonicus* contained significantly more Ni (8-fold more) than those collected from flowers of *Myodocarpus fraxinifolius*, a non-hyperaccumulator. Our results show that some insects feed on Ni hyperaccumulator plants and that their feeding mobilizes Ni into local food webs.

Key words biomagnification, food web, heavy metal, herbivory, hyperaccumulation, metal tolerance
DOI 10.1111/j.1744-7917.2006.00094.x

Introduction

The archipelago of New Caledonia contains an unusually high level of biodiversity (Mittermeier *et al.*, 1996). Unfortunately, its biodiversity is severely threatened as a result of human activities (McNeely, 2003; Jaffré *et al.*, 1998; Bouchet *et al.*, 1995). As a consequence of both these features, it has received global recognition as one of 25 'biodiversity hotspots' tabulated by Mittermeier *et al.* (1999). Botanically, New Caledonia is also renowned for its relatively large number of Ni hyperaccumulator plant species. Nickel hyperaccumulators contain extremely elevated Ni concentrations, more than 1 000 μg/g on a dry mass basis (Brooks *et al.*, 1977). Until recent studies of the ultramafic flora of Cuba discovered a large number of Ni hyperaccumulators there (Reeves *et al.*, 1996, 1999), more Ni hyperaccumulators were known from New Caledonia (almost 50 taxa) than from any other location (Reeves, 2003). New Caledonian Ni hyperaccumulators include the remarkable tree *Sebertia acuminata* Pierre ex Baillon (Sapotaceae), the latex of which contains as much as 26% Ni on a dry mass basis (Jaffré *et al.*, 1976). Unfortunately, the ultramafic flora of New Caledonia, which is particularly rich in endemic species (Jaffré *et al.*, 1998), is not well protected from human impact (Jaffré, 2005).

Because of their extreme Ni concentrations, Ni hyperaccumulators are a chemically unique resource for herbivores. Laboratory experiments with non-native herbivorous insects have shown that tissues of Ni hyperaccumulators can be toxic or deterrent and thus can

Correspondence: Robert S. Boyd, Department of Biological Sciences, 101 Life Sciences Building, Auburn University, Auburn, Alabama 36849-5407, USA. Tel: 334 844 1626; fax: 334 844 1626; email: boydrob@auburn.edu

protect hyperaccumulators against some natural enemies (Boyd, 1998, 2004). However, it is likely that some native herbivores feed on hyperaccumulator plants without ill effect. Boyd (1998) predicted this could stem from three tactics: avoidance, tolerance and dietary dilution. Avoidance could occur if metal concentrations are low in certain plant tissues and a herbivore targets those for feeding. Tolerance is the physiological adaptation of a herbivore to a high-metal diet. Boyd (1998) suggested this could occur in two ways: (i) a herbivore avoids uptake of metals as they move through its gut; or (ii) a herbivore could be genuinely metal-tolerant and take up metal into its tissues without ill effect. Finally, dietary dilution would allow a herbivore to consume some hyperaccumulator plant tissues by mixing those with tissues from non-hyperaccumulator plants to reduce its metal intake to below harmful levels.

Surveys of Ni hyperaccumulator plants for native herbivores have only recently been conducted and some Ni-tolerant insect herbivores have been encountered. A study of arthropods associated with the Ni hyperaccumulator *Streptanthus polygaloides* Gray (Brassicaceae) from California, USA found two mirid bugs (Heteroptera) that feed on the species and contain elevated body Ni levels (Wall & Boyd, 2002). One, *Melanotrichus boydi* Schwartz & Wall, is an apparent specialist on *S. polygaloides* and contains 770 μg Ni/g dry mass (Schwartz & Wall, 2001). The other, *Coquilletia insignis* Uhler, has a broader host range but those collected from *S. polygaloides* contained 500 μg Ni/g (Boyd *et al.*, 2004). Surveys of insects associated with the South African Ni hyperaccumulator *Berkheya coddii* Roessler (Asteraceae) have enumerated a number of apparently Ni-tolerant insects (Boyd *et al.*, 2006; Mesjasz-Przybylowicz & Przybylowicz, 2001). Most important of these is *Chrysolina pardalina* Fabricius (Chrysomelidae), which feeds exclusively on this species and can contain up to 2 650 μg Ni/g in adults (Mesjasz-Przybylowicz & Przybylowicz, 2001). Thus, it is clear that some native herbivorous insects are metal-tolerant and can utilize Ni hyperaccumulators as food sources.

One ecosystem consequence of herbivory on metal hyperaccumulator species is mobilization of metals into local food webs. A test of this hypothesis in Portugal (Peterson *et al.*, 2003), comparing Ni levels in arthropods from sites with and without the Ni hyperaccumulator *Alyssum pintodasilvae* Dudley (Brassicaceae), showed elevated Ni levels in arthropods collected from ultramafic sites. This matter is of interest to our understanding of ultramafic ecosystem function, but also is potentially important to applied uses of metal hyperaccumulator plants. These plants can be used for phytoextraction, removal of metals from high metal soils (Pilon-Smits, 2005), and the consequences of mobilizing metals from soils to plants and thence to herbivores may be an environmental concern (Whiting *et al.*, 2004).

There are more than 320 Ni hyperaccumulator species known (Reeves, 2003), yet to our knowledge only *S. polygaloides* from California and *B. coddii* from South Africa have been surveyed for Ni-tolerant arthropods. Thus, we know of no surveys conducted in the tropics, although many Ni hyperaccumulators are found there (see review by Reeves, 2003). In particular, we know of no surveys conducted in New Caledonia. To our knowledge, the report of Sagner *et al.* (1998) that the sap of *S. acuminata* was repellent to *Drosophila melanogaster* L. (Diptera: Drosophilidae) is the only published information on herbivore interactions with New Caledonian hyperaccumulator plants. The objective of this research was to survey arthropods at a New Caledonian field site that hosts at least six species of Ni hyperaccumulating plants. We conducted some generalized sampling but mainly focused on insects associated with two of the more abundant Ni hyperaccumulator species present on the site, *Sebertia acuminata* and *Hybanthus austrocaledonicus* (Vieill.) Schinz & Guillaumin *ex* Melchior (Violaceae).

Materials and methods

Study site

Insects were sampled from the Parc Provincial de la Rivière Bleue near the southern end of the main island (Grande Terre), during March 19–24, 1996 and again between December 15, 1996 and January 5, 1997. This park contains protected areas of humid tropical forest studied by Jaffré & Veillon (1991). The study location was a stand of humid forest at Kaori Géant, a site with an exceptionally large *Agathis lanceolata* Lindl. (Araucariaceae) tree and is the location of studies on the Ni hyperaccumulator *Psychotria douarrei* (Beauvis.) Däniker (Rubiaceae) by Boyd *et al.* (1999) and Davis *et al.* (2001). The site was also used to study Ni enrichment of surface soil by *Sebertia acuminata* (Boyd & Jaffré, 2001). Six Ni hyperaccumulator species co-occur at this site (Boyd *et al.*, 1999). The shrub layer contains *Psychotria douarrei*, *Hybanthus austrocaledonicus* and *Casearia silvana* Schltr. (Flacourtiaceae). Three Ni hyperaccumulating tree species are also present: *Homalium guillainii* (Vieill.) Briq., *Geissois hirsuta* Brongn. & Gris (Cunoniaceae) and *Sebertia acuminata*.

Community-level sampling

Community-level sampling was conducted via pitfall traps, night lighting, and sweep netting on non-hyperaccumulating hosts. Night lighting was conducted on the evenings of March 22 and December 28, 1996. Four portable UV lights with accompanying 2.3 m^2 light sheets were hung in a square array such that each light represented the corner of an approximately 50 m × 50 m square. Insects were collected throughout the night and killed in ethyl acetate kill jars after which samples were sorted, exemplars were mounted, and the remaining material stored in plastic containers for metal analysis. During both sampling trips two parallel transects of 10 pitfall traps each were placed 20 m apart in our study area. Transects were approximately 100 m long with each pitfall trap separated by 10 m. Traps consisted of 11.5 cm diameter × 10.5 cm deep plastic containers filled 1/4 full with dilute ethylene glycol. Specimens were sieved from the March traps, rinsed in water, sorted, air dried, and stored in plastic containers for metal analysis. Traps from the December sample were destroyed by two successive cyclones and specimens were not retrievable.

Specimens were sorted by morphotype (defined as specimens similar enough in general appearance so that they probably were members of a single species) and representatives of each were pinned and labeled for later identification to the lowest taxonomic level that could be readily attained. Upon returning from the field, specimens were air-dried for at least 72 h at 67°C and weighed. Individuals of the same morphotype weighing less than 50 mg were combined to create samples of at least that mass for analysis. Many of our morphotypes could not be analyzed for Ni concentration because too few specimens were collected to provide sufficient mass for analysis. Voucher specimens are deposited in the personal collection of M. Wall (San Diego Museum of Natural History).

Insects associated with hyperaccumulators

Two Ni hyperaccumulating species, *Sebertia acuminata* and *Hybanthus austrocaledonicus*, were targeted for sampling during the 1996−1997 trip. Over 100 fallen fruits of *Sebertia acuminata* were collected in the field. Damage from feeding by fly larvae was observed on some fruits. Fruits were categorized as immature undamaged (hard and green in color), mature undamaged (soft and dark colored) and fruits that were damaged by larval feeding (clearly and heavily damaged by larvae). Larvae were dissected from damaged fruits. In order to evacuate food retained in the gut, larvae were stored in containers containing moistened toilet paper (which many larvae consumed). Some larvae died but others formed puparia. A third of the puparia were frozen for metal analysis. Remaining puparia were kept in plastic containers until eclosion, upon which adults were separated from pupal cases and both adults and pupal cases were frozen for metal analysis. Nickel concentration of the collected *Sebertia* fruits was also determined. Fruits in each category were dissected into fractions containing the fleshy pericarp, the seed coats and the seed contents. Samples were dried and combined into 4−5 samples of each portion for each of the three categories of fruits (immature undamaged, mature undamaged, damaged). *Hybanthus austrocaledonicus* was sampled via sweep-netting and visual inspection. Captured insects were killed in ethyl acetate kill jars and transferred into plastic containers for later metal analysis.

Nickel analysis of arthropod and Sebertia *fruit samples*

Arthropod samples were digested in borosilicate glass test tubes using 3−5 mL of concentrated nitric acid at 110°C for 6−8 h, after which time most of the liquid had evaporated. The residue was then redissolved in 3−5 mL of 1 mol/L hydrochloric acid at 110°C for 2−4 h. The solutions were diluted with distilled water to a volume of 10 mL for samples less than 100 mg or 25 mL for samples over 100 mg. Both 10 and 25 mL reagent blanks were made and processed with every batch of samples in order to detect any contamination generated by the technique. Nickel concentration in solutions was determined with an atomic absorption spectrophotometer (Instrumentation Laboratory, IL 251). Analysis of *Sebertia* fruit samples followed a protocol similar to that described for arthropod samples except that samples were dry-ashed (Boyd & Davis, 2001).

Results

Community-level sampling

While night-lighting and pitfall trapping brought in many species of insects, most were either individuals or were too few to make metal analysis possible using the atomic absorption technique. Nonetheless, we were able to gather adequate material to conduct Ni analysis (Table 1) of two morphotypes of cicadas (Hemiptera: Cicadidae), a burrowing bug (Hemiptera: Cynidae), *Polycarmes punctatissimus* (Montrouzier) (Hemiptera: Pentatomidae) and a pyralid moth (Lepidoptera: Pyralidae). We also report Ni values for a few morphotypes for which we only gathered enough biomass for one sample. These include two beetles, a cricket, and a terrestrial amphipod (Table 1). Most of the samples from pitfall trapping contained less Ni than specimens of Heteroptera collected from hyperaccumulators (see below), but the beetle Nit-1 taken from pitfall traps was exceptional in containing 240 μg Ni/g (Table 1).

Table 1 Nickel concentrations (μg/g dry mass) of arthropod taxa collected from Rivière Bleue, New Caledonia.

Taxa	N	Mean	SD	Collection notes
Diptera (Tephritidae)				
Bactrocera psidii (larvae)	5	420	66	From *Sebertia acuminata* (Ni hyperaccumulator)
B. psidii (pupae)	2	160	43	From *Sebertia acuminata* (Ni hyperaccumulator)
B. psidii (pupal cases)	2	72	5	From *Sebertia acuminata* (Ni hyperaccumulator)
B. psidii (adults)	3	65	27	From *Sebertia acuminata* (Ni hyperaccumulator)
Hemiptera				
Cic-1 (Cicadidae)	5	20	15	Night lighting March 1996
Cic-2 (Cicadidae)	4	3	4	Night lighting December 1996/January 1997
Cyn-1 (Cynidae)	3	20	18	Pitfall trapping
Pentatomidae				
Utana viridipuncta (adults)	2	750	360	From *Hybanthus austrocaledonicus* (Ni hyperaccumulator)
U. viridipuncta (exuviae)	1	130	n/a	From *Hybanthus austrocaledonicus* (Ni hyperaccumulator)
U. viridipuncta (nymphs)	3	2 600	460	From *Hybanthus austrocaledonicus* (Ni hyperaccumulator)
Polycarmes punctatissimus	2	26	6	Night lighting December 1996
Orthoptera				
Ort-1	1	91	n/a	Pitfall trapping
Coleoptera				
Nit-1 (Nitidulidae)	1	240	n/a	Pitfall trapping
Sca-1 (Scarabaeidae)	1	58	n/a	Pitfall trapping
Hymenoptera (Apidae)				
Apis mellifera	3	40	20	From *Hybanthus austrocaledonicus* flowers (high Ni)
Apis mellifera	3	5	4	From *Myodocarpus fraxinifolius* flowers (low Ni)
Lepidoptera				
Pyr-1 (Pyralidae)	3	13	17	Night lighting March 1996
Amphipoda				
Amp-1	1	79	n/a	Pitfall trapping

Insects associated with hyperaccumulators

Although there were clearly at least three larvae (two Diptera and one Coleoptera) associated with the fruits of *S. acuminata*, only one dipteran species, *Bactrocera psidii* (Froggatt), was abundant enough for Ni analysis. Because we were able to rear *B. psidii* larvae to adulthood, we ultimately were able to quantify Ni in *B. psidii* larvae, pupae, pupal cases, and adults (Table 1). One-way analysis of variance (ANOVA) of the *B. psidii* data showed significant variation in Ni concentration among the categories sampled ($F_{3,8} = 41, P < 0.000\,1$), with larvae containing greater Ni levels than adults, pupae and pupal cases (Fisher's Protected Least Significant Difference test, $\alpha < 0.05$, Table 1).

Nickel concentrations of *S. acuminata* fruits were elevated (Table 2). Two-way ANOVA showed that fruit category ($F_{2,34} = 4.9, P = 0.014$), fruit portion ($F_{2,34} = 69, P < 0.000\,1$) and the interaction ($F_{4,34} = 3.2, P = 0.025$) all significantly affected Ni concentration. Generally, mature undamaged fruits had lesser Ni concentrations than young or damaged fruits and seed coats had lesser Ni concentrations than samples of pericarp or seed contents (Table 2). The significant interaction term stemmed mainly from the relatively low Ni concentrations of seed coats in mature undamaged and damaged fruits (700 μg/g) and the hyperaccumulator levels of Ni (> 1 000 μg/g) in all other tissues analyzed, including seed coats from immature undamaged fruits (Table 2).

Two insect species were collected in association with *Hybanthus austrocaledonicus*. *Apis mellifera* L. workers, collected as they foraged on *H. austrocaledonicus* flowers (Table 1), contained significantly more Ni (8-fold more) than conspecifics foraging on *Myodocarpus fraxinifolius* Brongn. & Gris (Araliaceae), a non-hyperaccumulator from an adjacent habitat (t-test: $t = 2.9$, df = 4, $P = 0.04$). Nymphs and adults of *Utana viridipuncta* Bergroth (Table 1) also were collected from *H. austrocaledonicus*. They were observed feeding on developing fruits of this plant species. The Ni concentration of nymphs was significantly greater (3.4-fold) than that of adults (t-test: $t = 4.7$, df = 3, $P = 0.019$).

Table 2 Nickel concentrations (μg Ni/g dry mass) in fallen fruits of *Sebertia acuminata*.

Fruit description	Fruit portion		
	Pericarp	Seed coat	Seed contents
Immature undamaged	5 300 (700, 5)	1 800 (970, 4)	4 500 (540, 5)
Mature undamaged	4 000 (480, 5)	700 (52, 5)	4 500 (360, 5)
Damaged	6 900 (400, 5)	700 (140, 5)	5 270 (230, 4)

Fruits were categorized as immature, mature and those with feeding damage from which insect larvae were collected. Fruits were partitioned into pericarp, seed coat and seed contents for analysis. Values are means (SE, N).

Discussion

Boyd (1998) predicted that herbivores of hyperaccumulating species can successfully attack these plants via three strategies: (i) avoidance, where relatively low-metal tissues are sought and consumed; (ii) diet dilution, where a generalist herbivore combines both hyperaccumulator and nonhypera-ccumulator species in its diet; and (iii) tolerance, where a herbivore possesses features that allow it to ingest relatively high-metal food with no apparent ill effect. Our results provide two examples of tolerance. *Bactrocera psidii* larvae were fruit miners that ingested high-Ni *Sebertia* fruit tissues as they tunneled through the fleshy pericarp. As larvae they contained considerable Ni (420 μg Ni/g). Because these larvae were able to evacuate their guts, this high Ni value suggests that the tissues of the larvae were high in Ni. Yet the larvae had much less Ni in their tissues than is found in *Sebertia* fruit pulp (Table 2), implying that this fly possesses mechanisms that immobilize Ni in the gut or otherwise limit uptake. We also found that *B. psidii* adults contained significantly less Ni (64 μg/g) than did the larvae (420 μg/g). This result suggests that considerable Ni is shed from the bodies of larvae during metamorphosis. *Utana viridipuncta* feeds using piercing-sucking mouthparts and was remarkable for its high whole body Ni concentration: nymphs contained much more Ni (more than 6-fold more) than *B. psidii* larvae. The mean Ni concentration of *U. viridipuncta* nymphs was 2 600 mg Ni/g (Table 1), a very high value. However, these insects may have had considerable Ni in their guts and hence we do not know how much Ni was present in their tissues. In general, Ni hyperaccumulators contain elevated Ni levels in all plant organs (Reeves, 2003). Leaves of *H. austrocaledonicus* are very high in Ni (> 13 000 μg/g, Jaffré & Schmid, 1974) and it is therefore likely that the developing fruits upon which *U. viridipuncta* fed also contained much Ni. Thus, it is reasonable to conclude that *U. viridipuncta*, like *B. psidii*, is able to limit Ni uptake into its body from its high Ni food.

Some prior studies of metal concentrations of arthropods collected from ultramafic soils (e.g., Davison *et al*., 1999; Peterson *et al*., 2003) have combined taxa into extremely broad categories (orders or families). Our results (Table 1), showing the wide variation in Ni concentrations between species of Pentatomidae, and between life stages of the same species (*B. psidii* and *U. viridipuncta*), highlight the importance of partitioning samples to the finest level possible. Unfortunately, the techniques and equipment that we employed were neither precise nor accurate enough at extremely small sample weights and many morphotypes could not be analyzed.

One concern about toxic substances in food webs is whether their concentrations increase with increasing trophic level (biomagnification). The biomagnification index of Laskowski (1991) is $B = C_n/C_{n-1}$, 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. Our data for *B. psidii* larvae (420 μg Ni/g) and the fruits on which they were feeding (6 900 μg Ni/g) suggest a value for B for the *B. psidii*/*S. acuminata* relationship of 0.06, which is very low (van Straalen & Ernst, 1991). The value would be still less (0.009) if we used the mean Ni concentration of adult *B. psidii* (65 μg Ni/g) to calculate this index. Thus, we find no evidence for Ni biomagnification, confirming results of the few studies of herbivores that feed upon Ni hyperaccumulators (Boyd & Wall, 2001; Mesjasz-Przybylowicz *et al*., 2004).

Although we did not find bioaccumulation of Ni, we conclude that Ni is being mobilized into the food web on our study site by herbivores that feed on Ni hyperaccumulators. Some of the Ni in the bodies of the herbivores reported here (Table 1) is probably transferred to their predators, as was shown by Boyd and Wall (2001) for predators of the high-Ni bug *M. boydi* from California, USA. Our study also includes results from a flower-visiting species (*Apis mellifera*) that is not native to New Caledonia (Kato & Kawakita, 2004). These results are intriguing because they suggest that floral visitors of hyperaccumulator species also may mobilize metals. This result parallels that of Wall and Boyd (2002), who reported significantly elevated whole body Ni concentrations from two species of bees, *Apis mellifera* and *Bombus vandykei* (Frison), collected while visiting flowers of the Ni hyperaccumulator *Streptanthus polygaloides*, relative to samples collected from non-hyperaccumulator flowers. Relatively few analyses of floral metal concentrations of hyperaccumulator species have been conducted, but the available data show floral concentrations of metals are at hyperaccumulator levels, for example Jaffré *et al.* (1976) for *S. acuminata*. However, we are unaware of analyses of metal concentrations in floral rewards (nectar or pollen) from any metal hyperaccumulator species. Our data suggest that floral rewards of *H. austrocaledonicus* are elevated in Ni concentration and result in elevated Ni values of visiting honeybees.

Finally, our study illustrates the scientific value of New Caledonia's hyperaccumulators and their relationships with native arthropods. Unfortunately, these relationships are under continuing threat from human activities even in protected areas. For example, the little fire ant, *Wasmannia auropunctata* (Roger), considered one of the worst invasive pest ants (Lowe *et al.*, 2000), was discovered in New Caledonia in 1972 (Fabres & Brown, 1978). It has spread through the main island and by 1997 had appeared in the Rivière Bleue provincial park (Le Breton *et al.*, 2003), where our study was conducted. *Wasmannia auropunctata* can have large negative impacts on native ants (Le Breton *et al.*, 2003, 2005) and other native fauna (Jourdan *et al.*, 2001). The continued spread of *W. auropunctata* may threaten some of the arthropods associated with Ni hyperaccumulators in the forests of Rivière Bleue before their ecological interrelationships can be fully explored. This is especially worrisome because the inventory of New Caledonia's biodiversity is incomplete, as evidenced by recent descriptions of new taxa of insects (e.g., Reid & Smith, 2004; Pellens, 2004) and even terrestrial vertebrates (e.g., Sadlier *et al.*, 2004). Thus, these human impacts (including the spread of *W. auropunctata*) have the potential to destroy arthropods that have evolved unique relationships with Ni hyperaccumulating plants before those relationships are fully explored.

Acknowledgments

We wish to thank Auburn University for providing travel funds for this research. Insect identifications were kindly provided by Allen Norrbom of the USDA-ARS Systematic Entomology Laboratory (*Bactrocera psidii*) and David Rider of North Dakota State University (Pentatomidae). We also thank two anonymous reviewers for helpful comments on the original manuscript.

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Accepted February 24, 2006