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ORIGINAL PAPER

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Arbuscular mycorrhiza of *Berkheya coddii* and other Ni-hyperaccumulating members of Asteraceae from ultramafic soils in South Africa

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Abstract The occurrence of arbuscular mycorrhiza (AM) in nickel-(Ni)-hyperaccumulating plants of the Asteraceae family growing on Ni-enriched ultramafic soils in South Africa was surveyed. All plants were found to be consistently colonised by AM fungi, with the abundant formation of arbuscules. *Berkheya coddii*, which is an important species for phytomining, formed well-developed mycorrhiza under greenhouse conditions. Plants cultivated under greenhouse conditions and inoculated with native fungi had a higher shoot biomass and Ni content than noninoculated plants. Inoculation of *B. coddii* with *Glomus intraradices* (BEG) was successful, but only plants with abundantly developed arbuscules showed increased yield. In other cases, shoot biomass was similar to noninoculated plants. Dense depositions localised on top of the arbuscules were often observed in roots collected from the field and from pot cultures.

Keywords Ni hyperaccumulation · *Berkheya coddii* · Asteraceae · Arbuscular mycorrhiza · Phytomining

Introduction

There has been a growing interest in the hyperaccumulation of heavy metals by plants due to the commercial potential of phytoremediation for cleaning up contaminated soil (Baker et al. 1994, 2000; Salt et al. 1998), and as a method to mine metals from low-grade ore bodies (Nicks and Chambers 1998; Brooks et al. 1998; Chaney et al. 1997, 2000). Ni-hyperaccumulation is defined as the ability of plants to take up and sequestrate in any aboveground parts more than 1000 μg Ni per g dry matter. Moreover, the Ni concentration in shoots should exceed that in roots and the soil (Baker and Brooks 1989). Almost 400 species of Ni-hyperaccumulating plants have been discovered so far (Salt et al. 1998; Reeves and Baker 2000), mostly within Euphorbiaceae, Brassicaceae and Asteraceae. In studies on the mechanisms of heavy-metal tolerance in hyperaccumulating plants, their interactions with rhizospheric organisms have been generally neglected (Salt and Kramer 2000). Despite several reports of arbuscular mycorrhiza (AM) colonising plants on heavy metal-rich soils, such as serpentines (Goncalves et al. 1997; Hopkins1987), or strip mines (Pawlowska et al. 1996; Orłowska et al. 2002), heavy metal-accumulating plants so far have been assumed to be nonmycorrhizal.

The main objective of the present study was to survey mycorrhizal colonisation of Ni-hyperaccumulating plants of the Asteraceae family occurring on ultramafic outcrops of the Mpumalanga Province in South Africa (Morrey et al. 1989, 1992). The capacity of these species to accumulate metal to high concentrations in leaves (Anderson et al. 1997; Robinson et al. 1997; Brooks et al. 1998; Mesjasz-Przybylowicz et al. 1996, 2001a, 2001b; Augustyniak et al. 2002) makes them potentially valuable for phytoextraction. Special attention has focused on *Berkheya coddii* Roessler, a species already known as a high biomass phytoremediation crop (Salt et al. 1998) and model for a possible economic phytomining system (Brooks and Robinson 1998; Brooks et al. 1998; Robinson et al. 1999). The plant was cultivated under laboratory conditions and preliminary results on the Ni content of shoots of mycorrhizal and nonmycorrhizal plants obtained.

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Materials and methods

Roots of *Berkheya coddii* Roessler, *B. zeyheri* (Sond. & Harv.) Oliv. & Hiern subsp. *rehmanii* (Thell.) Roessler var. *rogersiana* (Thell) Roessler, *Senecio coronatus* (Thunb.) Harv. and *Senecio* sp. aff. *S. anomalochrous* Hilliard, all belonging to the Asteraceae, were collected in October (pre-flowering period) and February to April (flowering and seed production) in 2000 and 2002 from sites

Table 1 Mycorrhizal frequency (F%), relative mycorrhizal root length (M%), and relative arbuscular richness (A%) estimated in Ni-hyperaccumulating plants. Different letters beside the data in columns indicate statistically significant differences at $P<0.05$

	Origin of material	F%	M%	A%
Senecio ***anomalochrous***	Kaapsehoop	100b	82c	78b
Berkheya coddii	Songimvelo	86b	54ab	29a
B. coddii	Agnes Mine	92b	45a	38a
B. zeyherii	Kaapsehoop	100b	77b	74b
B. zeyherii	Songimvelo	20a	19a	19a
S. coronatus	Kaapsehoop	100b	87c	87b

in Kapsehoop, Agnes Mine, and Songimvelo, Mpumalanga Province, South Africa. The soil was shallow and skeletal, containing ca. 0.3–0.7% Ni, 0.5–8.8% total organic carbon, with a pH of 4.2–7.2. Despite the occurrence of potentially toxic levels of Ni, a relatively species-rich vegetation was present (Morrey et al. 1989). A total of 46 samples of roots were analysed for mycorrhizal colonisation. The roots were prepared according to the modified method of Phillips and Hayman (1970). After careful washing with tap water, the roots were softened in 10% KOH for 24 h, washed in water, bleached in H_2O2 containing NH_3 (10:1) for a few minutes, again washed in water, acidified in 5% lactic acid in water for 12–24 h, stained with 0.01% aniline blue in 5% lactic acid for 24 h at room temperature and stored in 5% lactic acid. Mycorrhiza frequency (F%), relative mycorrhizal root length (M%), and, relative arbuscular richness (A%) were assessed (Trouvelot et al. 1986, http://www.dijon.inra.fr/bbceipm/Mychintec/Mycocalc-prg/download.html).

Nonsterilised *B. coddii* seeds were germinated on wet filter paper for 4–10 days and seedlings were transplanted into 500-ml pots containing soil from ultramafic sites at Agnes Mine, characterised by 1070 mg kg^{-1} total Ni, 300 mg kg^{-1} exchangeable Ni (extracted in 0.02 M di-ammonium EDTA), 7.2 mg kg^{-1} Ca, 18.2 mg kg^{-1} Mg, 0.5–8.8% total organic carbon and pH 6.5. The soil was mixed with granulated expanded clay (3:1). The cultures were kept in sunbags (Sigma) at a photosynthetic photon flux density of ca 78 μmol s^{-1} m^{-2} and a light regime of 12/12 h, and were watered according to Walker and Vestberg 1994. The substratum was pasteurised prior to planting and inoculated or not with *Glomus intraradices* (BEG) or with fungi obtained from roots or the rhizosphere of *B. coddii* in cultures grown on nonsterilised African ultramafic soil containing a crude inoculum of spores, extraradical mycelium and pieces of plant roots. Each experimental group (inoculated with *Glomus intraradices*, inoculated with native fungi and noninoculated) included 36 plants. The shoots for biomass estimation and the roots for mycorrhiza studies were collected after 2 months growth. Mycorrhizal colonisation was analysed individually for each plant. Mycorrhizal dependency was calculated according to Plenchette et al. (1983) as the ratio of the difference between the dry mass of AM-inoculated and noninoculated plants. Shoots for the analysis of Ni content (pilot study) were selected from plants in pots of similar mycorrhization levels and pooled. The shoots were washed with double-distilled water, dried to constant weight at 85°C and weighed. The dried and milled plant material was subsequently mineralised in a 4:1 mixture of ultra-pure concentrated HNO_3 and $HClO_4$ (Merck) (Grodzińska 1978). The total concentration of Ni was determined with AAS (Varian 20BQ).

Statistical data analysis was carried out with the non-parametric Kruskal-Wallis and Mann-Whitney tests ($P<0,05$) using Statistica (version 5.0) software, and the correlation analysis with Statgraphics (version 5.0).

Results

AM colonisation of roots collected from the field

AM was evident in all investigated root samples collected from three different sites in Mpumalanga Province.

Table 2 Mycorrhizal frequency (F%), relative mycorrhizal root length (M%), relative arbuscular richness (A%), nickel (Ni) content of shoots (μg per g dry wt.) and mean shoot dry weight (g) of *Berkheya coddii* inoculated (AM) or not (no AM) with native arbuscular mycorrhiza or *Glomus intraradices* and cultivated under laboratory conditions. Different letters besides the data in columns indicate statistically significant differences at $P<0.05$

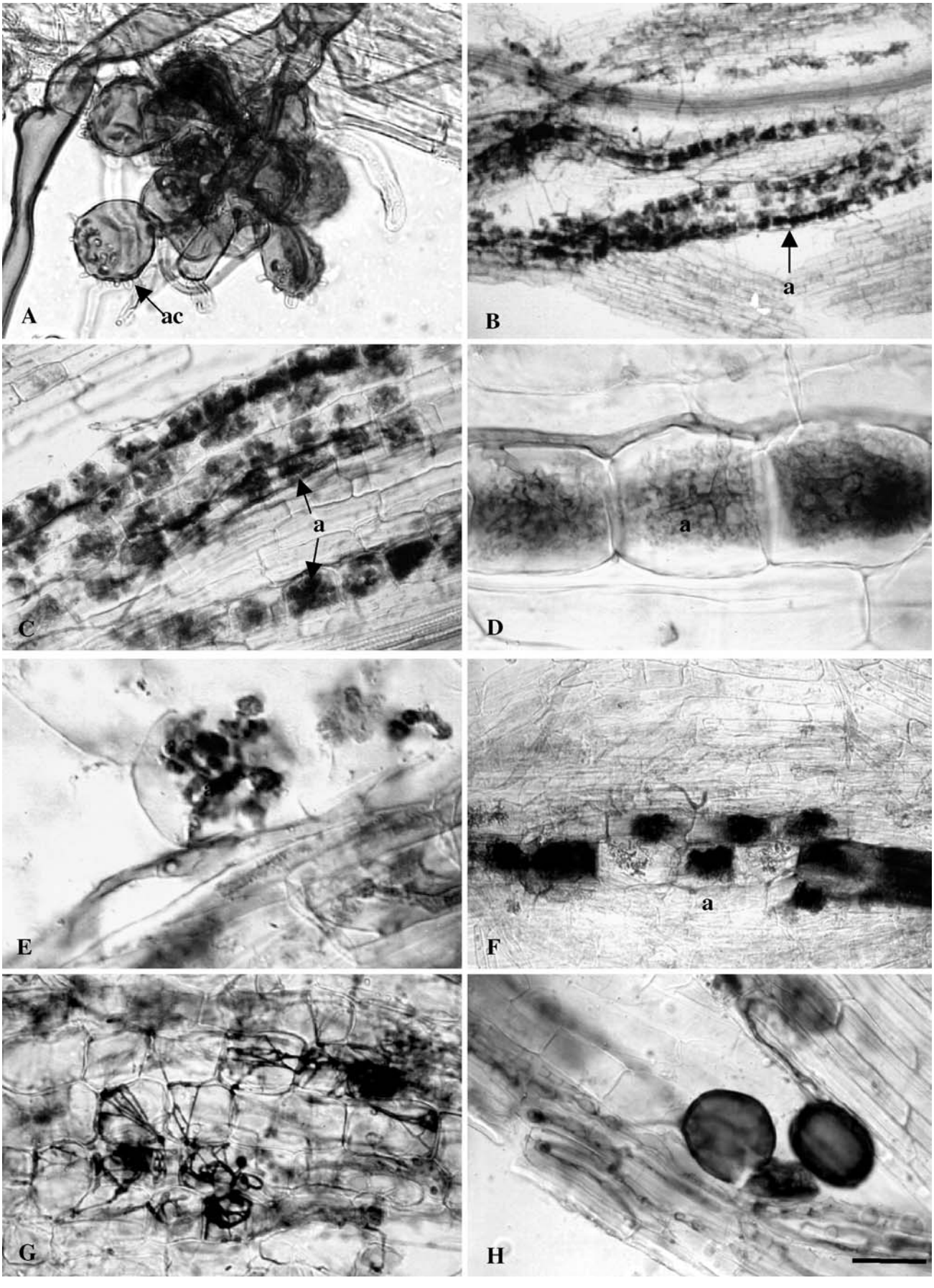
Inoculum	F%	M%	A%	Shoot dry wt.	Ni content
Native AM	93.5a	45.8a	45.3a	0.11a	7,206
G. intraradices	85.2ab	39.4a	33.1a	0.1a	13,204
G. intraradices	34.6b	10b	4.0b	0.07b	6,565
no AM	0.0	0.0	0.0	0.06b	4,953

Abundant spores and auxiliary cells of *Gigaspora* sp. were found in the plant rhizosphere (Fig. 1A). Usually, the degree of mycorrhizal colonisation in randomly selected root fragments was high (Table 1). Well-developed intraradical mycelium, formation of intracellular coils and abundant arbuscules (Fig. 1B–D), characteristic of *Arum*-type colonisation, were visible. Many arbuscules observed in the plant species analysed were surrounded by dense intracellular depositions, ranging in colour from yellow to black (Fig. 1E, F). The coarse mycelium was often accompanied by the fine endophyte *Glomus tenue* (Greenhall) Hall (Fig. 1G). Both arbuscular fungi were often colonising the same parts of the roots. Despite the common presence of the small vesicles of *Glomus tenue*, vesicles with a larger diameter (Fig. 1H), formed by coarse mycelium, were rare.

Greenhouse experiment

Specimens of *B. coddii* grown under laboratory conditions and inoculated with crude inoculum originating from South African ultramafic soil were highly mycorrhizal (Table 2; Fig. 2A, B). Both coarse and fine endophytes were present. Abundant and well-developed arbuscules were usually present. The height of plants cultivated

Fig. 1A–H Arbuscular mycorrhiza of Ni-hyperaccumulating plants collected from ultramafic soils in South Africa. **A** Auxiliary cells often seen close to roots of Ni-hyperaccumulating plants. **B** Mycorrhiza of *Senecio coronatus*., **C** Typical arbuscules common in *Berkheya coddii*. **D** Abundant development of arbuscules in *B. coddii*. **E, F** Depositions over arbuscules. **G** Mycelium and vesicles characteristic for *Glomus tenue*. **H** Vesicles formed by coarse AM mycelium; *bars* **A**, **H** 30 μm, **B**, **F** 15 μm, **C**, **E** 10 μm, **D**, **G** 60 μm (*a* arbuscule, *ac* auxiliary cells)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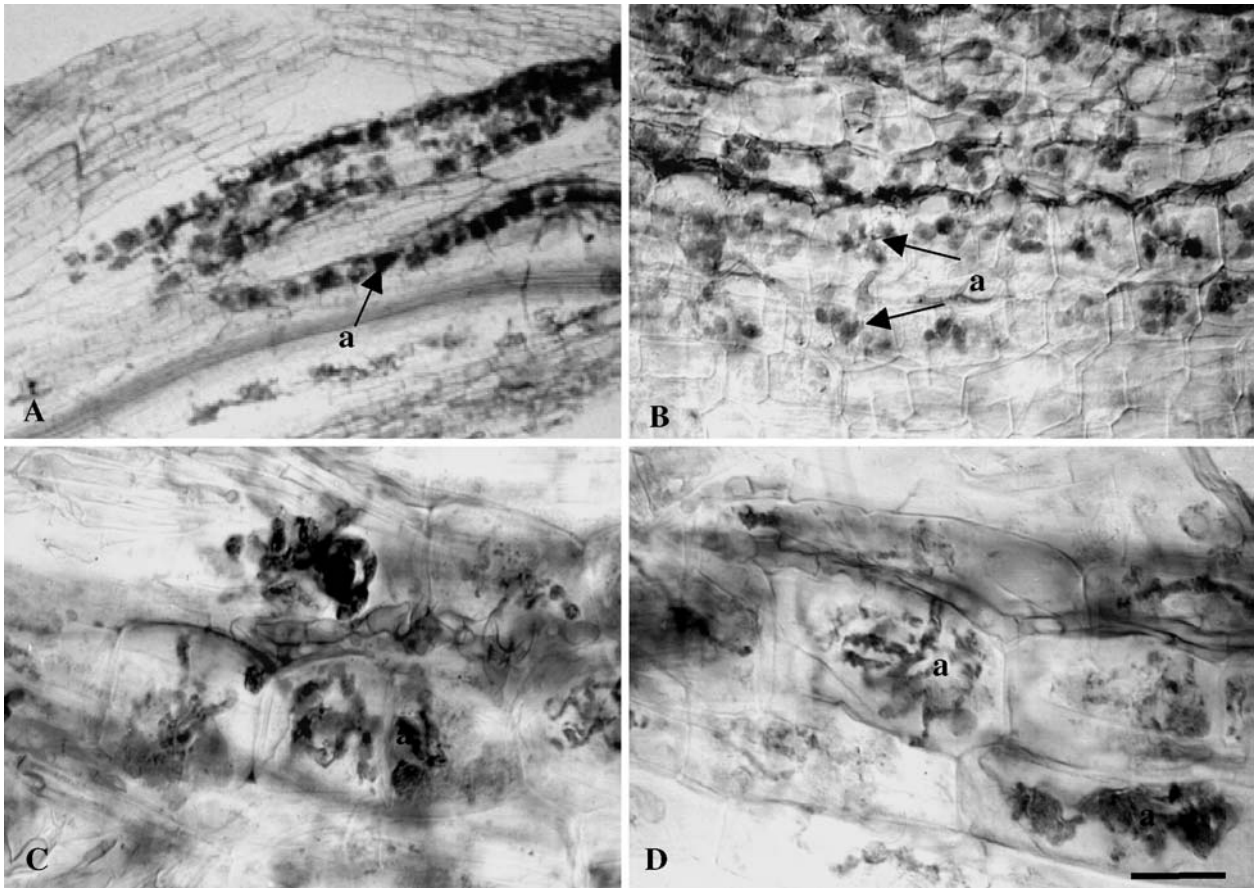

Fig. 2A–D Arbuscular mycorrhiza of *B. coddii* cultivated under laboratory conditions. **A, B** Abundant development of arbuscules within roots inoculated with AM fungi from ultramafic soil. **C, D** Depositions over arbuscules; *bars* **A** 50 μm, **B** 15 μm, **C**, **D** 15 μm (*a* arbuscule)

under greenhouse conditions in the presence of crude inoculum was at least double that of noninoculated plants, and the mean dry weight and Ni content of the shoots was about 30% higher (Table 2). Positive correlations between shoot height and mycorrhizal colonisation level (P = 0.046) and between shoot dry weight/shoot height and arbuscular richness (at P = 0.03 and 0.015, respectively) were found.

Results obtained for plants inoculated with *Glomus intraradices* were not uniform. Even plants in the same pots were either strongly mycorrhizal with similar high mycorrhiza frequencies and rich development of typical, well-branched arbuscules, or these parameters were much lower and well-developed arbuscules were rare. In the first case (plants with well-developed arbuscules), plants showed increased shoot growth and the highest content of Ni in shoots. Mycorrhizal dependency calculated for this group of plants was over 0.6. In the case of plants with poorly developed or no arbuscules, shoot biomass was similar to that of noninoculated plants; however, an increase in Ni content in shoots was observed (Table 2).

Dense depositions over arbuscules, similar to those observed in roots from the field, were found in ca. 50% of roots from pot cultures (Fig. 2C, D).

Discussion

All four Ni-hyperaccumulating representatives of Asteraceae included in the present study, like most other members of the family (Warcup and McGee 1983; Harley and Harley 1987; Warcup 1990; Mahendra and Deepak 1999), were found to form AM symbiosis in natural stands. The mycorrhizal parameters were either similar (*B. coddii*) or different (*B. zeyherii*) in plants of the same species collected from different stands. This could be due to factors such as soil characteristics, AMF propagule quality/quantity and plant genotype. It is also interesting that, besides other AMF species, *Gigaspora* was also present in soil containing high levels of Ni, while most studies on mycorrhiza and heavy metals have focused on *Glomus* (Leyval et al. 1997).

To our knowledge, this is the first report of the abundant occurrence of mycorrhiza in metal-hyperaccumulating plants. *Berkheya coddii*, the most interesting species, can accumulate Ni up to 3.8% of dry biomass of leaves under natural conditions (Augustyniak et al. 2002) and yields higher than most other hyperaccumulators. This species can be cultivated easily either mycorrhizal or nonmycorrhizal. As shown in the present work, well-developed mycorrhization (including arbuscule forma-

tion) increased not only the shoot biomass of the plant but also strongly increased the Ni content of shoots. The highest Ni shoot content of mycorrhizal plants was 1.3% of dry weight, while in nonmycorrhizal plants it was below 0.5%. This greatly contrasts with the conventional opinion that the presence of AM reduces the uptake of trace elements occurring in excessive amounts (Leyval et al. 1997).

Plants cultivated in the presence of a crude inoculum from the ultramafic soil contained less Ni than the highly mycorrhizal *Glomus intraradices*-inoculated plants, but more than the poorly mycorrhizal *Glomus intraradices*-inoculated plants. Ni uptake seems to depend not only on the level of colonisation but also on characteristics of the fungal species or strains. Differences in mycorrhizal colonisation level, richness of arbuscules, shoot biomass and Ni content between plants grown in the same substratum and inoculated with the same fungal strain also suggest diversity among individual plants in the response to mycorrhizal fungi.

Berkheya coddii may serve as an excellent experimental plant, useful for studying heavy-metal uptake, representing an example contradictory to non-hyperaccumulating plants, which usually contain less heavy metals in shoots than roots. For non-hyperaccumulating species, it has been suggested that growth is facilitated by the selective immobilisation of heavy metals within root tissues containing the fungal cells (Kaldorf et al. 1999). It is not known whether a similar mechanism exists in the hyperaccumulators. The occurrence of poorly developed arbuscules or their absence in the case of fungal strains originating from nonpolluted places may suggest a negative reaction of the non-adapted fungus to toxic levels of Ni, or indicate plant reaction towards the fungus. The phenomena at the interface between plant and fungus are so far not known and the above-described system may be valuable for expanding our knowledge. The appearance of dense depositions over arbuscules could indicate a reaction of the plant towards the fungus, or that substances are transported into the plant cells.

The discovery of the mycorrhizal status of *B. coddii* and the improvement of yield by AM fungi may lead to the optimisation of existing models of an economically feasible phytomining system (Brooks et al. 1998). Although mycorrhiza has been found to improve phytoremediation practices (Jeffries et al. 2002), the use of mycorrhiza in phytoextraction of heavy metals has so far been neglected.

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