

Variation in Nickel Content in the Nickel-Hyperaccumulating Shrub *Psychotria douarrei* (Rubiaceae) from New Caledonia¹

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ABSTRACT

Plants that hyperaccumulate Ni contain > 1000 ppm (dry wt.) in their tissues. Variation of Ni content within hyperaccumulating plant species is poorly explored. Using the Ni-hyperaccumulating shrub *Psychotria douarrei*, we documented variation of leaf Ni levels within individual shrubs, and variation with respect to plant size and leaf age. Plant size did not correlate significantly with leaf Ni content, and leaf Ni content did not correlate significantly with soil Ni content. Older leaves contained twice as much Ni as younger leaves. Older leaves also contained greater concentrations of Ca, Fe, and Cr but less K, P, and Cu. Five elements (Zn, Pb, Co, Mn, Mg) showed no significant variation due to leaf age. We also examined the effect of leaf age on epiphyll cover, finding increased epiphyll cover on the upper surface of older leaves. The dominant leafy liverwort epiphyll had a relatively high Ni content (400 ppm), suggesting that epiphylls of Ni hyperaccumulators obtain some Ni from host leaves. Individual shrubs differed in mean leaf Ni content almost two-fold (14,900–27,700 ppm). Variation among branches within individuals also ranged widely; however, this intraplant variability was not strongly correlated with the mean leaf Ni content of an individual shrub. We concluded that Ni contents in leaves of *P. douarrei* vary considerably due to leaf age, among individual shrubs, and among branches within a shrub.

Key words: elemental defense; epiphylls; epiphytes; leaf age; leafy liverwort; New Caledonia; nickel hyperaccumulation; plant size; *Psychotria douarrei*; serpentine soil.

PLANTS AND HERBIVORES ARE INVOLVED IN AN ANTAGONISTIC RELATIONSHIP in which benefit to the herbivore is derived at the expense of the plant; however, although plants are sessile, they are not defenseless. Plant defenses against herbivory may include phenological (*e.g.*, timing of bud break) and physical defenses (*e.g.*, spines, trichomes). Plants also defend themselves chemically, through a wide variety of compounds generally called secondary chemicals (Ehrlich & Raven 1967). Secondary chemical defenses are almost exclusively made by a plant's biochemical machinery (Berenbaum 1995). Some plants, however, concentrate large amounts of certain elements from the soil (Baker & Brooks 1989) and may, by virtue of their unusual elemental composition, be defended from herbivores (Boyd 1998). These plants, termed hyperaccumulators by Brooks *et al.* (1977), may contain elevated quantities of metals (*e.g.*, Ni, Zn, Cu).

Nickel is the metal most often hyperaccumu-

lated by plant species (Baker & Brooks 1989), with a tissue concentration of at least 1000 ppm Ni (dry wt.) considered the minimum level for classifying a plant as a Ni hyperaccumulator (Baker & Brooks 1989). Plants that hyperaccumulate Ni occur on serpentine soils in many areas of the world, including a number of locations in the tropics (Brooks 1987). For example, the South Pacific tropical archipelago of New Caledonia is renowned for having a greater diversity of Ni-hyperaccumulator species than any country in the world (Reeves 1992). Recent work from Cuba (Reeves *et al.* 1996), however, has uncovered an even larger number of Ni hyperaccumulators from that New World tropical island. It seems likely that more Ni hyperaccumulators from the tropics will be discovered as research progresses (Reeves 1992, Reeves *et al.* 1996).

Although unusually high levels of Ni in some plants have been known to science for almost 50 years (Brooks 1987), the survival value of hyperaccumulation has been the subject of much speculation but little research (Boyd & Martens 1992). We recently have shown that Ni may function as

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a chemical defense for Ni-hyperaccumulating plants. We have documented the acute toxicity of Ni in hyperaccumulator plants to insect herbivores (Boyd & Martens 1994, Martens & Boyd 1994) and a bacterial pathogen (Boyd *et al.* 1994), and the inhibition of a fungal pathogen (Boyd *et al.* 1994). Besides plant-herbivore and plant-pathogen interactions, Boyd and Martens (1998) suggest that other ecological processes may be affected by Ni hyperaccumulation. Plant mutualisms such as pollination, seed dispersal, and mycorrhizae, may function differently for Ni-hyperaccumulating plants. Plant to plant interactions also may be affected via elemental allelopathy (Boyd & Martens 1998). This involves the postulated enrichment in Ni of surrounding surface soil by decomposition of shed hyperaccumulator biomass, possibly resulting in the exclusion of less Ni-tolerant plant species from these Ni-enriched areas (Wilson & Agnew 1992). Finally, the soil microfauna/microflora may be affected by high-metal biomass, with possible effects on nutrient cycling (Boyd & Martens 1998). Knowledge of the degree of variation that exists in the Ni content of Ni hyperaccumulators will become important fundamental information as research on these topics proceeds.

Some information already exists regarding variability in the Ni contents of hyperaccumulator species. This information usually consists of ranges of Ni levels in samples used to identify whether or not a particular taxon is a Ni hyperaccumulator (*e.g.*, Baker *et al.* 1985, Reeves 1992). Fewer studies have documented variation within populations. Recent work on Ni hyperaccumulators in the genus *Streptanthus* (Kruckeberg & Reeves 1995) and on Zn contents of *Thlaspi caerulescens* J. *et* C. Presl (Baker *et al.* 1994) have shown considerable variation in metal levels. Variability on the scale of individual plants within a population is relatively unexplored.

The objective of this study was to document the variation in Ni content of a Ni hyperaccumulator. We examined the effects of plant size and leaf age on Ni content, and documented the degree of variability in Ni content within individual plants. We also noted epiphylls colonizing the leaves of the species selected for study. To our knowledge, epiphylls have not been reported previously for any Ni hyperaccumulator, and we hypothesized that they might absorb metals from their host plant and thus have high metal contents. We therefore investigated the influence of leaf age on epiphyll cover and documented epiphyll elemental composition.

STUDY SITE AND SPECIES

The field site for this research, conducted in late August 1995, is in the Parc de la Riviere Bleue in New Caledonia. The park, found near the southern end of the island, contains protected areas of humid tropical forest that have been studied by Jaffré and Veillon (1991). The exact location for our study was a stand of humid forest at the Kauri Geant, where an exceptionally large *Agathis lanceolata* Lindl. tree is located. This site was adjacent to one of the study plots used by Jaffré and Veillon (1991). Several species of Ni hyperaccumulators co-occurred in two layers of the vegetation (Jaffré & Veillon 1991). The shrub layer contained *Psychotria douarrei* (Beauvis.) Däniker, *Hybanthus austrocaledonicus* (Vieill.) Schinz & Guillamin *ex* Melchior, and *Casearia silvana* Schltr. The tree layer contained the Ni hyperaccumulators *Homalium guillaumii* (Vieill.) Briq. and *Geissois hersuta* Brongn. A third tree species present was the remarkable Ni hyperaccumulator *Sebertia acuminata* Pierre *ex* Baillon, also known as "Seve bleue" for its blue latex containing 26 percent Ni (dry wt; Jaffré *et al.* 1976).

Psychotria douarrei is notable among Ni hyperaccumulators because extremely high levels of foliar Ni have been recorded for this species. Jaffré and Schmid (1974) reported Ni contents of up to 4.7 percent (47,000 ppm) Ni (dry wt.) in leaves of this shrub, setting a record that only recently was exceeded by a newly discovered Ni hyperaccumulator from Cuba (Reeves *et al.* 1996). Indeed, Jaffré and Schmid (1974) found abundant Ni in all *P. douarrei* tissues examined, ranging from as little as 2000 ppm in a sample from the wood of secondary branches to 92,500 ppm in a sample from the bark of the roots (Jaffré & Schmid 1974).

METHODS

PLANT SIZE.—Individuals within a relatively dense portion of the population that contained a wide range of individual plant sizes were utilized for this study. The 22 plants selected were apparently vigorous, had not been damaged by recent treefalls, and were not growing under the canopy of a Ni-hyperaccumulating tree species. Stem diameter was measured to the nearest millimeter *ca* 8 cm above the soil surface. Diameter was measured at this height because *P. douarrei* varies in architecture and may have several main stems; however, in all shrubs we examined, multiple main stems diverged at heights > 10 cm. Thus, measuring stem diameter

below that level allowed us to represent total plant size with a single number. At least three undamaged leaves of medium age (described below) were collected from each plant, taking leaves from more than one branch if they were available. Leaves covered with large amounts of epiphylls were avoided when possible. Leaves were placed in paper sacks and dried at ca 60°C for several days. They then were ground and a sample was used for elemental analysis (procedure described below).

A soil sample was collected from as close to the base of each sampled shrub as possible. Litter was removed to expose mineral soil, and a sample of the upper 8 cm of soil was removed. Soil samples were dried, ground, and a subsample used to quantify extractable Ni. Soil samples were extracted using the DTPA extractant of Lindsay and Norvell (1978) adjusted to pH 7.0. The DTPA extract was analyzed for Ni using an atomic absorption spectrophotometer (Instrumentation Laboratory, IL 251). Data were analyzed by Spearman rank correlations to explore the relationship between plant size and leaf Ni, leaf Ni and soil Ni, and plant size and soil Ni.

LEAF AGE.—The influence of leaf age on elemental composition was determined by collecting leaves of varying ages for analysis. Medium- to large-sized shrubs (1 to 2-m tall) in the study area were selected for sampling. We observed that branches varied in the number of nodes with attached leaves, ranging from two to at least seven pairs of leaves. Leaves were classified into the following age categories: “young leaves” were those that ranged from 25 to 100 percent of fully expanded size but were yellow-green in color and relatively soft and flexible; “old leaves” were defined as those located farthest from the young leaves on a branch; and “medium-aged leaves” were fully expanded leaves of dark green color that were intermediate in position between young and old leaves on a given branch.

We collected at least four leaves of each age category from each of 12 shrubs. The leaves were placed in paper sacks and dried for several days at 60°C. Leaf samples were ground and analyzed for elemental composition as described below. Element data were analyzed by a randomized complete block one-way Analysis of Variance (ANOVA), where “shrub” was used as the blocking factor. In this way, variation due to differences among shrubs could be separated from that due to leaf age. A separate ANOVA was performed for each element quantified, and Fisher’s protected least significant

difference (PLSD) test was used for post hoc means separations (Abacus Concepts 1992).

EPIPHYLLS.—In the field, we noticed that leaves of *P. douarrei* were colonized by epiphylls. Epiphylls almost exclusively inhabited the upper blade surface, and their abundance seemed to increase on older leaves. To document this initial observation, we examined each leaf collected for the leaf age study and visually estimated the percentage of the upper surface that was covered by epiphylls. Cover was estimated to the nearest 5 percent, except when cover was low (<5%), in which cases we estimated cover to the nearest whole percent. These data were averaged to obtain the mean percent leaf area occupied by epiphylls for leaves in each age class of each shrub sampled. The mean values were transformed by the arc-sine square root transformation so they would better meet the assumptions underlying ANOVA (Zar 1996). The effect of leaf age on epiphyll cover was analyzed by a randomized complete block ANOVA, with “shrub” as the blocking factor to statistically remove variation among individual shrubs. Post hoc means separations used Fisher’s PLSD test.

A single composite sample of the epiphylls found on *P. douarrei* leaves was separated from the leaves for elemental analysis. This sample was composed of the predominant epiphyll encountered, a leafy liverwort. Epiphylls were removed from heavily colonized leaves that had been collected for the leaf age study. Epiphylls were removed after the leaves had been dried, as we found it relatively easy to flake the dried epiphylls from the leaf surfaces. Analyses of elemental content were performed as described below.

VARIATION WITHIN INDIVIDUALS.—We collected leaves from several branches on the same individual shrubs to determine the extent to which Ni content varied at this level. Fourteen shrubs that had not been used in previously described studies were selected. All selected shrubs were medium or large in size. Leaves of medium age (defined above) were harvested from between two and five branches from each shrub, avoiding branches bearing leaves that were heavily covered with epiphylls or damaged. The number of branches used for an individual shrub varied due to variation in both shrub size and the presence of branches with suitable leaves, but samples from most shrubs (11) were collected from three branches. Leaves from each sampled branch were placed into separate paper sacks and dried for several days at 60°C. Analysis of Ni con-

TABLE 1. Effect of leaf age on elemental content and epiphyll cover of leaves of *Psychotria douarrei*. Data are means with SE in parentheses. N = 12 for elements; N = 14 for epiphyll cover. Differing superscripts denote means for a parameter that varied significantly due to leaf age; * indicates parameters for which the RCB ANOVA indicated a significant block effect ($P < 0.05$), denoting significant variation among shrubs.

Parameter	Leaf age category		
	Young	Middle	Old
Ni (%)*	1.54 ^a (1.8)	2.17 ^a (2.1)	3.15 ^b (4.7)
Ca (%)*	0.89 ^a (0.12)	1.10 ^a (0.076)	1.20 ^b (0.095)
Fe (ppm)	157.92 ^a (25)	178.18 ^b (15.0)	261.67 ^b (32.0)
Cr (ppm)	12.25 ^a (4.1)	17.25 ^a (2.4)	28.58 ^b (3.8)
K (%)	1.74 ^a (0.29)	0.66 ^b (0.08)	0.44 ^b (0.07)
P (ppm)	1367.17 ^a (271.0)	736.17 ^b (66.2)	634.08 ^b (26.7)
Cu (ppm)	20.92 ^a (4.2)	9.17 ^b (0.051)	7.67 ^b (0.36)
Zn (ppm)	96.58 (17.0)	86.00 (8.7)	91.67 (8.9)
Pb (ppm)	57.17 (11.0)	55.17 (5.4)	53.08 (4.9)
Co (ppm)	15.33 (2.0)	17.58 (1.4)	20.33 (1.7)
Mn (ppm)	71.33 (14.0)	87.00 (18.0)	55.75 (5.9)
Mg (%)	5.70 (0.89)	6.22 (0.37)	7.04 (0.9)
Epiphyll cover (%)*	0.11 ^a (0.73)	20.52 ^b (5.1)	62.16 ^c (6.0)

tent for each leaf sample was performed as described below. Nickel concentrations were analyzed by one-way ANOVA to determine if shrubs varied significantly in leaf Ni content.

PLANT ANALYSES.—Dried samples were ground and elemental analyses were performed for 12 elements. Plant samples were dry-ashed at 485°C, further oxidized with boiling 1 N/M HNO₃, dissolved in 1 N/M HCl, and analyzed for Ca, K, Mg, P, Cu, Fe, Mn, Cr, Pb, Co, and Zn using an inductively coupled argon plasma spectrometer (Jarrell Ash, ICAP 9000). Nickel was determined by analyzing the above extract with an atomic absorption spectrophotometer (Instrumentation Laboratory, IL 251).

RESULTS

PLANT SIZE.—Plant size did not influence leaf Ni content. Basal stem diameters of sampled shrubs ranged fifteen-fold, from 0.3 to 4.5 cm. Leaf Ni content varied much less (2.8-fold), with values ranging from 7700 to 21,600 ppm Ni (dry wt.). Spearman rank correlations of leaf Ni content and stem diameter revealed no significant relationship ($r_s = -0.082$, $N = 19$, $P = 0.729$). Soil extractable Ni ranged from 17.9 to 73.3 ppm, but there was no significant relationship between leaf Ni content and extractable soil Ni ($r_s = 0.242$, $N = 19$, $P = 0.305$). There also was no significant relationship between plant size and soil Ni level ($r_s = 0.131$, $N = 22$, $P = 0.548$).

LEAF AGE.—Leaf elemental composition varied sig-

nificantly with age for most of the elements quantified (Table 1). Four of the elements showed a pattern in which concentration increased with leaf age. These elements (Table 1) were: Ni, for which concentration increased two-fold between young and old leaves; Ca, which had a 1.4-fold increase in old compared to young leaves; Fe, with a 1.7-fold increase; and Cr, with the greatest increase (2.3-fold). Three elements showed a pattern in which concentration declined with increasing leaf age (Table 1). These elements were: K, for which concentration declined 4-fold as leaves aged; P, demonstrating a 2.2-fold decline; and Cu, with a 2.7-fold decrease. The remaining elements (Zn, Pb, Co, Mn, Mg; Table 1) did not vary significantly with leaf age. The ANOVA also indicated significant differences among shrubs (the blocking factor) for two elements: Ni and Ca (Table 1).

EPIPHYLLS.—Most of the epiphyll cover on leaves of *P. douarrei* was composed of leafy liverworts, with the only other epiphyll noted being a crustose lichen. Epiphyll cover increased dramatically with leaf age. Young leaves had very low epiphyll cover (<1%), but medium-aged leaves showed a 181-fold increase and old leaves a 550-fold increase (Table 1). ANOVA also indicated significant differences among shrubs (the blocking factor) for epiphyll cover (Table 1).

The sample of epiphylls removed from *P. douarrei* leaves for elemental analysis was exclusively composed of liverworts. Elemental levels, presented in Table 2, showed high levels of Ca, Mg, and K. The Ni content also was high; at 400 ppm,

TABLE 2. Elemental analysis of epiphylls (leafy liverworts) removed from leaves of *Psychotria douarrei*.

Element	Concentration (ppm dry wt.)
Ca	6900.0
Mg	6400.0
K	4600.0
P	500.0
Ni	400.0
Fe	130.0
Zn	44.0
Cr	34.0
Mn	32.0
Pb	24.0
Co	9.8
Cu	6.1

it was the fifth most abundant element present in the ash.

VARIATION WITHIN INDIVIDUALS.—Variation of leaf Ni content within single shrubs was also great. Mean values for the 14 shrubs ranged from 14,900 to 27,700 ppm Ni (Fig. 1). ANOVA revealed that shrubs did not vary significantly in Ni content ($F = 1.46$, $df = 13, 30$, $P = 0.192$). Inspecting the sizes of the confidence interval bars in Figure 1 indicates that leaf Ni content of some shrubs varied more than others. Spearman rank correlation showed no significant correlation between mean Ni levels and standard deviations ($r_s = 0.442$, $N = 14$, $P = 0.111$), indicating that variation of Ni content within a shrub was not correlated with its overall Ni hyperaccumulation level.

DISCUSSION

Our data showed considerable variation of Ni levels in *P. douarrei*, but only a portion of this variation was attributable to the factors we studied. Baker *et al.* (1985) studied the Ni hyperaccumulator status of the genus *Psychotria* in the Pacific Basin, including *P. douarrei*. They also reported large amounts of variation in their data, with a mean Ni content of 22,400 ppm foliar Ni and a standard error of 1110 ppm. Our data for leaves of *P. douarrei* (Table 1) fell within their range, but with greater levels of variation. Reeves (1992) pointed out that analytical technique, as well as possible soil contamination, can contribute to the variation in Ni levels reported in the literature, suggesting that high Fe (>500 ppm) and Cr (>50 ppm) values may be used as indicators of possible soil contamination in plant samples. Only two of our samples had higher val-

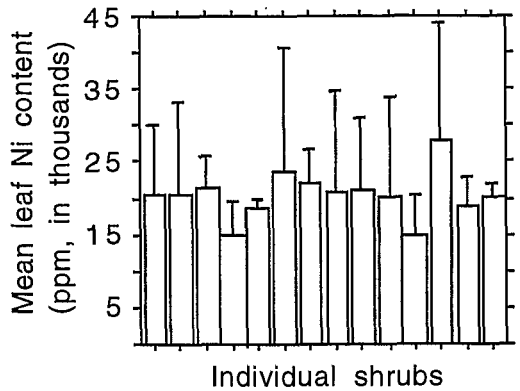


FIGURE 1. Mean Ni content and variability (represented by 95% confidence bars) for each *Psychotria douarrei* shrub tested. Shrub means are listed in the order of their field-assigned number from left to right on the X-axis.

ues for these elements. Jaffré (1980) noted that the soils in our study area have high Fe (40–55%) and Cr (2–5%) contents, so a small amount of contamination could have affected the Fe and Cr values of our samples. These soils, however, are also rather low in Ni (0.1–0.35%; Jaffré 1980); thus the Ni values we measured probably are relatively unaffected by possible soil contamination.

Very few other studies have focused on variability of Ni content among individual plants in a single population of hyperaccumulators. A recent example is that of Nicks and Chambers (1995), who studied intrapopulation variation in Ni content of the California annual Ni hyperaccumulator, *Streptanthus polygaloides*. They found that individuals varied from 3300 ppm to 7800 ppm Ni, a level of variability slightly larger than that documented in our study of *P. douarrei* (mean foliar Ni/shrub = 14,900–27,700 ppm; Fig. 1).

Leaf Ni levels of *P. douarrei* were not significantly affected by plant size (and thus, plant age), and did not correlate highly with soil Ni levels. This latter result was not surprising, as Ni hyperaccumulators can sequester high levels of Ni from soils with widely varying Ni contents (Morrison *et al.* 1980). Little prior work regarding the relationship between plant size (or age) and Ni levels has been reported. One recent study, however, using *Abyssum pintodasilvae* T. R. Dudley, a temperate zone herbaceous perennial, concluded that above-ground plant Ni content increased with age (de Varennes *et al.* 1996). A second study involving *Streptanthus polygaloides* showed that mean plant Ni

concentration decreased 50 percent when plants bolted to flower (Nicks & Chambers 1995).

We also discovered no correspondence between plant size and soil Ni levels. This result has some bearing on one of the suggested functions for Ni hyperaccumulation in plants: the "elemental allelopathy" hypothesis (Boyd & Martens 1998). This hypothesis, suggested by Baker and Brooks (1989), Gabrielli *et al.* (1991), and Wilson and Agnew (1992), speculates that Ni hyperaccumulators enrich the surface soil beneath their canopies with Ni released during decomposition of fallen leaves. This elevated soil Ni level then prevents less Ni-tolerant plants from growing close to the hyperaccumulator plant, providing the hyperaccumulator with lessened competition. Our research failed to find a positive correlation between soil Ni content and *P. douarrei* shrub size, implying that Ni enrichment of surface soils was not occurring in this case. Studies of other hyperaccumulating species are needed to determine if our result is representative of metal hyperaccumulators in general.

Elements in plant leaves vary in their mobility and hence in their concentrations in leaves of different ages. According to Hill (1980), phloem-immobile elements (*e.g.*, Ca) tend to accumulate steadily in a leaf through time, whereas phloem-mobile elements typically reach a peak concentration and then are withdrawn during leaf senescence. Still other elements show a variable pattern of accumulation; Hill (1980) referred to these as having variable phloem mobility. Our data for *P. douarrei* showed that Ni, Ca, Fe, and Cr contents increased with leaf age (Table 1). We therefore suggest that they are all phloem-immobile elements in *P. douarrei*. Early work with Ni accumulation in various plants showed that accumulation patterns varied with leaf age, depending on the species used (Mishra & Kar 1974), but to our knowledge no metal hyperaccumulator species has been examined prior to our work. Recent research using the Ni hyperaccumulator *Abyssum lesbiacum* Candargy has documented the importance of histidine as a complexing agent for transport of Ni through the xylem from the root to the shoot (Kramer *et al.* 1996). Our results suggest that once Ni is incorporated into shoot tissues of hyperaccumulators, it becomes relatively immobile.

The defensive function of hyperaccumulated metals has been demonstrated (Boyd & Martens 1994, Boyd *et al.* 1994, Martens & Boyd 1994, Pollard & Baker 1997), leading to the question of how metal defenses can be characterized according to recent classifications of plant defenses. The tem-

poral pattern of Ni accumulation in *P. douarrei* leaves suggests that Ni is acting as an immobile defense (*sensu* Coley *et al.* 1985), building up over time as a leaf ages. In this sense, Ni is defensively similar to plant structural defenses or secondary defenses such as tannins (Howe & Westley 1988). Recent studies of metal hyperaccumulators (*e.g.*, Vazquez *et al.* 1994, Mesjasz-Przybyłowicz *et al.* 1996) indicate that Ni is concentrated in epidermis tissue and cell walls. This positioning of Ni away from the protoplasts of leaf cells might explain the immobility of Ni in *P. douarrei* leaves. Once Ni is deposited into the cell wall, the metal may no longer be accessible to the cell for remobilization into the phloem.

Our results also imply that young leaves are relatively less well-defended by Ni, but they certainly are not undefended. Young leaves contained 1.5 percent Ni (dry wt.; Table 1), a fairly high amount for plant tissue and well above the 1000 ppm threshold used to define Ni hyperaccumulation (Baker & Brooks 1989). It has been predicted by Martens and Boyd (1994) and Boyd (1998) that some specialist herbivores have evolved tolerance to Ni-based defenses, and that some may even be able to sequester Ni for defense against their own predators. The high levels of Ni present in leaves of *P. douarrei*, both young and old, suggest that herbivores that naturally feed on this species might be good candidates for investigations of Ni sequestration. Furthermore, Ni is probably not the only defensive material present in *P. douarrei* leaves. Members of the genus *Psychotria* are known to produce secondary chemicals (*e.g.*, tannins [Sagers & Coley 1995] and alkaloids [Solis *et al.* 1997]) and these may defend relatively young tissues. Practically nothing is known of interactions between elemental defenses and secondary chemicals in protecting metal hyperaccumulating plants (Boyd 1998). The interplay of elemental and secondary chemical defenses is in great need of experimental investigation.

The relatively high Ni value of the epiphylls collected in this study suggests that hyperaccumulator species may affect the epiphytes growing on them through chemical means. Proctor *et al.* (1989) concluded that Ni was excreted into senescing leaves of plants growing on ultrabasic rocks in Malaysia. They did not report epiphyll data, and although trunk epiphytes were quantified in general, a specific value was not reported for the one tree species included in their study for which foliar Ni values approached hyperaccumulation levels. This species, *Shorea tenuiramulosa*, contained 460–

1000 ppm Ni, much less than the Ni levels shown here for *Psychotria douarrei*. That Ni leached from leaves was the source of the high Ni level of our epiphyll sample is suggested by the location of Ni within hyperaccumulators. Vazquez *et al.* (1994) and Mesjasz-Przybyłowicz *et al.* (1996) documented high metal levels in epidermal tissues and cell walls. This placement of Ni at leaf and cellular surfaces may increase its likelihood of becoming available to epiphylls. Further study of epiphyte–Ni hyperaccumulator relationships will be of great interest, given the important role that epiphytes can play in nutrient cycles of tropical ecosystems (Coxson & Nadkarni 1995).

Our data show that the metal content of a Ni-hyperaccumulating species can vary greatly and at several ecological scales. This variability demonstrates the difficulty of summarizing metal content for a hyperaccumulating species based on only a small number of plants or using leaves of undoc-

umented age. Much of the literature regarding Ni hyperaccumulators (*e.g.*, Reeves *et al.* 1996) has been directed toward discovery of new hyperaccumulating species. This broad objective has not allowed finer-scale questions to be addressed. As research begins to focus on smaller-scale patterns, consistent patterns of variability may help define the role of elemental defenses in the ecological interactions between metal hyperaccumulators and other organisms.

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