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REGULAR ARTICLE

Relationships between Ni-hyperaccumulation and mycorrhizal status of different endemic plant species from New Caledonian ultramafic soils

H. Amir · N. Perrier · F. Rigault · T. Jaffré

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Abstract For a long time, Ni-hyperaccumulating plants have been considered to be non-mycorrhizal species. However, two recent publications have reported arbuscular mycorrhizal fungi (AMF) colonisation in Ni-hyperaccumulators. In this work, 9 endemic Ni-accumulators of unknown mycorrhizal status, from New Caledonia, were studied. All were mycorrhizal, but some were poorly colonised by the symbiots. Only AMF were observed. We analysed the relationships between Ni-hyperaccumulation ability and AMF colonisation of the plants. The roots of the three strongest hyperaccumulators, namely *Sebertia acuminata*, *Psychotria douarrei* and *Phyllanthus favieri*, were characterised by a lower mycorrhizal colonisation than the others. Mycorrhizal density varied with the level of Ni concentration in soil and plant. Root-colonisation by AMF was negatively correlated with leaf Ni content and with extractable-Ni concentration in soil. The roots of Ni-hyperaccumulators and the soils collected under these plants clearly inhibited germination of AMF spores. Hence, it appears that mycorrhizal colonisation is inhibited above a certain threshold of Ni concentration in soil and plant and becomes either absent or very low. However AMF isolated from the roots of strong Ni-hyperaccumulators have developed a very high level of Ni-tolerance and are then able to colonize at least parts of their roots.

Keywords Arbuscular mycorrhizae · AMF spore germination · Nickel · Ni-hyperaccumulating plants · Ultramafic soils

H. Amir (✉) · N. Perrier
Unité ''Mécanismes Adaptatifs et Biomolécules des plantes endémiques de Mélanésie'',
Université de la Nouvelle-Calédonie,
BP R4, Noumea 98851, New Caledonia
e-mail: amir@univ-nc.nc

N. Perrier
UMR 161, Institut de Recherche pour le Développement, BP A5,
Noumea 98848, New Caledonia

F. Rigault · T. Jaffré
Laboratoire de Botanique et d'Ecologie Appliquées,
Institut de Recherche pour le Développement,
Centre de Nouméa, Noumea, New Caledonia

Introduction

In serpentine environments, plants have developed different strategies to avoid Ni-toxicity and can be classified into three different groups depending on their type of strategy: excluders, indicators, and hyperaccumulators (Baker 1981; Tomsett and Thurman 1988; Verkleij 1990). Ni-hyperaccumulating plants are characterized by their ability to accumulate more than 1000 μg g^{-1} (dry weight) of Ni in their leaves.

For a long time, Ni-hyperaccumulating plants have been considered as non-mycorrhizal species (Leyval

et al. 1997; Pawlowska et al. 2000; Coles et al. 2001). Indeed, in the past, studies of Ni-hyperaccumulators have concerned mostly non-tropical plants, especially Brassicaceae which are known as non-mycorrhizal (Smith and Read 1997; Wang and Qiu 2006). Recently, Turnau and Mesjasz-Przybylowicz (2003) have shown *Berkheya coddii* and three other Ni-hyperaccumulating Asteraceae from South Africa to be consistently colonized by arbuscular mycorrhizal fungi (AMF).

In New Caledonia, one of the floristically richest serpentine environments in the world, over 50 species of Ni-hyperaccumultating plants have been reported (Jaffré 1980). Their mycorrhizal status remains almost unknown: the only reported study concerned AMF colonisation of *Phyllanthus favieri* (Euphorbiaceae) a strong Ni-hyperaccumulator (Perrier et al. 2006). Some of these species are relatively abundant (Jaffré and Veillon 1991) and could be used in restoration programs.

New Caledonian ultramafic soils are characterized by P, N, K and Ca deficiencies (Brooks 1987). Despite their high metal contents, AMF are abundant in these soils and seem to play an important role in the adaptation of plants to this environment (Amir et al. 1997; Perrier et al. 2006). In other respects, it becomes more and more clear that, in a metal rich substrate, AMF can reduce the concentration of Ni in shoots and thereby increase metal-tolerance of plants (Heggo and Angle 1990; Hilderbrandt et al. 1999; Joner and Leyval 2001; Vivas et al. 2005).

When one consider the aforementioned elements, the relationships existing between Ni-hyperaccumulation by certain plants and their mycorrhizal status in ultramafic soils can be questioned. It seems clear that analysis of these relationships could help us to understand plant adaptation to toxic metalliferous soils. Several fundamental questions on these points include: are New Caledonian Ni-hyperaccumulators generally mycorrhizal? Is mycorrhizal colonisation of strong hyperaccumulators as consistent as that of weak accumulators? Does the mycorrhizal status of each Ni-hyperaccumulating species vary depending on Ni concentration in leaves or soil? If there is an inhibition of mycorrhizal colonisation, how can it be explained? If AMF are present in Ni-hyperaccumulator roots, are they more tolerant to Ni than other AMF isolates from ultramafic soils?

To address these questions, nine Ni-accumulators from New Caledonia were studied and the relationships between Ni-hyperaccumulation and different mycorrhizal parameters investigated.

Materials and methods

Study sites and studied plant species

Nine plant species with different levels of Ni-accumulation were chosen. They were selected because they are easy to collect or because they are strong hyperaccumulators. They were sampled in 3 different sites, located in Rivière Bleue, Mont Koghis and the Koniambo Massif. The average annual temperature of these sites ranges between 18°C and 21°C, with a maximum of 30°C and a minimum of 10°C.

The Rivière Bleue site is a tropical humid forest in the south of the main island (60 km east of Noumea), with an altitude of 160 m and an annual precipitation of 3,180 mm. Samples were collected at points located within 3–4 km of the «grand Kaori». The main soil studied is an alluvial lateritic soil (oxisol), relatively deep, lying on peridotite.

The Koghis site is a tropical humid forest located 20 km north of Noumea. The altitude of the site is 400 m, and average annual precipitation 1,750 mm. The sampling points are about 300 m from the restaurant «Auberge du mont Koghis». The soil is a brown hypermagnesian clayey soil formed on serpentinised peridotite.

The third site, in the Koniambo Massif (in the north of the main island), is at an altitude of 710 m and has annual precipitation of 1,800 mm. The sampling site is located in a tropical humid forest dominated by *Nothofagus balanse*. The soil is a colluvial lateritic soil (oxisol) lying on unweathered peridotite.

Some chemical characteristics of soil samples of the three sites are shown in Table 1. The plant species sampled in each of the three sites and their ability to accumulate Ni are presented in Table 2.

AMF isolates used

Five *Glomus* sp. isolates, all from New Caledonian soils, were used for studies on spore germination in relation to Ni-hyperaccumulation. FSCtAc was

Table 1 Some chemical characteristics of representative samples of the studied soils

Characteristics	Rivière Bleue: alluvial lateritic soil (oxisol)	Mont Koghis: brown hypermagnesian soil	Koniambo massif: colluvial lateritic soil (oxisol)
Org. C (%)	4.99	3.17	10.55
Total N(%)	0.38	0.26	0.43
C /N	12.8	12.2	24.5
pH (H_2O)	6.0	6.7	5.7
P ($\mu g\ g^{-1}$)	509	487	393
Ca (%)	0.05	0.07	0.04
Mg (%)	0.41	5.53	0.32
Fe (%)	33.45	34.11	50.12
Mn (%)	0.67	0.85	0.22
Ni (%)	0.73	0.939	0.22
Co (%)	0.07	0.119	0.02
Mg DTPA[a]	/	2527	818
Mn DTPA	613	653	1213
Fe DTPA	189	310	143
Ni DTPA	354	108	246
Co DTPA	116	76	120

[a] Extractable with dimethylene triaminopentaacetique acid or DTPA ($\mu g\ g^{-1}$)

Table 2 List of the studied plant species and their ability to accumulate Ni

	Species	Location of the sampling sites	Ni in leaves[a] ($\mu g\ g^{-1}$)
Weak accumulators	*Pancheria alateroides* (Cunoniaceae)	Rivière Bleue	349 ± 92
	Cloezia artensis (Myrtaceae)	Rivière Bleue	528 ± 254
Moderate hyper-accumulators	*Geissois pruinosa* (Cunoniaceae)	Rivière Bleue and Mont Koghi	3,900 ± 2,080
	Geissois hirsuta (Cunoniaceae)	Rivière Bleue and Mont Koghi	5,290 ± 2,120
	Homalium kanaliense (Flacourtiaceae)	Rivière Bleue	14,720 ± 3,430
Strong hyper-accumulators	*Hybanthus austro-caledonicus* (Violaceae)	Rivière Bleue	20,540 ± 6,760
	Phyllanthus favieri (Euphorbiaceae)	Mont Koniambo	21,750 ± 8,230
	Psychotria douarrei (Rubiaceae)	Rivière Bleue	23,220 ± 9,210
	Sebertia acuminata (Sapotaceae)	Rivière Bleue	25,280 ± 7,120

[a] Mean of 8–10 values for each plant species

isolated from a dry forest non-ultramafic soil collected at Pointe Maa (just north of Noumea). T2R3 and SFONL were isolated from lateritic ultramafic soils collected respectively in Koniambo massif and Ouenarou (near Rivière Bleue). «Psychot1» and «Sebert1» were isolated from roots of *Psychotria douarrei* and *Sebertia acuminata* at Rivière Bleue. The two latter isolates were used to check if their Ni-tolerance is higher than three isolates collected under non-hyper-accumulating plants. FSCtAc, (non-ultramafic isolate) was used in all spore germination experiments because of its relatively low Ni-tolerance.

General approach

During the first stage, 8–10 individuals of each of the 9 plant species were sampled and characterised for the following parameters: total Ni content in leaves, Ni-DTPA in soil under plant, type of mycorrhizae, mycorrhizal frequency and mycorrhizal density.

During the second stage, one moderate Ni-hyperaccumulator (*Geissois pruinosa*) and 3 strong hyperaccumulators (*Phyllanthus favieri, Psychotria douarrei* and *Sebertia acuminata*) were studied in more detail. In this case, 18–40 individuals (depending on the difficulty to find and collect the roots) were sampled for each species. The same parameters as above were measured; but we also documented the arbuscule frequency and the number of viable spores in the soil under each plant. A statistical analysis of the correlations between these different parameters was then carried out.

During the third stage, AMF spore germination in contact with sterile sand containing different concentrations of Ni, or in contact with soils or root crush of Ni-hyperaccumulating plants was assessed to check if there was inhibition of spore germination in these conditions.

Root sampling and assessment of mycorrhizal colonisation

Depending on the objective (see above) 8–40 haphazardly chosen individuals of each plant species were selected per site. The area explored for each site and each species was about 1 ha and the distance between the individuals was 10–30 m. The fine roots were sampled and placed in plastic bags. To avoid confusion between the roots of different species, the plants were dug up if they were small and the roots were followed from the main root to the finer roots, which were sampled; then the plant was replanted. For bigger plants, the roots were traced carefully, from the main root to the finer roots. The roots were kept refrigerated until treatment. In the laboratory the roots were prepared following Koske and Gemma (1989) procedure. After being thoroughly rinsed with water, they were immersed in a 10% KOH bath at 90°C for 90 min, a few drops of H_2O_2 were added after 1 h to lighten the colour. They were then rinsed again and stained by immersion in a trypan blue bath for 15 min at 90°C. For each sample, 30 haphazardly selected stained segments (approximately 1 cm long) were observed under the microscope. A root was considered arbuscular mycorrhizal when arbuscules, vesicles or hyphal coils were present. Mycorrhizal frequency percentage (F) mycorrhizal density percentage (M) and arbuscule density percentage (A) were determined according to the Trouvelot et al. (1986) method.

Soil and leaf sampling, and nickel analyses

Soils under plants were sampled in each of the 3 sites. The surface under each plant was scraped to eliminate the litter, and the soil was taken from a 15 cm deep top layer. In a few cases, soil was also taken 10 m away from the plant to avoid the influence of Ni-rich litter. After a thorough mix, all samples were sieved through a 2 mm mesh before treatment. For each sampled plant, a few healthy representative leaves were collected on three different branches using an automatic CHNOS analyser (Thermoquest Finnigan). All chemical analyses were performed at the chemistry laboratory of IRD Noumea. The leaves were washed thoroughly with water and then dried under neon light. Total C and N were measured.

The total Ni content in leaves (expressed in $\mu g\ g^{-1}$ of dry leaf) and the diethylene triamine pentacetic acid (DTPA) extractable Ni in soils (expressed in $\mu g\ g^{-1}$ of dry soil) were performed following the Lindsay and Norvell (1978) method, by measurement with an ICP-OES (Inductively Couple Plasma—Optical Emission Spectroscopy).

AMF spore counts

The number of viable AMF spores present in each soil sample was determined after wet-sieving and centrifugation. The spores were extracted from three 50 g aliquots of dry soil. The aliquots were sieved through 1 mm and 38 μm sieves. The fraction collected between the two sieves was centrifuged for 5 min at 1,000 *g*. The pellets were then resuspended in 50% sucrose and centrifuged for 1 min at 1,000 *g*. AMF spores were then recovered from the supernatant, which was poured through a 38 μm sieve, thoroughly rinsed with water and retransfered to filter paper for counting. Only spores filled with intact cytoplasm and which were not black were recorded.

Bioassay of Ni-tolerance of AMF spore germination

Aliquots of 30 g of fine sand autoclaved 1 h at 120°C were placed into 5 cm diameter Petri dishes. A cellulose filter membrane (0.45 μm, 47 mm diameter) was applied on the surface of the sand of each plate. Eighty AMF spores obtained after wet sieving and centrifugation (see above), were placed on the membrane. Distilled water or Ni solution (NiSO4) was then added to the sand until saturation caused the membrane to stick to the sand by water capillarity. Four Ni concentrations were used: 0, 10, 20, 30 μg g^{-1}. Three replicates of each treatment were prepared. The Petri dishes were then sealed with parafilm and incubated 30 days at 25°C. The percentage of germinated spores was determined at 45× magnification. The same technique was used to test the influence of soils on spore germination but the sand was replaced by the tested soil. In another experiment, roots of a Ni-hyperaccumulating plant were crushed roughly with a hand grinder; then 1 g of root crush was spread on the surface of a fine layer of sand just under the cellulose membrane.

Data analysis

Two types of statistical analyses were performed using the computer program Kyplot v2.0 beta 13. To study the relationships between Ni-accumulation and mycorrhizal status, total correlations between Ni-accumulation parameters and mycorrhizal parameters were analysed. ANOVA (Newman-Keuls test) was used to compare values of different treatments, especially for AMF spore germination data.

Results

Mycorrhizal status of the 9 studied plants in relation to Ni in soil and leaves

The nine studied Ni-hyperaccumulating plants were characterised by very different Ni contents in leaves (Fig. 1a). The lower values (for *Pancheria alateroides* and *Cloezia artensis*) were under the minimal level which define Ni-hyperaccumulation, whereas the higher values (*Psychotria douarrei* and *Sebertia acuminata*) exceeded 2.2%. The DTPA-extractable Ni concentrations in soil under plants (Fig. 1b) were lower than 50 μg g^{-1} for the 2 weak accumulators, and varied from 84 μg g^{-1} to 326 μg g^{-1} for the moderate hyperaccumulators. Among the four strong hyperaccumulators, only *Phyllanthus favieri* had a relatively moderate level of DTPA-Ni in soil under plants (132 μg g^{-1}); the values measured for the others were above 750 μg g^{-1}.

A highly significant correlation (r = 0.823) between Ni content in leaves and DTPA-Ni in soil under plant was recorded. The roots of the 9 plant species were all more or less colonised by AMF (Fig. 1c). No other types of fungal symbiots were observed. Mycelium and vesicles were present. Arbuscules and coils were generally present in the same root fragments. Values of root colonisation by AMF were high for the weak and moderate accumulators (>40%), and very low (<12%) for the strongest hyperaccumulators. The negative correlation between M and Ni content in leaves was highly significant (r = 0.886), whereas the correlation between M and DTPA-Ni in soil under plant was not significant but close to the significance level (P = 0.06).

Correlations between mycorrhizal parameters and Ni-hyperaccumulation for *Geissois pruinosa*

The values of Ni content in leaves of *G. pruinosa* were very variable depending on the Ni concentration in soil (Table 3). Indeed, a few soil samples did not contain detectable extractable Ni, whereas others contained more than 600 μg g^{-1} Ni. Root colonisation by AMF was also quite variable: M varied from 8.5% to 95%. Arbuscules were generally present but not frequent. An average number of 57 AMF spores per 100 g of soil under plant was recorded.

The correlation between M an leaf Ni content was negative and highly significant, whereas the correlation of M with the DTPA-Ni in soil under plant was close to the significance level. The correlations obtained with the other mycorrhizal parameters (F, A and spore numbers) were not significant.

Correlations between mycorrhizal parameters and Ni-hyperaccumulation for *Phyllanthus favieri*

The values of leaf Ni content of *P. favieri* varied from 7,800 μg g^{-1} to 42,200 μg g^{-1} and those of DTPA-Ni in soil under plant from 25 μg g^{-1} to

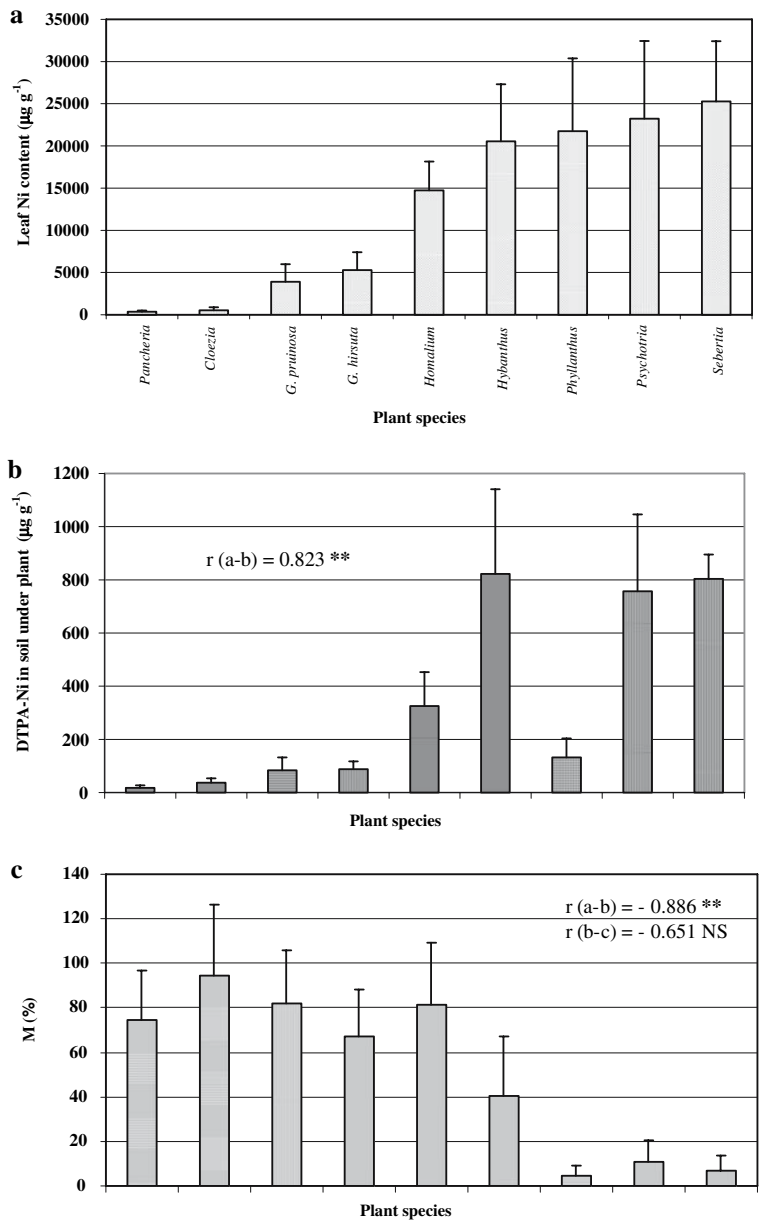

Fig. 1 Relationships between leaf Ni content, DTPA-Ni in soil under plants and mycorrhizal intensity (M) of the 9 studied species (means of 8–10 samples for each value); G = *Geissois*. The coefficient *r* indicates the total linear correlation between the three parameters; *r* (**a**, **b**): correlation between Leaf Ni content and DTPA-Ni; *r* (**a**–**c**): correlation between Leaf Ni content and M; *r* (**b**, **c**): correlation between DTPA-Ni and M; ** = significant at $P < 0.01$; NS = non significant

225 µg g^{-1} (Table 4). Three root samples (13% of the total number of samples) did not show any AMF colonisation, but most of them were highly colonised.

Significant negative correlations were recorded between M and leaf Ni content ($r = -0.580$), M and DTPA-Ni in soil under plant ($r = -0.464$), F and Ni content in leaves ($r = -0.662$).

Correlations between mycorrhizal parameters and Ni-hyperaccumulation for *Psychotria douarrei*

The minimal and maximal values of Ni content in leaves were respectively 5,300 and 35,530 µg g^{-1}, with a mean of 17,800 µg g^{-1} (Table 5). Nine plant samples (20% of the total number of samples) did not show any AMF colonisation in their roots and all had

Table 3 Variation of Ni-hyperaccumulation in *Geissois pruinosa* (25 samples), DTPA extractable Ni in soil under plant and mycorrhizal parameters of the plant

	Leaf Ni content (μg g^{-1})	DTPA-Ni in soil (μg g^{-1})	F (%)	M (%)	A (%)	Number of AMF spores/100 g soil
Maximal value	10,040	604	100	95.0	24.2	244
Minimal value	49	0	83.3	8.5	0	64
Mean	3,290	98	99.3	60.2	4.2	57
Total correlations with						
Leaf Ni content		0.642***	NS	−0.516**	NS	NS
DTPA-Ni in soil			NS	−0.362 NS	NS	NS

Total correlations between the parameters are reported

NS: non significant; ** significant at $P < 0.01$; *** significant at $P < 0.001$

Table 4 Variation of Ni-hyperaccumulation in *Phyllanthus favieri* (21 samples), DTPA extractable Ni in soil under plant and mycorrhizal parameters of the plant

	Leaf Ni content (μg g^{-1})	DTPA-Ni in soil (μg g^{-1})	F (%)	M (%)	A (%)	Number of AMF spores/100 g soil
Maximal value	42,210	225	100	70.9	ND	734
Minimal value	7,810	25	0	0	ND	12
Mean	19,400	115	76.6	41.4	ND	199
Total correlations with						
Leaf Ni content		0.564*	−0.662**	−0.580**		NS
DTPA-Ni in soil			−0.348 NS	−0.464 *		NS

Total correlations between the parameters are reported

NS: non significant; * significant at $P < 0.05$; ** significant at $P < 0.01$; ND: not determined

Table 5 Variation of Ni-hyperaccumulation in *Psychotria douarrei* (43 samples), DTPA extractable Ni in soil under plant and mycorrhizal parameters of the plant

	Leaf Ni content (μg g^{-1})	DTPA-Ni in soil (μg g^{-1})	F (%)	M (%)	A (%)	Number of AMF spores/100 g soil
Maximal value	35,530	1,560	100	70	58.3	110
Minimal value	5,300	25	0	0[a]	0	6
Mean	17,800	469	85.1	30.2	22.8	46
Total correlations with						
Leaf Ni content		0.566***	−0.487***	−0.328*	−0.371*	NS
DTPA-Ni in soil			−0.429**	−0.461**	−0.397**	NS

Total correlations between the parameters are reported

[a] M (%) = 0 for 9 plant samples (20%) all containing more than 20,000 μg g^{-1} Ni in their leaves. NS: non significant; * significant at $P < 0.05$; ** significant at $P < 0.01$; *** significant at $P < 0.001$; ND: not determined

more than 20,000 μg g^{-1} Ni in their leaves. The other plant samples were more or less colonised by AMF and showed arbuscules in the root cortex.

The high number of samples studied for this easy to collect plant allowed a more robust statistical analysis so that more significant correlations were

obtained: all the mycorrhizal parameters were negatively and significantly correlated to leaf Ni content and to DTPA-Ni in soil under plant.

Correlations between mycorrhizal parameters and Ni-hyperaccumulation for *Sebertia acuminata*

Ni content in leaves of *S. acuminata* varied from 10,490 μg g^{-1} to 41,280 μg g^{-1} and DTPA-Ni in soil under plant from 572 μg g^{-1} to 883 μg g^{-1} (Table 6). Nine samples (50% of the total number of samples) did not show any AMF colonisation; among them 7 had more than 25,000 μg g^{-1} Ni in their leaves. The other 9 samples were generally very weakly colonised: only one with M > 12%. Among these 9 last samples 7 had less than 16,000 μg g^{-1} Ni in their leaves.

F was negatively and significantly correlated with leaf Ni content (Table 6). The correlation between M and leaf Ni content was very close to the significance level ($P = 0.055$).

Influence of soils collected under hyperaccumulators on AMF spore germination

Figure 2 shows the effects of soils on AMF spore germination. About 60% of the spores germinated on sand with distilled water. A solution of 10 μg g^{-1} Ni reduced the germination to 26%. A non-ultramafic soil, with 1 μg g^{-1} Ni did not affect on spore germination. The value of germination was 30.5% when spores were in contact with an ultramafic soil having 34 μg g^{-1} DTPA-Ni. Soils collected under *Geissois pruinosa* and *Psychotria douarrei*, characterised by a relatively high level of DTPA-Ni (respectively 110 and 278 μg g^{-1}), induced a strong inhibition (Fig. 2). The inhibition induced by the soils was greater for soil with greater extractable Ni.

Influence of root crushes of Ni-hyperaccumulators on AMF spore germination

Root crushes of *Geissois pruinosa* and *Psychotria douarrei* inhibited AMF spore germination (Fig. 3). The inhibition was more important for *P. douarrei* and was related to the concentration of total Ni in roots; among the 2 root samples of this plant the sample from Rivière Bleue, with 7,733 μg g^{-1} total-Ni, reduced the germination to only 4%.

Concentrations of total and available Ni in roots of Ni-hyperaccumulators

The nickel absorbed by the roots was not totally in inactivated and non-mobile forms after the roots were crushed (Table 7). For *G. pruinosa*, about 25% of the Ni was extractable with KCl. The root crush of *P. douarrei* released 80% of the Ni in mobile form, whereas for *S. acuminata* the entire Ni concentration was extractable with KCl.

Tolerance to Ni of 2 AMF isolates from roots of hyperaccumulators in comparison with other isolates

The effect of three Ni concentrations on AMF spore germination varied according to each isolate tested (Fig. 4). Isolates FSCtAc and T2R3 were less

Table 6 Variation of Ni-hyperaccumulation in *Sebertia acuminata* (18 samples), DTPA extractable Ni in soil under plant and mycorrhizal parameters of the plant

	Leaf Ni content (μg g^{-1})	DTPA-Ni in soil (μg g^{-1})	F (%)	M (%)	A (%)	Number of AMF spores/100 g soil
Maximal value	41,280	883	100	52.0	49.6	124
Minimal value	10,490	572	0	0	0	20
Mean	24,330	714	31.2	6.28[a]	5.82	54
Total correlations with						
Leaf Ni content		0.374 NS	−0.735***	−0.457 NS	−0.386 NS	NS
DTPA-Ni in soil			NS	−0.378 NS	NS	NS

Total correlations between the parameters are reported

[a] M (%) = 0 for 9 samples (50%), only one sample with M > 12%. NS: non significant; *** significant at $P < 0.001$; ND: not determined

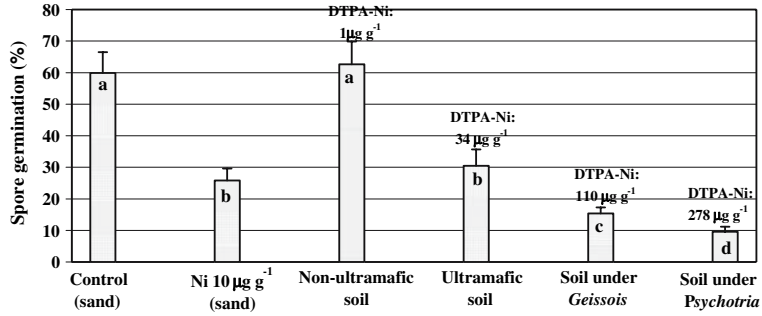

Fig. 2 Influence of soils collected under *Geissois pruinosa* and *Psychotria douarrei* on AMF spore germination (isolate FSCtAc) in comparison with other soils and with a solution of 10 µg g^{-1} Ni. Ni-DTPA concentrations of the soils are shown on the top of the histograms. Different letters indicate significant differences at $P \leq 0.05$ (ANOVA, Newman-Keuls test)

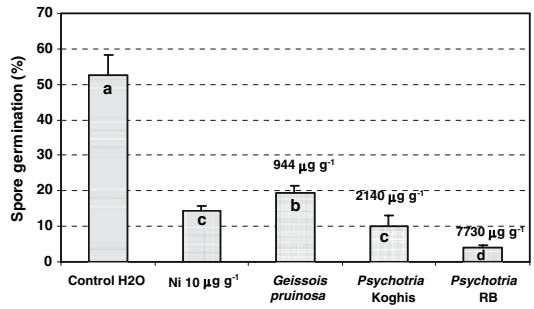

Fig. 3 Influence of 3 samples of root crush (*Psychotria douarrei* from mont Koghis, or Rivière Bleue (RB) and *Geissois pruinosa* from mont Koghis) on AMF spore germination (strain FSCtAc) in comparison with a solution of 10 µg g^{-1} Ni and a control of H2O. Total Ni concentrations of the root crushes are shown at the top of the histograms. Different letters indicate significant differences at $P \leq 0.05$ (ANOVA, Newman-Keuls test)

tolerant: germination percentage was about five times lower in the presence of Ni in comparison with the control without Ni. SFONL was moderately tolerant, whereas Psychot1 and Sebert1 (isolated respectively from *P. douarrei* and *S. acuminata)* were highly tolerant to Ni. Indeed, for these latter two, the germination percentage was not affected by Ni at the highest concentration used.

Table 7 Concentrations of total Ni and available Ni (extractable with KCl) in crushed roots of 3 Ni-hyperaccumulating plants (means of four values with standard errors)

	Total-Ni (µg g^{-1})	KCl–Ni (µg g^{-1})
Geissois pruinosa	1,010 ± 311	252 ± 66
Psychotria douarrei	7,730 ± 2,110	6,170 ± 877
Sebertia acuminata	14,540 ± 3,740	15,680 ± 3,080

Discussion

The ability of Ni-accumulating plants to absorb and store nickel is highly variable (Brooks 1987; Reeves 1992). The nine plant species studied here are representative of this diversity. A highly significant positive correlation between leaf Ni content and DTPA-Ni in soil under plants was highlighted for all the series of the studied samples. Boyd et al. (1999) analysed the relationships between leaf Ni content and DTPA-Ni in soil under *Psychotria douarrei* and did not find a significant correlation, but this could be due to the lower number of samples they studied. It is known that metal hyperaccumulators increase the toxicity of the soil underneath them, through their Ni-rich litter (Schlegel et al. 1991; Boyd and Jaffré 2001), and roles of this plant strategy (for example chemical defense against pathogens and competition with other plants) have been hypothetised (Boyd et al. 1994; Boyd and Jaffré 2001; Jhee et al. 2005).

The nine endemic Ni-accumulators studied were all more or less mycorrhizal, but only AM were observed. Apart from *Phyllanthus favieri*, which was previously reported as mycorrhizal (Perrier et al. 2006), the mycorrhizal status of all these species was documented for the first time. The first report of mycorrhizal colonisation of Ni-hyperaccumulating plants was by Turnau and Mesjasz-Pzybylowicz (2003) who showed AMF colonisation of *Berkheya coddii* and three other Ni-accumulating Asteraceae from South Africa. Another recent study concerned mycorrhizal colonisation of the Zn-hyperaccumulator *Thlaspi praecox* in metal polluted soils (Vogel-Mikus et al. 2005). In the light of these results, it is clear that

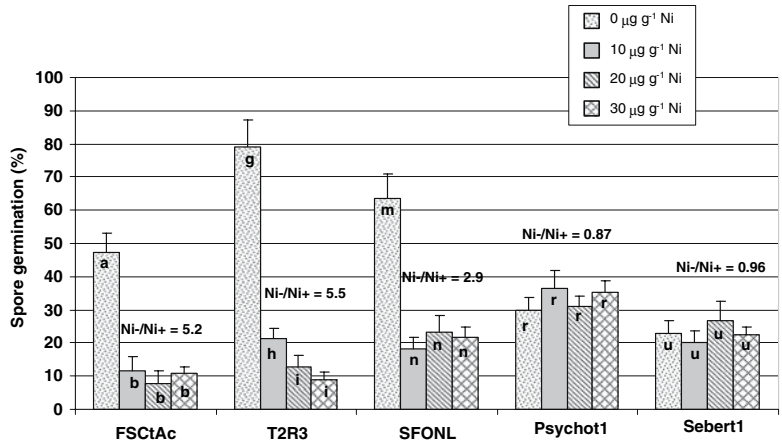

Fig. 4 Influence of different Ni-concentrations on AMF spore germination of two AMF isolates collected under hyperaccumulating plants (Psychot1 and Sebert1), in comparison with three other isolates. Ni−/Ni+ indicates, for each isolate, the ratio between the control without Ni and the average of the three Ni-treatment values, and show the tolerance of the isolates. For each isolate, different letters indicate significant differences at $P \leq 0.05$ (ANOVA, Newman-Keuls test)

metal-hyperaccumulating plants are not non-mycorrhizal, as was generally assumed in the past (Leyval et al. 1997; Pawlowska et al. 2000; Coles et al. 2001). However, our study clearly shows the strongest hyperaccumulating plants are less colonised by AMF than weak or moderate accumulators. Thus, *Sebertia acuminata*, commonly called «blue sap tree» because it contains the highest known level of Ni in its latex (Jaffré et al. 1976), was poorly colonised by AMF: 50% of the studied samples were not colonised, the other samples showed generally less than 12% mycorrhizal density (M). Another strong hyperaccumulator, *Psychotria douarrei*, showed AMF colonisation varied largely according to the levels of Ni concentrations in plant and soil. Therefore, what factors determine the inhibition of AMF colonisation of the strong hyperaccumulators?

The correlation analysis on variables studied in natural conditions requires a relatively high number of samples to reduce the heterogeneity due to the numerous factors, which influence the variables. In this study, negative correlations between nickel concentrations in soil and leaves and mycorrhizal parameters were recorded in all cases, but with various significance levels. The correlations were particularly clear for *P. douarrei* because of the high number of samples studied (43). These results mean that high levels of nickel in leaves and/or in soil are related to low levels of mycorrhizal colonisation.

Correlation does not necessarily indicate a causal relationship, but allows us, as a first step, to propose the following hypothesis: inhibition of root colonisation by mycorrhizal fungi may occur for high levels of Ni-hyperaccumulation and/or high levels of Ni in soil under plants due to Ni-hyperaccumulation. If this hypothesis is correct, soils taken from under plants, or roots of Ni-hyperaccumulators should inhibit germination or growth of AMF. In fact, soils collected under *G. pruinosa* or under *P. douarrei* induced a strong inhibition of AMF spore germination (Fig. 2). An ultramafic soil containing a lower level of DTPA-Ni caused a significantly lower inhibition, whereas a non-ultramafic soil did not affect spore germination. Only 10% of the spores germinated in contact with the soil collected under *P. douarrei* and containing 278 µg g^{-1} DTPA-Ni, which was 6 times lower than the control (sand). Inhibition of AMF spore germination by heavy metals in polluted soils has already been reported by Leyval et al. (1995) who also noticed that mycorrhizal parameters tended to be negatively correlated with total heavy metal concentrations (especially Cd, Zn and Pd) in soil. The concentrations of Ni in New Caledonian ultramafic soils are highly variable. DTPA extractable concentrations generally vary from 1 µg g^{-1} to 400 µg g^{-1} (Brooks 1987; Amir and Pineau 2003), but can reach 1,500 µg g^{-1} in the 15 cm upper soil under strong Ni-hyperaccumulating plants (as shown in Table 5). However, this concentration generally decreases in

the deeper soil (results not shown). We noted that the inhibition induced by the 10 μg g^{-1} Ni solution in sand was similar to that obtained with a soil containing 34 μg g^{-1} DTPA-Ni. Indeed, DTPA-Ni soil does not measure the available and active fraction of Ni, but the releasable fraction, so that only a part of this fraction is active at any time.

It was not possible to test the direct effect of roots on AMF spore germination under field conditions, but crushed roots had significant negative effects on the germination. The roots with a high content of total-Ni induced a greater inhibition: only 4% of the spores in contact with root crush of *P. douarrei* collected from Rivière Bleue germinated. The roots of *S. acuminata* could not be tested in this experiment because of the difficulty to obtain a sufficient quantity of fine roots of this tree (roots are very deep in the soil) but, as shown in Table 7, 80–100% of the root Ni of the two strongest hyperaccumulators is in available form (extractable with KCl). Inside hyperaccumulator organs, Ni is generally complexed with organic acids, amino-acids (histidine) or nicotianamine (Krämer et al. 1996; Sagner et al. 1998; Ouerdane et al. 2006). These latter authors have found all these forms not only in the shoots but also in the roots and xylem of *Thlaspi caerulescens*. Organic acid complexes such as citrate and malate were found in *Psychotria douarrei* (Kersten et al. 1980) and in *Sebertia acuminata* (Lee et al. 1977; Sagner et al. 1998; Perrier et al. 2004). It must be noted that the root crush in the present experiment could also have released Ni stored in the cell vacuoles (Perrier et al. 2004), which is normally inactive in non-crushed roots. However, in natural conditions, the metal stored in the vacuoles is continuously released with the exfoliation of the dead cells, so that Ni concentration in the rhizoplane and in the rhizosphere is higher than in the bulk soil and can prevent the initiation of the root colonisation by AMF. Indeed, Bathia et al. (2004) reported that a high proportion of nickel is stored inside epidermal cells. In addition, these authors outlined that inside tissues Ni is present in a highly soluble and mobile form. Thus, the results of the two germination tests confirm that AMF development may be inhibited in soil under Ni-hyperaccumulators and in contact with their roots. It is interesting to note that in the roots of the moderate hyperaccumulator *G. pruinosa* only 20% of the total Ni was extractable with KCl, whereas the two strong hyperaccumulators contained more than 80% of KCl extractable Ni (Table 7). This could mean that the storage of Ni in the root in unavailable forms is reduced when the level of nickel is very high. This needs to be further investigated, and more precisely, it would be beneficial to identify the chemical forms of Ni inside the roots and in the rhizosphere.

Despite the clearly toxic levels of Ni in the soil and in the root surface of Ni-hyperaccumulators, at least some AMF colonisation was observed. This can be explained by the high Ni-tolerance level of the AMF associated with these plants. Indeed, the two isolates from roots of *P. douarrei* and *S. acuminata* are clearly more tolerant to Ni than the three other isolates tested. These two isolates are indifferent to concentrations of Ni up to 30 μg g^{-1} and are able to colonize at least parts of roots of Ni-hyperaccumulators when Ni content do not exceed about 20,000 μg g^{-1} in leaves. Tolerant AMF isolates to Cd and Zn have already been reported (Gildon and Tinker 1981; Weissenhorn et al. 1994), but Ni-tolerance of AMF has not been reported before.

Current research is dedicated to the identification by DNA sequencing of hyperaccumulator-related AMF isolates and to their better characterisation, including the study of their effects on plants. The relation between the hyperaccumulation phenomenon and mycorrhizal status also requires further investigation, in particular by comparison of pairs of plant species of the same genus (a Ni-hyperaccumulator and a non-accumulator), for example *Geissois pruinosa* and *G. montana*, or *Psychotria douarrei* and *P. baillonii*.

References

Amir H, Pineau R (2003) Relationships between extractable Ni, Co and other metals and microbiological characteristics of different ultramafic soils from New Caledonia. Aust J Soil Res 41:1–14

Amir H, Pineau R, Violette Z (1997) Premiers résultats sur les endomycorhizes des plantes de maquis miniers. In: Jaffré T, Reeves RD, Becquer T (eds) The ecology of ultramafic and metalliferous areas. ORSTOM Edition, Noumea, pp 79–85

Baker AJM (1981) Accumulators and excluders strategies in the response of plants to heavy metals. J Plant Nutr. 3:643–654

Bathia N, Walsh KB, Orlic I, Siegele R, Ashwath N, Baker AJM (2004) Studies on spatial distribution of nickel in

leaves and stems of the metal hyperaccumulator *Stackhousia tryonii* using nuclear microprobe (micro-PIXE) and EDXS techniques. Func Plant Biol 31:1061–1074

Boyd RS, Jaffré T (2001) Phytoenrichment of soil Ni content by *Sebertia acuminata* in New Caledonia and the concept of elemental allelopathy. South Afr J Sci 97:535–538

Boyd RS, Jaffré T, Odom JW (1999) Variation of nickel content in the nickel-hyperaccumulating shrub *Psychotria douarrei* (Rubiaceae) from New Caledonia. Biotropica 31:403–410

Boyd RS, Shaw JJ, Martens SN (1994) Nickel hyperaccumulation defends *Streptanthus polygaloides* (Brassicaceae) against pathogens. Am J Bot 81:294–300

Brooks RR (1987) Serpentine and its vegetation. A multidisciplinary approach. Dioscorides Press, Portland, Oregon

Coles KE, David JC, Fisher PJ, Lappin-Scott HM, Macnair MR (2001) Solubilisation of zinc compounds by fungi associated with the hyperaccumulator *Thlaspi caerulescens*. Bot J Scot 51:237–247

Gildon A, Tinker PB (1981) A heavy metal-tolerant strain of a mycorrhizal fungus. Trans Brit Mycol Soc 77:648–649

Heggo A, Angle JS (1990) Effects of vesicular-arbuscular mycorrhizal fungi on heavy metal uptake by soybeans. Soil Biol Biochem 22:865–869

Hilderbrandt M, Kaldorf M, Bothe H (1999) The zinc violet and its colonisation by arbuscular mycorrhizal fungi. J Plant Physiol 154:709–717

Jaffré T (1980) Etude écologique du peuplement végétal des sols dérivés de roches ultrabasiques en Nouvelle-Calédonie. ORSTOM Press, Paris

Jaffré T, Brooks RR, Reeves RD (1976) *Sebertia acuminata*: a nickel accumulating plant from New Caledonia. Science 193:573–580

Jaffré T, Veillon JM (1991) Etude floristique et structurale de deux forêts denses humides sur roches ultrabasiques en Nouvelle-Calédonie. Bull Mus Natn Hist Nat 12:243–273

Jhee EM, Boyd RS, Eubanks MD (2005) Nickel hyperaccumulation as an elemental defense of *Streptanthus polygaloides* (Brassicaceae): influence of herbivore feeding mode. New Phytol 168:331–344

Joner EJ, Leyval C (2001) Time-course of heavy metal uptake in maize and clover as affected by root density and different mycorrhizal inoculation regimes. Biol Fertil Soils 33:351–357

Kersten WJ, Brooks RR, Reeves RD, Jaffré T (1980) Nature of nickel complexes in *Psychotria douarrei* and other nickel-accumulating plants. Phyochemistry 19:1963–1965

Koske RE, Gemma JN (1989) A modified procedure for staining roots to detect VA Mycorrhizas. Mycol Res 92:486–505

Krämer U, Cotter Howells JD, Charnock JM, Baker AJM, Smith JAC (1996) Free histidine as a metal chelator in plants that accumulate nickel. Nature 379:635–638

Lee J, Reeves RD, Brooks RR, Jaffré T (1977) Isolation and identification of a citrato-complex of nickel from nickel-accumulating plants. Phytochemistry 16:1503–1505

Leyval C, Singh BA, Joner EJ (1995) Occurrence and infectivity of arbuscular mycorrhizal fungi in some Norwegian soils influenced by heavy metals and soil properties. Wat Air Soil Pol 84:203–216

Leyval C, Turnau K, Haselwandter K (1997) Effect of heavy metal pollution on mycorrhizal colonisation and function, physiological, ecological and applied aspects. Mycorrhiza 7:139–153

Lindsay WL, Norvell WA (1978) Development of a DTPA soil test for zinc, iron, manganese and copper. Soil Sci Soc Am J 42:421–428

Ouerdane L, Mari S, Czernic P, Lebrun M, Lobinski R (2006) Speciation of non-covalent nickel species in plant tissue extracts by electrospray Q-TOFMS/MS after their isolation by 2D size exclusion-hydrophilic interaction LC (SEC-HILIC) monitored by ICP-MS. J Anal Atom Spectr 21:676–683

Pawlowska TE, Chaney RL, Chin M, Charvat I (2000) Effects of metal phytoextraction practices on the indigenous community of arbuscular mycorrizal fungi at a metal contaminated landfill. Appl Environ Microbiol 66:2526–2530

Perrier N, Amir H, Colin F (2006) Occurrence of mycorrizal symbioses in the metal-rich lateritic soils of the Koniambo Massif, New Caledonia. Mycorrhiza, 57:572–582

Perrier N, Colin F, Jaffré T, Ambrosi JP, Rose J, Bottero JY (2004) Nickel speciation in *Sebertia acuminata*, a plant growing on a lateritic soil of New Caledonia. C R Geosciences 336:567–577

Reeves RD (1992) The hyperaccumulation of nickel by serpentine plants. In: Baker AJM, Proctor J, Reeves RD (eds) The vegetation of ultramafic (serpentine) soils. Intercept, Andover, pp 253–277

Sagner S, Kneer R, Wanner G, Cosson J-P, Deus-Neumann B, Zenk MH (1998) Hyperaccumulation, complexation and distribution of nickel in *Sebertia acuminata*. Phytochemistry 47:339–347

Schlegel HG, Cosson JP, Baker AJM (1991) Nickel-hyperaccumulating plants provide a niche for nickel-resistant bacteria. Bot Acta 104:18–25

Smith SE, Read DJ (1997) Mycorrhizal symbiosis. Second edn. Academic Press, San Diego, London

Tomsett EB, Thurman DA (1988) Molecular biology of metal tolerances in plants. Plant Cell Environ 11:383–394

Trouvelot A, Kough JL, Gianinazzi-Pearson V (1986) Mesure du taux de mycorhization VA d'un système radiculaire. Recherche de méthodes d'estimation ayant une signification fonctionnelle. In: Gianinazzi-Pearson V, Gianinazzi S (eds) Physiological and genetical aspects of mycorrhizae. INRA Edition, Paris, pp 217–221

Turnau K, Mesjasz-Przybylowicz J (2003) Arbuscular mycorrhiza of *Berkheya coddii* and other Ni-hyperaccumulating members of Asteraceae from ultramafic soils in South Africa. Mycorrhiza 13:185–190

Verkleij JAC (1990) In: Shaw AJ Heavy metal tolerance in plants: evolutionary aspects. CRC Press, Boca Raton, FL

Vivas A, Barea JM, Azcon R (2005) Interactive effect of *Brevibacillus brevis* and *Glomus mossae*, both isolated from Cd contaminated soil, on plant growth, physiological mycorrhizal fungal characteristics and soil enzymatic activities in Cd polluted soil. Environ Pollut 134:257–266

Vogel-Mikus K, Drobne D, Regvar M (2005) Zn, Cd and Pb accumulation and arbuscular mycorrhizal colonisation of pennycress *Thlaspi praecox* Wulf. (Brassicaceae) from the

vicinity of a lead mine and smelter in Slovenia. Environ Pollut 133:233–242

Wang B, Qiu YL (2006) Phylogenetic distribution and evolution of mycorrhizas in land plants. Mycorrhiza 16:299–363

Weissenhorn I, Glashoff A, Leyval C, Berthelin J (1994) Differential tolerance to Cd and Zn of arbuscular mycorrhizal (AM) fungal spores from heavy-metal polluted soils. Plant Soil 167:189–196