Arbuscular mycorrhizal colonization and growth of *Eremanthus incanus* Less. in a highland field

M.C. Pagano, M.N. Cabello, M.R. Scotti

Institute of Biological Sciences, Federal University of Minas Gerais, Belo Horizonte, Brazil

ABSTRACT

This paper focuses on *Eremanthus incanus* Less. (Asteraceae), a common species of highland regions in Brazil. The effect of arbuscular mycorrhizal (AM) inoculation on plant growth (height and diameter) was evaluated. Roots were examined from individuals randomly selected from undisturbed areas of highland vegetation and from an experimental restored site. Results showed that *E. incanus* presented high AM colonization both in restored and undisturbed sites. Moreover, AM colonization was significantly higher in the inoculated treatment than in the non-inoculated one. The species presented *Arum*-type colonization and frequent production of vesicles, especially in the restored site. Arbuscular mycorrhizal inoculation stimulated plant growth (height and diameter). Ten AM fungi (AMF) taxa were found in the studied rooting zones: *Acaulospora spinosa*, *A. elegans*, *A. foveata*, *Acaulospora* sp., *Gigaspora margarita*, *Glomus* sp., *Dentiscutata biornata*, *D. cerradensis*, *Dentiscutata* sp. and *Racocetra verrucosa*. These results revealed that AMF is a common and important component in highland vegetation in Brazil, and should be included in future restoration programs.

Keywords: arbuscular mycorrhizal fungi; *Eremanthus incanus*; plant growth; highlands; Brazil

A complete understanding of tropical plant life histories should include traits related to AM formation. *Eremanthus incanus* Less. (candeia, candeinha), common in southeast Brazil, in the States of Minas Gerais and São Paulo, was selected to be studied. This arboreal species which reaches 3 to 5 m in height is common in Minas Gerais highland fields. This plant is presumed to be a threatened species and attempts to study its characteristics are important for conservation and management because this species is subject to intense exploratory pressure (Velten and Garcia 2007).

Arbuscular mycorrhizal fungi (AMF) are the main components of the microbial soil community, and their diversity is associated with plant community structure and functioning; AMF diversity also seems to be very important to determine productivity and plant diversity in natural environments (Van der Heijden and Sanders 2003). AMF provide phosphate to their host plants, being particularly important in soils with low P content (Bohrer et al. 2004); their sporulation is related to host species.

The State of Minas Gerais is characterized by a hilly relief with elevations ranging from 79 to 2890 m, and its natural vegetation needs urgent strategies for conservation (Versieux and Wendt 2007). This fragile region has suffered from human impact on a large scale, and conservation action must be developed to protect fauna and flora.

Highland fields, also termed rupestrian fields, have shrubby, tortuous and sclerophyllous vegetation or open grasslands and replace the cerrado (savanna) vegetation at 1000 m altitude. Plants grow in stones, in sandy soils and present varied adaptations (Giulietti and Pirani 1988). Typical transition forests composed predominantly of *Eremanthus* spp. (Asteraceae) are commonly found (Rizzini 1997).

One of the highland regions, the extreme southern part of Espinhaço Range, is called 'Quadrilátero Ferrífero', due to exposed iron oxide deposits; it provides a habitat for many saxicolous species (Rizzini 1997). This is a region with high biodiversity, presenting endemic and endangered species. Soils are shallow, acid, nutrient-poor, and have excessively drained sands that are highly erodable. The mycorrhizal associations of native species growing at highland fields have been scarcely studied. Nogueira et al. (2005) showed

the mycorrhizal status of some orchids; Pagano and Scotti (2009) showed AM colonization of two other species (*Paepalanthus bromelioides* and *Bulbostylis* sp.); and Matias et al. (2009) reported AM colonization for *Centrosema coriaceum* and estimated the spore numbers in the rhizosphere of *C. coriaceum* and *Tibouchina multiflora*, native species from this biome.

The main objective of this study was to investigate the effect of inoculation with AMF and the mycorrhizal associations of *Eremanthus incanus* Less. (Asteraceae), a native pioneer species in highlands of southeastern Brazil.

Due to the urgent need for conservation of this biome and the absence of any report for mycorrhizal colonization of this plant species (Wang and Qiu 2006), this paper describes the AM associations of a native species to inform about conservation and revegetation actions in the region. This is the first detailed report ever published on the AM status of this species.

MATERIALS AND METHODS

Study area. The study area is located in the iron mines region (43°30'W, 19°10'S), Minas Gerais State, in southeastern Brazil (Figure 1). The local landscape is highland fields, showing small woody plants which support environmental stress (high day and night temperature oscillation and high irradiance) (Rizzini 1997). Therefore, highly xerophytic vegetation with high plant species diversity and endemism is found (Rizzini 1997). These areas, termed rupestrian fields, have shrubby, tortuous and sclerophyllous vegetation or open grasslands

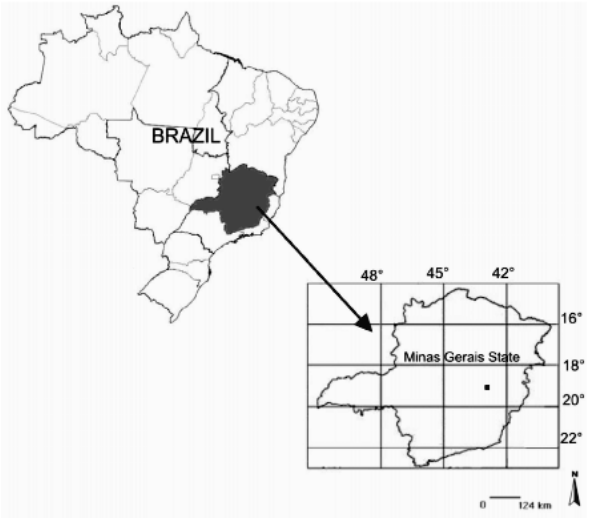

Figure 1. Map showing the location of the sampling site in the southern of Brazil

and replace the cerrado (savanna) vegetation from around 900–1000 m altitude (Giulietti and Pirani 1988). The climate is tropical montane (Aw type) with mean annual temperature varying between 17 and 18.5°C, and mean annual precipitation between 1450 and 1850 mm. Precipitation falls primarily between December and March.

This study was conducted at a highland field in restoration (43°26'W, 19°53'S) 1100 m a.s.l., in São Gonçalo do Rio Abaixo, Minas Gerais State, and in an undisturbed adjacent site.

Treatments and experimental design. The experiment was set up in a degraded area used to keep iron-ore products. *Eremanthus incanus* plants were cultivated in the experimental site (0.6 ha). Seedlings were transplanted during the rainy season in 2001–2002 and mixed with other 4 plant species. The experimental design consisted of 12 replicate blocks (each 24 × 6 m). Two plants per plot randomized by species within the plot with 2-m spacing between individual seedlings were used. Sixteen plants of *E. incanus*/block were used, thus mixed plantation in proportions of 2:10 (2 plants of *E. incanus* mixed with 10 plants of other 4 species/plot) was surveyed.

The treatments were: 1 – *E. incanus* with complete fertilization; 2 – *E. incanus* with complete fertilization (except for phosphorus) + inoculation with AMF. Complete fertilization consisted in P (218 kg/ha) using triple superphosphate, K (2 kg/ha) as KCl, Mg (4.5 kg/ha) as $MgSO_4 \cdot 7\,H_2O$, Zn (13.6 kg/ha) as $ZnSO_4 \cdot 7\,H_2O$, Mo (1.5 kg/ha), as $Mo_7O_2 \cdot 4\,H_2O$, urea (222 kg/ha) following Somasegaran and Hoben (1985) and was applied at the beginning of the plantation. For inoculated treatment 50% of phosphorus fertilization was used.

Arbuscular mycorrhizal fungi were inoculated by placing into each pot 1 ml of suspension composed by 100 spores/ml in a total of 3 species (*Gigaspora margarita*, *Dentiscutata heterogama* and *Glomus etunicatum*) using 33% of each species. Mycorrhizal fungi used were from the BHCB – UFMG collection.

Site and soil characteristics. Soil samples were collected in 2003 from an undisturbed site in a highland field of southeastern Brazil, Minas Gerais State. Soil samples were kept in plastic bags, labeled, sealed and transported to the IMA (Instituto Mineiro de Agronomia) Agropecuary Chemical Laboratory (Brazil). Soils were air-dried and sieved with a sifter of 2 mm. Organic matter and phosphorus content (colorimetric method) was also determined. Potassium (K^+), calcium (Ca^{2+}), magnesium (Mg^{2+}) and sodium (Na^+) were determined by atomic absorption spectrometry (Atomic Absorption spectrophotometer 6800, Analytical

Instruments Division Kyoto Japan, SHIMODZOU corporation, www.shimodzou.com).

Soil texture was determined by the hydrometer method and soil pH was measured in H_2O. Exchangeable K, Mg and Ca were determined by atomic-absorption spectrometry using 1N ammonium acetate as extracting solution. Exchangeable Al was extracted with 1M KCl solution and determined by titration with NaOH.

Analysis of plant growth under field conditions. Plant height and diameter of *E. incanus* were measured in field over 2 years (2002–2003), within the dry and the rainy period. Growth data in both treatments were compared by the Tukey's test ($P < 0.05$) following ANOVA.

AM fungal distribution. Field work was carried out from March 2003 to December 2003, when visits were conducted to collect rhizospheric soils and plant material. Material was collected in March (rainy season), July (dry season), September (late dry season) and December (rainy season) 2003 for analysis.

Rhizospheric soils were collected for analysis of AM spores. Spores were extracted from 100 g soil. AM spores were recovered from soil samples of each treatment in the field, separated by wet sieving, decanting and sucrose centrifugation (Pagano and Scotti 2009), and the analysed data were expressed as number of spores/1 g of dry soil. Only healthy spores were counted. Each spore type was mounted sequentially in PVLG (polyvinyl-lacto-glycerol) and Melzer's reagent for identification. Identification was based on spore colour, size, surface ornamentation and wall structure, with reference to the descriptions provided by the International collection of vesicular and AMF (http://invam.caf.wvu.edu) and the original species descriptions. Numbers of species were counted and spore numbers were square rooted, transformed and statistically analyzed, and means were compared by the Tukey's test ($P < 0.05$).

The roots of three or more plants randomly selected were excavated and only those attached to the plant were used; these were fixed in FAA solution (5 ml of formaldehyde, 5 ml of acetic acid, 90 ml of ethyl alcohol) until samples could be processed. Roots were stained and assessed for mycorrhizal infection as follows. Roots were taken from the FAA, washed several times in tap water and bleached in 10% (w/v) KOH overnight and then heated to approximately 90°C in a water bath for 1 h. The cooled root samples were washed and stained with 0.05% trypan blue (Pagano and Scotti 2009). Roots were cut into 1 cm segments and thirty one-cm-root fragments were examined per sample for their AM status under a compound microscope (100–1000 X). Quantification of mycorrhiza colonization was done noting the presence and absence of each of the fungal structures (intraradical hyphae and vesicles), and results were expressed as percentage of each structure in colonized segments. Also the intensity of AMF colonization was recorded (Pagano and Scotti 2009). These data were arcsin $(x/100)^{1/2}$ transformed and statistically analyzed, and means were compared by the Tukey's test ($P < 0.05$).

RESULTS AND DISCUSSION

The soil was sandy loam (0–30 cm depth). Some basic properties of the soil were as follows: soil was strongly acid, base saturation was low, and P content very low. The texture of the fine soil showed low content of clay and higher percent of silt (Table 1). The bare soil, before restoration, did not contain any AM fungal propagules.

The survival index of *E. incanus* plants one year after plantation (63%, data not showed) was similar to the survival index reported for other plant species from this biome (Matias et al. 2009). Mortality

Table 1. Chemical analysis of the soil from highland field studied

Soil property [a]	
pH (H_2O) 1:1	4.2
Soil organic C (%)	1.79
Available P (mg/kg of soil)	1.7
Available K^+ (mg/kg)	175
Exchangeable Al^{3+} (cmol (+)/kg)	0.62
Exchangeable Ca^{2+} (cmol (+)/kg)	0.40
Exchangeable Mg^{2+} (cmol(+)/kg)	0.19
CEC (cmol (+)/kg)	12.91
Base saturation (%)	7.82
Texture (%)[b]	
Coarse sand	37.10
Fine sand	26.16
Clay	7.38
Silt	29.36

[a]mean of two measures from one composite sample. SOM colorimetric method, Organic carbon: SOM/1.724, CEC – cation exchange capacity, extracted by 1M potassium chloride; [b]particle size distribution: coarse sand 2–0.2 mm, fine sand 0.2–0.02 mm, silt 0.02–0.002 mm and clay < 0.002 mm

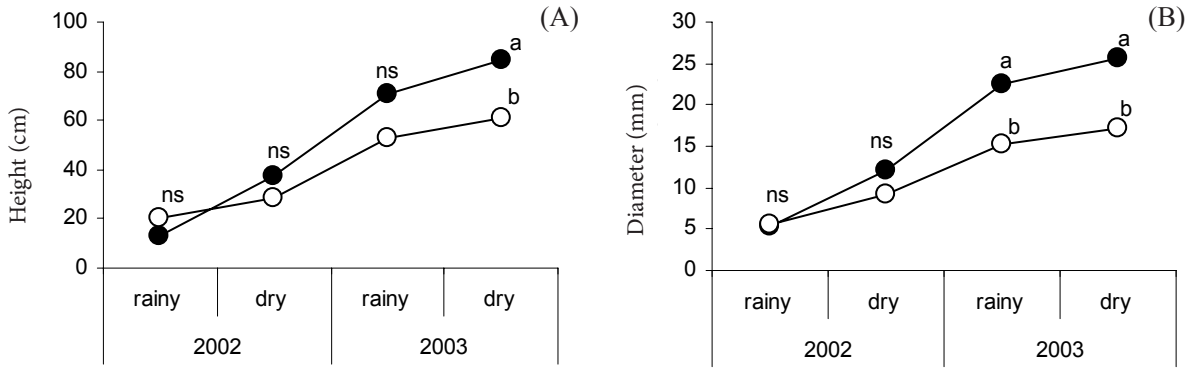

Figure 2. Effect of AM fungal inoculation on height (A) and diameter (B) growth of *E. incanus* after 2 years of field plantation. Treatments: ○ uninoculated; ● inoculated. Different letters indicate significant differences as determined by Tukey's HSD test ($P \leq 0.05$). ns – not significant

should occur because of typical limitation problems of plants in disturbed areas such as removal of the topsoil and the eventual exposure to the ever-present population of biotic mortality agents.

Figure 2 shows growth parameters for *E. incanus* under both treatments. Inoculated plants were considerably taller than uninoculated ones at the second year of growth.

Assessments of percentage of AM root length for *E. incanus* are shown in Table 2. In general, the extent of root colonization varied from about 90 to 100%. The uninoculated treatment presented native AM colonization and the values of frequency of colonization (% F) were not significantly different from inoculated *E. incanus* plants or plants under natural conditions (Table 2).

Typical AM structures such as intraradical hyphae, vesicles, and extraradical hyphae were observed in the roots of *E. incanus*, whereas arbuscules were not observed. Oblong vesicles (25–31 × 41–52 µm) were often observed within *E. incanus* root segments. Moreover, roots had root hairs and *Gigaspora* like auxiliary cells with spines in extraradical hyphae associated with them.

Regarding inoculation, *E. incanus* inoculated plants showed higher intensity of colonization (Figure 3) and frequency of extraradical hyphae (% Eh, Table 2) than uninoculated plants and plants under natural conditions. In natural conditions a significant decrease in vesicles (% V) values was also observed in roots of *E. incanus* (Table 2).

Higher colonization levels are not always indicative of enhanced growth responses because it is yet not clear if mycorrhizal function is reduced to the same extent as mycorrhizal colonization (Aerts 2003); however, in this study as well as in others reports (Matias et al. 2009) the intensity of AM colonization was related to higher plant growth.

Figure 4 shows the AMF sporulation at the studied *E. incanus* rhizosphere. In relation to spore numbers, *E. incanus* under inoculation showed significantly higher spore number (per 100 g soil) of *Gigaspora* than uninoculated plants. The average AM spore number (*E. incanus* in the experimental site) was 401–514 per 100 g air-dried soil. In *E. incanus* rhizosphere AM richness attained ten species (Table 3).

The species richness was lower (5 species) in inoculated plants than in uninoculated ones. This can be due to the more competitive inoculated AMF species, and to the fact that plant species respond differently to AMF resulting in alterations in AMF composition (Hart and Klironomos 2003).

Table 2. Mycorrhizal status of *E. incanus* in restored and preserved highland fields, Brazil

Site		AM (%)	V (%)	PC	Eh (%)	Ac
Restored	*E. incanus*	99.15[a]	65[a]	iv, rh	28[b]	–
	E. incanus inoculated	100[a]	60.8[a]	ov	67[a]	+
Natural	*E. incanus*	90[a]	25[b]	ov	20[b]	–

AM – arbuscular mycorrhizal colonization; V – vesicles, PC – pattern of AM colonization: iv – irregular vesicles; ov – oval vesicles; rh – root hair; Eh – extraradical hyphae; Ac – auxiliary cells. + presence, – absence. Different letters (compare means in column) indicate significant differences as determined by Tukey's HSD test ($P \leq 0.05$)

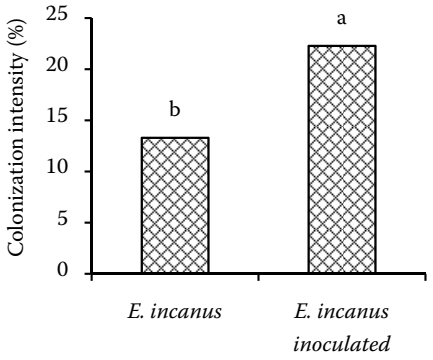

Figure 3. Intensity of AM root colonization of *E. incanus* under different treatments. Different letters indicate significant differences as determined by Tukey's HSD test ($P \leq 0.05$) ▦ *Scutellospora*

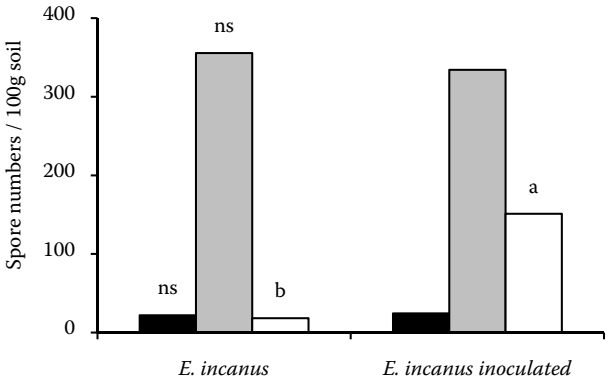

Figure 4. Spore number (per 100 g soil) from each of the arbuscular mycorrhizal fungi genus distinguished in the soil samples from the two treatments. Different letters indicate significant differences as determined by Tukey's HSD test ($P \leq 0.05$). ns – not significantly different. ■ *Glomus*, ■ *Acaulospora*, □ *Gigaspora*,

As would be expected, there were differences in AM species in the rhizosphere of plants under experimental and natural conditions. Under natural conditions five AM species were also observed; however, only two species were in common (Table 3).

In our study, ten taxa of AM fungi (Figure 5) were distinguished in the rooting zone soil samples, of which 7 were identified at the species level and 3 at the genus level (Table 3). Of the ten taxa, one belonged to the genus *Glomus*, four to *Acaulospora*, three to *Dentiscutata*, one to *Racocetra* and one to *Gigaspora*. Throughout this paper, we used the recent revision of *Scutellospora* by Oehl et al. (2008) since it is more practical for spore identification. Lugo et al. (2008) also found ten AMF species in the Puna highlands (Argentina), and notably two species (*A. spinosa* and *D. biornata*) were in common.

It has been frequently observed that in arid ecosystems four AMF genera are commonly found: *Acaulospora*, *Gigaspora*, *Glomus* and *Scutellospora* (Tao and Zhiwei 2005, Lugo et al. 2008). Three of them (*Acaulospora*, *Glomus* and *Scutellospora*) were reported by Gai et al. (2006) for semiarid Tibetan grasslands.

Table 3. AM spore diversity in *E. incanus* rhizosphere in a highland field in Brazil

AMF Species	Restored site		Natural site
	E. incanus	*E. incanus* inoculated	*E. incanus*
Gigasporaceae			
Gigaspora margarita Becker & Hall	X	X	X
Racocetraceae			
Racocetra verrucosa (Koake & C. Walker) Oehl, F.A.Souza & Sieverd.	X	X	–
Dentiscutataceae			
Dentiscutata biornata (Spain, Sieverd. & S. Toro)Sieverd., F.A.Souza & Oehl	X	–	–
Dentiscutata cerradensis (Spain & J.Miranda) Sieverd, F.A.Souza & Oehl	–	X	–
Dentiscutata sp. 1	–	–	X
Acaulosporaceae			
Acaulospora sp. 1	X	–	X
Acaulospora spinosa Walker & Trappe	X	–	–
A. elegans Trappe & Gerdemann	X	–	–
A. foveata Trappe & Janos	–	–	X
Glomeraceae			
Glomus spp. #	X	X	X

#total spores of *Glomus*

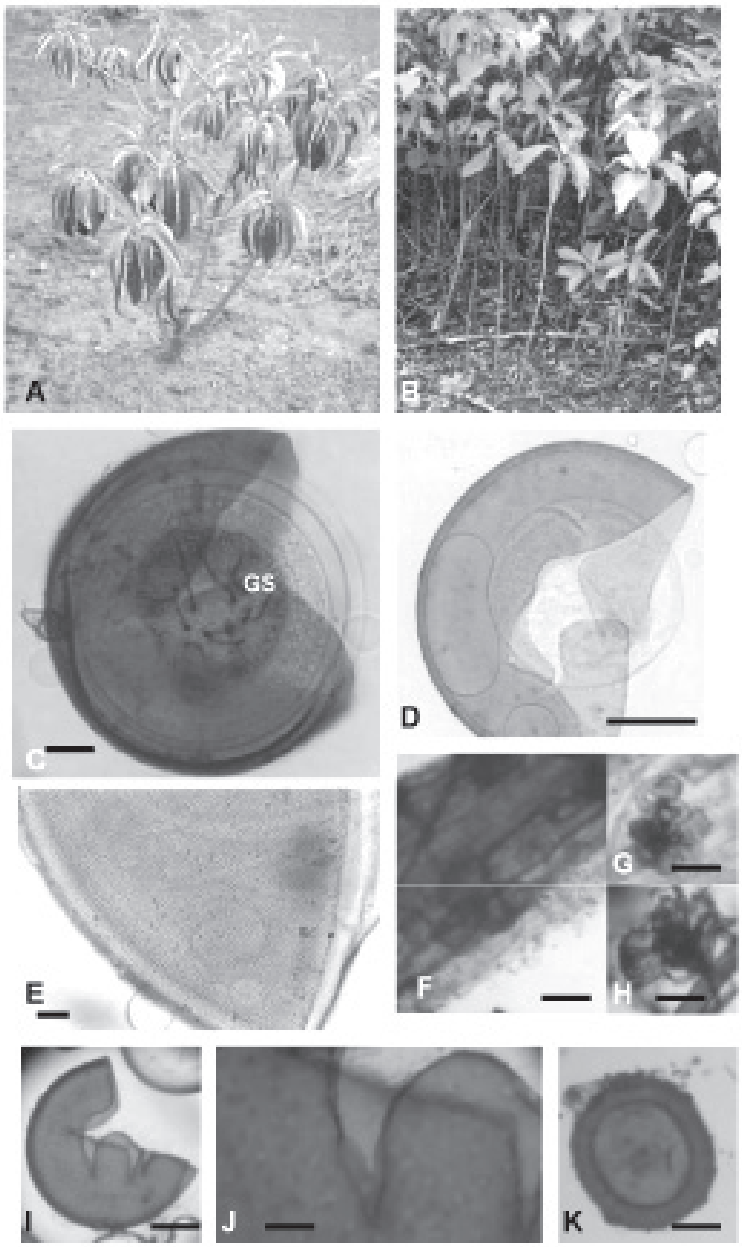

Figure 5. AM spores and colonization of the studied species. A: *E. incanus* in experimental site. B: seedlings in preserved site. C: *Dentiscutata biornata* spores showing the germination shield (GS). D: *Racocetra verrucosa*. E: *Acaulospora spinosa*. F: colonized root showing hypha often growing in parallel to each other, connected by distinct 'h' branching pattern. G–H auxiliary cells in *E. incanus* root. I–J: *A. elegans*, J: detail of ornamentation. K: *Glomus* sp. in PVLG. Bars for C, D = 100 µm; E, J = 10 µm G–H = 25 µm, F, I, K = 50 µm

In our study, *A. spinosa* and *D. cerradensis* were the most common species. It is surprising that *A. spinosa* was reported for arid sites in China (Tao and Zhiwei 2005) and arid sites with altitude in Argentina (Lugo et al. 2008).

In our study, *Gigaspora margarita* was found in both treatments, and also in the undisturbed area. Presence of *Gigaspora*-like auxiliary cells, with narrow projections observed in *E. incanus* roots (Figure 5), suggests a possible effectiveness of *G. margarita* spore inoculation. This AM species has a worldwide distribution and is commonly used as inoculum.

In other studies in natural highland fields (Matias et al. 2009, Pagano and Scotti 2009), higher spore number of *Glomus* was observed. In the present study, a higher spore number of *Acaulospora* than *Glomus* was found in the restored site (Figure 4), suggesting an abiotic or biotic effect on the AMF composition. The predominance of Acaulosporaceae could be associated with presence of pioneer plant species as reported by Córdoba et al. (2001) in foredunes. Some authors showed that *Acaulospora* tended to be more frequent in the worse (eroded) sites (Carpenter et al. 2001).

It is known that most *Scutellospora* species were described in warmer climates characterized by pronounced rainfall and a dry season (Tchabi et al. 2008). In our study, we recovered four previously named *Scutellospora* species, one in the pristine site and the rest in the restored site. *Dentiscutata biornata* (previously named *Scutellospora biornata*, Figure 5) seems to be a common AM species in highland fields. A different morphotype

of *Dentiscutata* was present in the more pristine site (Table 3).

E. incanus was shown to form a persistent soil seed bank, showing higher potential for regeneration in habitats subjected to disturbance (Velten and Garcia 2007), and the additional fact that *E. incanus* plants are AM-dependent in natural and restored highland field ecosystem supplies important information for restoration programs.

We can conclude that this highland field plant species contains natural AM fungal species richness that can be affected by land use (mining), and that restoration using this plant may be facilitated with AM fungal inoculation (spores).

Acknowledgements

Marcela Pagano is grateful to the Minas Gerais State Agency for Research and Development (FAPEMIG) (Process 311/07) for Post-doctoral scholarships granted from 2008 to 2009. Marta N. Cabello is a research scientist from the Comisión de Investigaciones Científicas (CIC) Bs. As.-Argentina. We would also like to thank Dr. Adriana Pagano (Graduate Program in Linguistics and Applied Linguistics – UFMG) for proofreading of the manuscript.

REFERENCES

Aerts R. (2003): The role of various types of mycorrhizal fungi in nutrient cycling and plant competition. In: Van der Heijden M.G.A., Sanders I.R. (eds.): Mycorrhizal Ecology. Springer, Berlin, 117–133.

Bohrer K., Friese C.F., Amon J.P. (2004): Seasonal dynamics of arbuscular mycorrhizal fungi in differing wetland habitats. Mycorrhiza, *14*: 329–337.

Carpenter F.L., Mayorga S.P., Quintero E.G., Schroeder M. (2001): Land-use and erosion of a Costa Rican Ultisol affect soil chemistry, mycorrhizal fungi and early regeneration. Forest Ecology and Management, *144*: 1–17.

Córdoba A.S., Mendonça M.M. de, Stürmer S.L., Rygiewicz P.T. (2001): Diversity of arbuscular mycorrhizal fungi along a sand dune stabilization gradient: a case study at Praia da Joaquina, Ilha de Santa Catarina, South Brazil. Mycoscience, *42*: 379–387.

Gai J.P., Feng G., Cai X.B., Christie P., Li X.L. (2006): A preliminary survey of the arbuscular mycorrhizal status of grassland plants in southern Tibet. Mycorrhiza, *16*: 191–196.

Giulietti A.M., Pirani J.R. (1988). Patterns of geographic distribu8 tion of some plant species from the Espinhaço Range, Minas Gerais and Bahia, Brazil. In: Heyer W.R., Vanzolini P.E. (eds.): Proceedings of a workshop on Neotropical distribution patterns. Academia Brasileira de Ciências, Rio de Janeiro, 39–69.

Hart M.M., Klironomos J.N. (2003): Diversity of arbuscular mycorrhizal fungi and ecosystem functioning. In: Van der Heijden M.G.A., Sanders I.R. (eds.): Mycorrhizal Ecology. Springer, Berlin, 75–92.

Lugo M.A., Ferrero M., Menoyo E., Estévez M.C., Siñeriz F., Anton A. (2008): Arbuscular mycorrhizal fungi and rhizospheric bacteria diversity along a altitudinal gradient in South American Puna grassland. Microbial Ecology, *55*: 705–713.

Matias S.R., Pagano M.C., Muzzi F.C., Oliveira C.A.,Carneiro A.A., Horta S.H., Scotti M.R. (2009): Effect of rhizobia, mycorrhizal fungi and phosphate-solubilizing microorganisms in the rhizosphere of native plants used to recover an iron ore area in Brazil. European Journal of Soil Biology.

Nogueira R.E., Pereira O.L., Kasuya M.C., Lanna M.C.S., Mendonça M.P. (2005): Mycorrhizal fungi associated to orchids growing in 'campos in Quadrilátero Ferrífero' region, Minas Gerais State, Brazil. Acta Botany Brasílica, *19*: 417–424.

Oehl F., de Souza F.A., Sieverding E. (2008): Revision of *Scutellospora* and description of five new genera and three new families in the arbuscular mycorrhiza-forming *Glomeromycetes.* Mycotaxon, *106*: 311–360.

Pagano M.C., Scotti M.R. (2009): A survey of the arbuscular mycorrhizaoccurrence in Paepalanthus bromelioides and Bulbostylis sp. in rupestrian fields, Brazil. Micologia Aplicada International, *21*: 1–10.

Rizzini C.T. (1997): Treaty of Phytogeography in Brazil: ecological, sociological and floristic aspects. 2nd Edition. Âmbito Cultural, São Paulo.

Somasegaran P., Hoben H.J. (1985): Methods in Legume-Rhizobium Technology. NIFTAL, Hawaii.

Tao L., Zhiwei Z. (2005): Arbuscular mycorrhizas in a hot and arid ecosystem in southwest China. Applied Soil Ecology, *29*: 135–141.

Tchabi A., Coyne D., Hountondji F., Lawouin L., Wiemken A., Oehl F. (2008): Arbuscular mycorrhizal fungal communities in sub-Saharan Savannas of Benin, West Africa, as affected by agricultural land use intensity and ecological zone. Mycorrhiza, *18*: 181–195.

Van der Heijden M.G.A., Sanders I.R. (2003): Mycorrhizal Ecology. Springer, Berlin.

Velten S.B., Garcia Q.S. (2007): Variation between three *Eremanthus* (Asteraceae) species in their ability to form a seed bank. Revista Brasileira de Botanica, *30*: 713–719.

Versieux L.M., Wendt T. (2007): Bromeliaceae diversity and conservation in Minas Gerais state. Brazil Biodiversity and Conservation, *16*: 2989–3009.

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

Received on May 25, 2009

Corresponding author:

Marcela Claudia Pagano, Federal University of Minas Gerais, Institute of Biological Sciences, Av. Antônio Carlos, 6627, Pampulha, CEP: 31270-901, Belo Horizonte, MG, Brazil
phone: + 55 31 968 140 66, fax: + 55 31 340 926 71, e-mail: marpagano@gmail.com