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# New insights into the mycorrhizal status of Cyperaceae from ultramafic soils in New Caledonia

Alexandre Lagrange, Marc Ducouso, Philippe Jourand, Clarisse Majorel, and Hamid Amir

**Abstract:** In New Caledonia, a hot spot of biodiversity, plants from the Cyperaceae family are mostly endemic and considered pioneers of the nickel-rich natural serpentine ecosystem. The aim of the study was to highlight the mycorrhizal status of these Cyperaceae and to bring new insights into the role of this symbiosis in plant tolerance to ultramafic soils. Nine Cyperaceae species were studied and presented evidence of root colonization by arbuscular mycorrhizas (AMs), with frequencies ranging from 8% to 57%. The highest level of AM colonization was observed in plants from the endemic dominant genus *Costularia*. Molecular evidence demonstrated the presence of *Glomus* species inside the roots. In a controlled greenhouse assay, AM inoculation of *Costularia comosa* grown under ultramafic conditions significantly enhanced plant growth, with an increase in biomass by up to 2.4-fold for shoots and 1.2-fold for roots, and also reduced nickel content in roots by 2.5-fold, as compared with the controls. All these data support our hypotheses (i) that a relationship exists between the mycorrhizal status of Cyperaceae and their habitat, and (ii) that AM have a positive role in plant tolerance to ultramafic soils (mineral nutrition and metal tolerance), suggesting the use of these pioneer plants with AM management as potential tools for nickel mine site rehabilitation in New Caledonia.

**Key words:** arbuscular mycorrhiza, Cyperaceae, ultramafic soil, New Caledonia.

**Résumé :** En Nouvelle-Calédonie, un point chaud de la biodiversité, les plantes appartenant à la famille des Cypéracées sont principalement endémiques et sont considérées comme pionnières de l'écosystème naturel de la serpentine riche en nickel. Cette étude visait à éclaircir le statut mycorrhizien de ces Cypéracées et d'apporter un nouvel aperçu de rôle de cette symbiose dans la tolérance des végétaux aux sols ultrabasiqes. Neuf espèces de Cypéracées ont été étudiées et la colonisation des racines par des mycorrhizes arbusculaires (MA) a été mise en évidence avec des fréquences variant de 8 % à 57 %. Le niveau de MA le plus élevé a été noté chez les plantes du genre endémique dominant *Costularia*. Les données moléculaires ont démontré la présence de *Glomus* sp. à l'intérieur des racines. Lors d'un essai en serre contrôlée, l'inoculation de MA chez *Costularia comosa* cultivée en conditions ultrabasiqes a significativement accru la croissance des plants, avec une augmentation de la biomasse des pousses de 2,4 fois et des racines de 1,2 fois, réduisant aussi le contenu en nickel des racines de 2,5 fois, tout cela par rapport au contrôle. Toutes ces données plaident en faveur de l'hypothèse (i) d'une relation entre le statut en mycorrhize des Cypéracées et leur habitat, et (ii) du rôle positif des MA dans la tolérance aux sols ultrabasiqes (nutrition minérale et tolérance aux métaux), suggérant que ces plantes pionnières parallèlement à la gestion des MA puissent constituer des outils potentiels de réhabilitation des sites miniers de nickel en Nouvelle-Calédonie.

**Mots-clés :** mycorrhizes arbusculaires, Cypéracées, sol ultrabasiqes, Nouvelle-Calédonie.

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## Introduction

New Caledonia is a tropical archipelago in the South Pa-

cific Ocean. One third of the main island is covered by ultramafic soils, a weathered product of serpentine bedrocks,

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characterized by low contents of the major nutrients (N, P, and K), an unbalanced Ca/Mg ratio, and the presence of iron oxides with high concentrations of heavy metals (Ni, Cr, Mn, and Co) (Latham 1985; Brooks 1987; Perrier et al. 2006a). As a result, specific biological endemic ecosystems have evolved, making the main island a biodiversity hot spot (Myers et al. 2000). Among noteworthy endemic plants colonizing this archipelago are sedges belonging to the Cyperaceae family, which are very abundant and considered the main pioneer plants of ultramafic maquis, where they constitute almost exclusively the herbaceous stratum (Jaffré 1992). According to Bruhl (1995), the Cyperaceae is a cosmopolitan family of monocotyledonous plants with about 5000 species. Ecologically, the Cyperaceae are also very important, as they are able to colonize a wide range of habitats at almost all latitudes (Bruhl 1995). However, the mycorrhizal status of this plant family is still unclear. In general, Cyperaceae are considered nonmycorrhizal (NM) or only weakly mycorrhizal (Meney et al. 1993; Miller et al. 1999; Wang and Qiu 2006), while several recent studies have reported arbuscular mycorrhizal (AM) associations within Cyperaceae (Amir et al. 1997; Muthukumar et al. 2004; Gai et al. 2006; Perrier et al. 2006b). In a recent review, Brundrett (2009) suggested that there is a continuum extending from fully NM to AM species in the Cyperaceae (a number of 4500 species were considered) resulting from continuing adaptation to variable habitat conditions. Brundrett suggested that the balance of information collected about the mycorrhizal status of Cyperaceae from various habitats (from arctic, alpine habitats to dry, warm arides or to wetlands and disturbed habitats) indicates that this plant family is predominantly NM, with only sporadic glomeromycotan fungus colonization in roots. Previous data about the mycorrhizal status of such sedges from New Caledonia reported that AM structures were observed at low intensity in 3 Cyperaceae species, suggesting they might contribute to plant adaptation to the extreme ultramafic soil conditions (Perrier et al. 2006b).

The aims of the study were (i) to evaluate the AM symbiotic status of Cyperaceae in New Caledonian ultramafic soils by extending the study to most Cyperaceae species dominating such an extreme ecosystem, and (ii) to test the role of AM symbioses on plant growth and survival in these soils. For this purpose, we investigated the occurrence of mycorrhizal colonization of 9 Cyperaceae species from New Caledonian ultramafic maquis ecosystems by microscopic observation to quantify the presence of AM structures within roots. We also performed a molecular analysis to identify the fungi associated with sedges. The relationships between the extent of AM colonization and plant growth, survival, and nutrient status was assessed in greenhouse experiments using *Costularia comosa* (C.B. Clarke) Kük, one of the most frequent cyperaceous species found in New Caledonia ultramafic soils.

## Materials and methods

### Study sites

Four sites located in ultramafic ligno-herbaceous maquis

ecosystems were chosen for this study (see Fig. S1<sup>2</sup>). Site 1 is located in Plum area (22°16'59"S, 166°39'12"E) on colluvial lateritic soil. Site 2, also composed of colluvial lateritic soil, is located on the Koniambo Massif (21°00'39"S, 164°49'08"E). Site 3 is located in the Plaine des Laes region (22°13'06"S, 166°52'49"E) on alluvial lateritic soil. Site 4 is located in the Prony area (22°19'28"S, 166°49'25"E) on nodular ferralitic soil. Jaffré (1992) and Perrier et al. (2006a, 2006b) have already reported a full description of the areas where the sites are located, including climate, geology, geomorphology, and vegetation structure of these ultramafic sites. Soils present in these 4 sites are highly weathered oxisols (Latham 1985; Perrier et al. 2006a) with an iron (Fe) concentration range from 290 to 535 g·kg<sup>-1</sup> with diethylene triamine pentaacetic acid extractable concentrations ranging from 81 to 238 mg·kg<sup>-1</sup>. The 3 other main characteristics of these slightly acidic soils (pH 5.2–6.1) are a strong deficiency in phosphorus and potassium (P<sub>total</sub>, 64–86 mg·kg<sup>-1</sup>; P <sub>Olsen</sub>, 1–5 mg·kg<sup>-1</sup>; K, 20–84 mg·kg<sup>-1</sup>), an unbalanced calcium to magnesium ratio (Ca, 0.1–1.5 g·kg<sup>-1</sup>; Mg, 3.1–88.4 g·kg<sup>-1</sup>), and a high content of putatively toxic heavy metals (Ni, 3.2–6.5 g·kg<sup>-1</sup>; Cr, 12.7–22.8 g·kg<sup>-1</sup>; Mn, 5.2–11.0 g·kg<sup>-1</sup>; Co, 0.4–1.1 g·kg<sup>-1</sup>) with diethylene triamine pentaacetic acid extractable concentrations as follows: Ni, 0.2–2.4 mg·kg<sup>-1</sup>; Co, 0.3–0.6 mg·kg<sup>-1</sup>; Cr, 0–0.8 mg·kg<sup>-1</sup>; Mn, 0.9–1.1 mg·kg<sup>-1</sup> (Perrier et al. 2006b). These soils are well drained and are characterized by successions of short periods of optimal moisture and variable periods of drought (Perrier et al. 2006a). Sedge species present in each site have been identified according to morphological features using the Noumea Herbarium, New Caledonia.

### Plant root sampling for microscopic observation and molecular analysis

One to 5 g (fresh mass) of thin roots per plant were collected and then fixed in a solution of 33% ethanol, 33% glycerol, and 34% water. Plants were sampled during the rainy season from January to April. Root identity was ascertained by tracing from the clump to the root tips. For each sedge species, 8 independent plants were randomly sampled. The presence of lateral swollen dauciform roots was directly observed by visual examination in the field and later confirmed by stereomicroscope observation in the laboratory.

### Staining, observation, and characterization of root fungal colonization

Plant root fungal colonization was investigated to assess both the level and frequency of mycorrhizal colonization. Roots were stained in Trypan blue (Koske and Gemma 1989) to assess the presence of AM colonization. For each individual sample, at least 25 root segments of 1 cm length were mounted in 10% glycerol on glass slides for examination with a light microscope. A root segment was considered to be mycorrhizal when arbuscules, vesicles, or hyphal coils were clearly visible. The frequency of AM colonization (*F* (%)) was calculated for each root sampled as follows: (the ratio of the number of mycorrhizal root fragments/the total number of root fragments) × 100. The intensity of AM colonization in the root system (*M* (%)),

<sup>2</sup>Supplementary data for this article are available on the Journal website at <http://cjm.nrc.ca>.

which reflects the abundance or the density of AM structures in the roots. Both *F* and *M* were determined according to Trouvelot et al. (1986).

### Molecular analyses

Molecular analyses were carried out to identify the mycorrhizal fungi colonizing the plant root tissues and to assess their diversity. We analysed thin root fragments of the 3 most frequent sedge species, *C. comosa*, *Costularia nervosa*, and *Costularia pubescens*. A total of 142 DNA samples were extracted for amplification: 30 DNA samples were extracted from 3 plants of *C. nervosa*, 60 DNA samples from 3 plants of *C. pubescens*, and 52 DNA samples from 2 plants of *C. comosa*. Total genomic fungal DNA was extracted from root fragments using the DNeasy Plant Qiagen kit (Qiagen, Courtaboeuf, France). An approximately 550-bp partial fragment of the DNA region encoding for 18S small subunit rDNA (SSU) was amplified with primers AM1 (5'-GTTTCCCCGTAAGGCGCCGAA-3') (Helgason et al. 1999) and NS31 (5'-TTGGAGGGCAAGTCTGGTGCC-3') (Simon et al. 1992). PCR reactions were performed as previously described by Helgason et al. (1999). PCR products were analysed by electrophoresis in 1% (*m/v*) agarose gel containing ethidium bromide. PCR products of 550 bp were purified using the Qiagen Gel Extraction kit (Qiagen) and pooled according to plant species and then cloned in the pGEMt vector in *Escherichia coli* by using the Promega pGEMt kit (Promega, Charbonnières, France). Then, 50 *E. coli* clones corresponding to each amplified DNA fragment pooled per plant were analysed as follows: after reamplification of the 550 bp DNA fragment by PCR, PCR products were purified and then sequenced using Big Dye Terminator chemistry and a ABI3730 capillary electrophoresis-based genetic analyser (Macrogen, Seoul, Korea). DNA sequence analyses were performed by using Chromas Pro 5.2 software (Technelysium Pty Ltd., Shannon, Ireland). A DNA consensus sequence was then deposited in the EMBL nucleotide database for assignment of accession numbers that are further reported.

### Greenhouse experiment

A greenhouse experiment was carried out to assess the effect of mycorrhizal inoculation (with AM) of Cyperaceae when growing in ultramafic soil conditions. *Costularia comosa* was chosen in the present study because we showed this species with a high mycorrhizal index. In addition, seeds of *C. comosa* were easily available with a germination rate above 50%. Seeds of *Costularia comosa* were sown in sterilized sand and grown for 1 month before the transfer of young plants into plastic pots (0.6 L) containing a sterilized (autoclave 30 min; 120 °C) mixture of 5 volumes of sieved (2 mm) lateritic topsoil from site 1, with 1 volume of commercial compost (N, 1.7 mg·g<sup>-1</sup>; P<sub>total</sub>, 150 mg·kg<sup>-1</sup>; P<sub>OlSen</sub>, 7 mg·kg<sup>-1</sup>; K, 139 mg·kg<sup>-1</sup>; terreau universel, Agrofino, France). Plants were cultivated for 12 months under greenhouse conditions (15–25 °C, daily watering to field capacity, with partial shading, 50% net). The addition of commercial compost was necessary because the young *C. comosa* plants are very fragile and many of them do not survive when transferred on a pure lateritic fine soil.

Two different AM inocula were prepared as follows.

(i) Strain SFONL (*Glomus etunicatum*), originally isolated from the rhizosphere of *Alphitonia neocaledonica* (Rhizaceae) in a lateritic soil located at Ouenarou, New Caledonia (22°10'26''S, 166°44'24''E) (Amir and Lagrange 2008). This SFONL strain is known for its capacity to promote plant growth (of *Alphitonia neocaledonica*, *Xanthostemon multiflorus*, and *Cloezia artensis*) under greenhouse conditions (Amir and Lagrange 2008). The SFONL strain was maintained via trap culture on sorghum. A partial 18S rDNA sequence of this strain is available under accession No. FM997956. *Costularia comosa* inoculation with SFONL strain was realized by coating 1-month-old roots with 5 mL of a suspension containing ca. 170 spores, during transfer into the plastic pots. (ii) Spores from the rhizosphere of *C. comosa* from site 1 were isolated (wet-sieving and decanting method) together with young roots to prepare a “natural inoculum”. This inoculum consisted of a 5 mL suspension of crushed roots (ca. 250 mg) containing ca. 115 spores and was used as previously described to inoculate *C. comosa*. Under these conditions, it was possible to compare the effects on plant growth of the natural inoculum with those obtained with SFONL strain.

Thirty *C. comosa* plants were inoculated with the SFONL strain, the natural inoculum, or distilled water as negative control and were grown in individual pots randomly distributed in the greenhouse. To ascertain the effect of these 2 mycorrhizal treatments, 30 sorghum plants (a highly mycotrophic species use as positive control) were treated in the same conditions and grown for 12 weeks.

*Costularia comosa* survival rates were measured after 2, 8, and 12 months of growth. After 12 months, the number and length of leaves and the dry masses of both leaves and roots were measured to assess the effects of AM inoculation on plant growth. In addition, the tissue content of the major plant mineral nutrients N, P, and K was measured as an indicator of AM effects on plant growth. Similarly, Ni content was quantified in plant leaves and roots: Ni content is used as one major ultramafic indicator of AM effect on plant adaptation to ultramafism. The Ni quantification was carried out the chemistry laboratory of IRD (LAMA IRD Noumea) as described in Perrier et al. (2006b). *F* and *M* were determined according to Trouvelot et al. (1986).

All data were subjected to a one-way analysis of variance (standard ANOVA). In addition, the results were also analysed with the multiple comparison test of Tukey–Kramer ( $p \leq 0.05$ ). XLSTAT computer software was used to carry out both ANOVA and correlation tests (Addinsoft 2005).

## Results

### Field mycorrhizal survey

The frequency of AM colonization (*F*) and the intensity of AM colonization (*M*) of the 9 studied species of Cyperaceae species are presented in Table 1. Among the 5 genera studied, *Costularia*, present on these 4 study sites, showed the greatest AM colonization (*F* = 32.4% and *M* = 5.8%) and did not show important changes in mycorrhizal frequency and intensity between the 4 sites. In contrast, the genera *Beaumea* and *Schoenus* presented weak AM colonization (*F* = 6.1% and *M* = 1.4%). The most frequently observed mycorrhizal structures were long, straight hyphae,

**Table 1.** Frequency (*F*) and intensity (*M*) of arbuscular mycorrhizal colonization within 9 sedge species collected in the field from 4 different ligno-herbaceous maquis sites.

Species		<i>F</i> (%)	<i>M</i> (%)
<i>Baumea deplanchei</i> Boeck.	Site 1	7.9±5.1	2.6±2.1
	Site 4	0	0
<i>Costularia arundinacea</i> Kuek	Site 3	43.2±16.1	6.7±4.2
<i>Costularia comosa</i> (C.B. Clarke) Kük	Site 1	29.3±10.4	6.2±3.1
	Site 2	21.5±12.2	1.6±1.1
<i>Costularia nervosa</i> Raynal	Site 1	32.6±13.4	7.3±3.9
	Site 3	57.1±23.7	11.8±5.8
	Site 4	22.2±11.8	3.8±2.7
<i>Costularia pubescens</i> Raynal	Site 1	28.3±9.8	5.2±3.1
	Site 4	25.0±16.8	3.8±2.0
<i>Gahnia aspera</i> (R.Br.) Spreng	Site 4	18.1±8.9	3.1±1.8
<i>Lepidosperma perteres</i> C.B. Clarke	Site 3	24.2±14.8	3.8±2.5
	Site 4	16.7±7.2	2.1±1.7
<i>Schoenus juvenis</i> C.B. Clarke	Site 1	10.0±7.8	6.1±4.7
	Site 4	19.7±13.9	2.7±1.8
<i>Schoenus neocaledonicus</i> C.B. Clarke	Site 1	8.7±6.1	0.7±0.6
	Site 4	8.1±5.9	2.1±1.8

**Note:** *F* = (number of mycorrhizal root fragments/total number of root fragments)×100; *M* = (95n<sub>5</sub> + 70n<sub>4</sub> + 30n<sub>3</sub> + 5n<sub>2</sub> + n<sub>1</sub>)/(total number of root fragments), where n<sub>5</sub> is the number of root fragments with more than 90% colonization, n<sub>4</sub> is the number of root fragments with 89%–50% colonization, n<sub>3</sub> is the number of root fragments with 49%–10% colonization, n<sub>2</sub> is the number of root fragments with 9%–1% colonization, and n<sub>1</sub> is the number of root fragments with <1% colonization). Values are the means ± standard deviation.

which followed the length of the root cortex. We also observed, although with a lower frequency, *Glomus*-type vesicles with a subspherical shape eventually compressed against the cell wall (Fig. 1B). Extraradical spores and intracellular arbuscules have also been observed (Figs. 1A and 1D). All 136 examined specimens developed dauciform roots, with some showing evidence of AM colonization (Fig. 1C).

#### Molecular identification of a *Glomus* species associated with AM in Cyperaceae

The DNA sequencing of the PCR 550-bp cloned products revealed one unique consensus DNA sequence showing strong homologies (blast homology 92%) to *Glomus* group B closely related to *Glomus etunicatum*, *G. claroideum* and to the SFONL strain used in this study. The DNA consensus sequence has been deposited in EMBL nucleotide database under accession No. FM997957.

#### Effects of AM inoculation on *Costularia comosa* root colonization; survival; growth; and N, P, K, and Ni contents

AM treatments were tested on sorghum used as positive controls. The analysis of AM colonization of sorghum revealed that all analysed root fragments presented mycorrhizal structures when treated with either natural or SFONL inoculum, with mycorrhizal intensity values (*M*) of 37% and 60%, respectively. In contrast, uninoculated sorghum plants never presented any mycorrhizal structures. This result indicated that our inoculum protocol and growth conditions allowed an efficient plant mycorrhizal colonization.

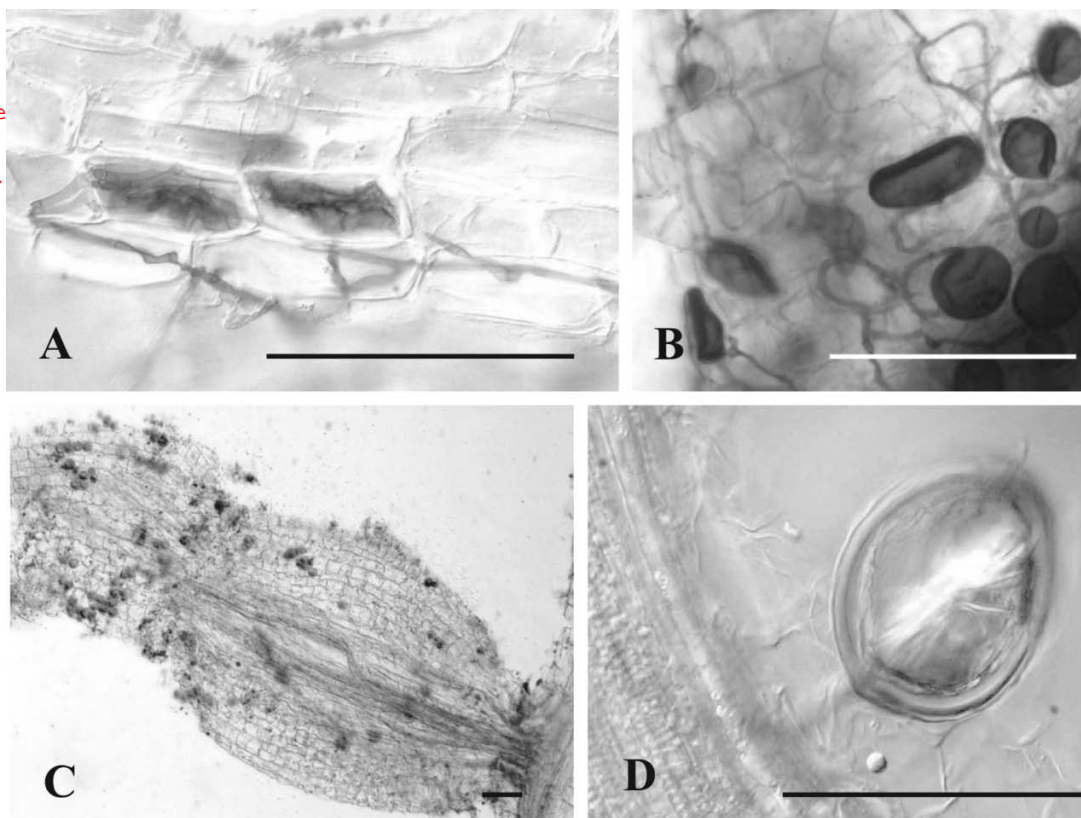
Table 2 shows *Costularia comosa* dry mass variations and

major element contents (N, P, K, and Ni) of shoots and roots of plants either inoculated with AM fungi (natural inoculum or strain SFONL) or uninoculated. *Costularia comosa* inoculated with SFONL strain showed a fungal root colonization frequency value (*F*) of 62% and an intensity value (*M*) of 5%. In contrast, the uninoculated control *C. comosa* showed *F* and *M* values of 12.7% and 1%, respectively, which were significantly different from those presented by roots inoculated with SFONL strain. Natural inoculum led to an intermediate mycorrhizal colonization with *F* and *M* values of 31% and 1.7%, respectively. As observed in situ, AM colonization was weak and structures mainly corresponded to extra- and intra-cortical AM hyphae and *Glomus*-type vesicles. The occurrence of vesicles was higher with the SFONL strain. Intracellular arbuscules were observed with the 2 inoculation treatments but at lower frequency.

The survival rates were different depending on inoculation treatments, with 42%, 58%, and 70%, respectively, for control, natural inoculum and strain SFONL treatments. We also observed that survival rates reached stable values 6 months after the beginning of the assay. We then analysed the effect of mycorrhizal colonization on plant growth parameters. SFONL inoculation increased root and shoot dry mass by 124% and 246%, respectively, when compared with controls (uninoculated plants). Similarly, the mean values of root and shoot biomass, in natural inoculum treatment, were enhanced by 48% and 92%, respectively, but the differences with the control were not statistically significant. Then, to appreciate the overall link between plant growth and mycorrhizal colonization, we assessed a total correlation test. Highly significant total positive correlations were obtained: *r* = 0.511 between shoot dry mass and *F* (*p* ≤

**Fig. 1.** Microscopic observation of arbuscular mycorrhizas in *Costularia comosa* after staining with Trypan blue (Koske and Gemma 1989) and mounting in 10% glycerol. (A) Arbuscules observed in a plant inoculated with strain SFONL, (B) *Glomus*-type vesicles in the cortex of plant root, (C) infection by arbuscular mycorrhizas in a dauciform root, and (D) a spore of *Glomus* sp. on the surface of root. The bars represent 50  $\mu\text{m}$ .

Author: fig. 1 will be published in black and white in print, but in colour on the Web.



**Table 2.** Dry mass (g) and N, P, K, and Ni contents ( $\mu\text{g}\cdot\text{g}^{-1}$ ) of shoots and roots of *Costularia comosa* inoculated with arbuscular mycorrhizal fungi (natural inoculum or strain SFONL) or uninoculated.

	Control (uninoculated)	Natural AM inoculum	SFONL strain ( <i>Glomus</i> )
<i>F</i> (%)	12.7 $\pm$ 11.0a	31.0 $\pm$ 13.0b	62.0 $\pm$ 17.0c
<i>M</i> (%)	1.0 $\pm$ 1.6a	1.7 $\pm$ 0.9a	5.0 $\pm$ 1.5b
Dry mass			
Shoots	0.089 $\pm$ 0.062a	0.171 $\pm$ 0.057a	0.308 $\pm$ 0.081b
Roots	0.106 $\pm$ 0.071a'	0.157 $\pm$ 0.045a'	0.238 $\pm$ 0.046b'
N			
Shoots	8600 $\pm$ 960a	8800 $\pm$ 790a	8300 $\pm$ 950a
Roots	4200 $\pm$ 620a'	4130 $\pm$ 545a'	3730 $\pm$ 586a'
P			
Shoots	433 $\pm$ 71a	386 $\pm$ 55a	415 $\pm$ 59a
Roots	253 $\pm$ 21a'	236 $\pm$ 32a'	232 $\pm$ 29a'
K			
Shoots	12 350 $\pm$ 1 088a	11 233 $\pm$ 2 055a	12 430 $\pm$ 1 683a
Roots	3450 $\pm$ 3940b'	3400 $\pm$ 350b'	5160 $\pm$ 476a'
Ni			
Shoots	21 $\pm$ 9a	32 $\pm$ 12a	21 $\pm$ 7a
Roots	1789 $\pm$ 198a'	1239 $\pm$ 127b'	718 $\pm$ 132c'

**Note:** Numbers followed by the same letter for each arbuscular mycorrhizal (AM) colonization parameter, each element, and plant shoot and root dry mass (shoot or root) are not significantly different at  $p \leq 0.05$ .

0.001);  $r = 0.417$  between root dry mass and  $F$  ( $p \leq 0.01$ );  $r = 0.486$  between shoot dry mass and  $M$  ( $p \leq 0.001$ );  $r = 0.405$  between root dry mass and  $M$  ( $p \leq 0.01$ ).

Variations in plant element content revealed that only K concentration increased significantly in roots of plants inoculated with SFONL, while K, N, and P concentrations in shoot were not affected by SFONL or natural inoculation. In contrast, the Ni content in roots was reduced significantly 2.5 times in SFONL-inoculated plants and 1.4 times in plants treated with natural inoculum, all when compared with controls.

## Discussion

### New Caledonian Cyperaceae species are generally mycorrhizal

Species belonging to the Cyperaceae family are generally considered not or weakly mycorrhizal (Meney et al. 1993; Miller et al. 1999; Wang and Qiu 2006). Our results showed the presence of AM in the roots of 9 Cyperaceae species examined from New Caledonia ultramafic ligno-herbaceous maquis. These observations are in accordance with previous studies showing a weak colonization of sedge roots by AM fungi forming more frequently intracortical hyphae and vesicles (Muthukumar et al. 2004; Gai et al. 2006; Perrier et al. 2006b). Indeed, AM colonization frequency and intensity varied greatly between genera and species. For example plants from the genus *Costularia*, the most abundant sedge genus in the ligno-herbaceous ultramafic maquis, presented the most developed AM colonization among the studied species. In contrast, plants from the genus *Baumea* presented the lowest level of AM mycorrhization. However, the level of mycorrhizal colonization of Cyperaceae from New Caledonia, even in the genus *Costularia*, was relatively low and intracellular arbuscules were observed but not frequent. The unique 18S rDNA consensus sequence we isolated is the first molecular proof, to our knowledge, of the presence of *Glomus* into the root of a Cyperaceae. In addition, it shows an absence of fungal diversity of mycorrhizal colonization, which might be related to the extreme conditions of the habitat (ultramafic soils). This result is also in agreement with previous data reviewed by Brundrett (2009) who reported that in the Cyperaceae, the only AM colonization noticed is mostly due to the sparse glomeromycotan fungi colonization.

### Role of arbuscular mycorrhizas in *Costularia comosa*

AM fungi are relatively abundant in New Caledonian ultramafic soils and seem to play a role in the adaptation of different plant species to ultramafic conditions (Amir et al. 1997; Perrier et al. 2006b; Amir and Lagrange 2008). The presence of AM at a low level of colonization in our observations on Cyperaceae raised the question of the functional effect of AM colonization on sedge survival and growth in ultramafic soils (low NPK nutrient contents, unbalanced Ca/Mg ratio, and presence of high heavy metals concentrations). Our results, obtained using plants grown in greenhouse, showed that even a weak AM colonization with a selected AM strain significantly improved *C. comosa* survival and growth. The effects of AM colonization on *C. comosa* are also supported by a highly significant positive

correlation between plant growth and AM colonization parameters. The presence of low levels of mycorrhization in the controls is probably due to cross-contaminations (only 6 plants were found mycorrhizal in a set of 30 plants), as currently occurs in greenhouse conditions when an experiment duration is over a few months. However, the results presented are means with high standard deviations and do not affect the statistical significance of the whole results. These highly significant correlations indicated that plants with the better growth were those presenting the higher mycorrhizal colonization, and this is true for all the treatments, even the natural inoculum, which did not show a significant difference with the control, because of the high variance of the values. If we consider the low concentrations of mineral elements needed by these plants (Lagrange 2009), a low level of root colonization is probably sufficient to increase the plant absorption, and consequently the plant growth, as observed in greenhouse experiment. These results clearly indicate that the AM symbiosis is functional in *C. comosa*. For species belonging to plant families generally considered NM or weakly mycorrhizal, Brundrett (2009) suggested that the presence of intraradical mycelium and vesicles is not sufficient to attest that the species is mycorrhizal, because their presence could be due to a saprophytic growth inside the plant without any functionality. In the case of *C. comosa*, the presence of arbuscules and the positive effect of AM inoculum on plant growth and survival in greenhouse conditions truly suggest that (i) the plant belongs to the AM group and (ii) the mycorrhizal colonization is functional. Lagrange (2009) performed a field experiment with *C. comosa* in ultramafic soil amended with P, N, and K. He reported that mycorrhizal colonization, including arbuscule frequency, was greatly enhanced by a moderate P addition. He concluded that the low concentrations of P, in the majority of ultramafic soils, reduce the efficiency of the mycorrhizal symbiosis in natural conditions for this plant, as suggested by Lambers et al. (2009) for Australian species. Thus, our data on Cyperaceae from New Caledonian ultramafic soils argue in favour of Brundrett's hypothesis that among plants from Cyperaceae family, there is a continuum between NM to AM varying to both genus and species levels and to habitats and environmental conditions (Brundrett 2009).

Mycorrhizal inoculation with AM is known to (i) improve plant growth by leading to an increase of total P and N accumulated in each plant, and by contributing to plant K uptake, and (ii) improve host plant heavy metal tolerance (Marschner 1995). In our experiment, concentrations of P and N were not clearly enhanced, but as SFONL-inoculated plants had a higher dry mass, they also contained more N, P, and K than uninoculated control plants, suggesting that AM colonization participate in nutrient assimilation. A significant increase of K concentration was observed in roots of *C. comosa* inoculated by SFONL. This suggests that the mycorrhizal strain might improve the plant K uptake, as previously reported by George et al. (1992) showing that hyphal structures contribute to about 10% of the total K in mycorrhizal *Agropyron repens*. In addition, inoculation by the SFONL strain significantly reduced Ni concentration in roots, suggesting a role of AM in Ni avoidance. While the underlying mechanism is still not known, the protective effect of AM against Ni is probably one of the factors favour-

ing the growth of mycorrhizal plants on ultramafic soil (Amir et Ducouso 2010).

### Multiple strategies of New Caledonian pioneer sedge to colonize an adverse environment

Our study showed a beneficial effect of AM colonization on *Costularia* development. We observed also that all samples of the 9 sedge species studied under field conditions and all samples of greenhouse experience developed dauciform roots. This root organ, which belongs to the so-called cluster roots, is characterized by lateral swellings that develop remarkably dense hairy root clusters (Shane et al. 2006). Dauciform roots are mostly found in particular genera of the Cyperaceae family (Lambers et al. 2006) and only appear when plants grow on a low-P soil (Miller 2005; Shane et al. 2005). These structures are considered a plant adaptation to survive stressful soil conditions, notably P deficiency (Lambers et al. 2008).

Therefore, New Caledonian sedge species developing on highly weathered ultramafic soil cumulate at least 2 adaptive structures favouring nutrient acquisition: arbuscular mycorrhizas and dauciform roots. Lambers et al. (2008) noticed that plants of extremely infertile soils are frequently nonmycorrhizal but sometimes compensate for this lack of symbiosis by developing cluster roots. However, Boulet and Lambers (2005) have reported the case of a species endemic to Australian ultramafic soils, *Hakea verrucosa* (Proteaceae), which cumulates the presence of AM and cluster roots. The additional effects of these 2 adaptation strategies could be responsible for the Cyperaceae pioneer behaviour allowing plants to survive and to colonize bare soils. These results designate endemic Cyperaceae and AM management as potential tools for the improvement of mine site rehabilitation in New Caledonia.

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