Efficacy and persistance of introduced AM fungi in rehabilitation processes.

Estaún V, Busquets M, Calvet C. Camprubí A.

IRTA Investigació i tecnologia agroalimentàries. Protecció Vegetal. Cabrils. Cra de Cabrils Km 2 , 08348 Cabrils (Barcelona) Spain

Introduction

In semiarid climates the establishment of a plant cover is the most important step in the restoration of degraded areas, such as quarries or urban wastelands, to avoid further degradation and desertification.. Under Mediterranean conditions the restoration becomes difficult because of the many constraints associated with the climate (dry periods followed by torrential rains) and the soil (shallow, stony soils with low organic matter and high pH). In most instances the plants used in these projects are grown in the nursery under supra-optimal conditions and do not present any mycorrhizal symbiosis. The success level of these re-vegetations is low (Sort & Alcañiz, 1996). The use of organic fertilizers such as sewage sludge has increased the success of the plants survival (Sort & Alcañiz, 1996), however it has as a draw-back considering the development of many volunteer plants that are non-mycorrhizal and extremely aggressive and can delay the targeted species growth and development, besides added problems of soil and water contamination. These problems have prevented the spread of this practice in sensitive areas. Mycorrhiza are reported to reduce the detrimental effects on plant growth of soil-associated stresses such as lack of nutrients, high pH and climate associated stresses such as drought and high temperatures (Requena *et al*, 2001; Caravaca *et al*, 2003; Alguacil *et al*, 2005), however the effect of the symbiosis under field conditions is sparsely documented and with diverse results (Clemente *et al*, 2005).
The establishment of a plant cover can be done transplanting shrubs and /or trees or casting seed in the areas to be revegetated. Both strategies are routinely used, depending on the accessibility of the area to be reclaimed and on economic issues. Some rehabilitation projects contemplate the use of a nurse crop of herbaceous species to stop the erosion process and improve the soil for future rehabilitation activities (Skousen and Zipper, 1996). In this paper we present the results of pre-transplant inoculation with AMF on the re-vegetation of two quarries and one urban wasteland using native shrubs and the establishment of an herbaceous crop directly sown with a hydroseeder as a first rehabilitation step. The urban small open space was included in the experiment due to the growing importance of these areas in reclamation projects.

Materials and methods

Quarry re-vegetation
The first quarry studied is located in Mont-Ral (Tarragona, Spain; 41º 16'N 1º07'E). The quarry has been mined for several decades for the extraction of stone blocks for ornamental use. The experimental area covered a terrace of 10.000m^2 formed by stocked limestone gravel and other debris. Topsoil originally retrieved from the area to allow the excavation and stored in piles was spread over the terrace to provide a substrate for revegetation. (Photo1)

Photo 1: Re-vegetation of the quarry with added top-soil

The second quarry studied is located in Castellar del Vallés (Barcelona Spain, 41º 36' N 2º03'E). The quarry has been mined extensively to obtain ground stone for building and production of cement. The experimental area was a dumping area with unusable ground rock debris with no added topsoil. (Photo2)

Photo 2: Re-vegetation of the quarry with no added top-soil

The plants used for the experiment were: *Lavandula angustifolia* Mill. and *Santolina chamecyparissus* L. for both quarries with *Juniperus phoeniceae* L. and *Thymus vulgaris* L., for the first quarry and *Anthyllis citisoides* L. and *Rosmarinus officinalis* L. for the second quarry The AM inoculum used was a mixture of roots and rhizosphere substrate of leek plants inoculated with *Glomus intraradices* Schenk &Smith BEG72 and grown in Terragreen. The growth parameters evolution was estimated as plant volume for *L. angustifolia*, *T. vulgaris*, *S. chamaecyparissus, R. officinalis* and *A. cytisoides* and as plant height for *J. phoenicia*. To assess mycorrhizal colonisation composite samples were taken from rhizosphere soil with a soil core borer in three points chosen at random for each of the repeated treatments of 6 plants, samples were observed under a binocular microscope to evaluate mycorrhizal colonization (Koske and Gema,1989; Giovanetti and Mosse, 1980)
To assess the diversity and persistence of the introduced AM fungus in the area after 23 months growth, eight 1cm root pieces of each of the root composite samples (inoculated and non-inoculated) were used for DNA extraction using a chelex extraction method (van Tuinen et al. 1998, Kjøller & Rosendahl 2000). Primary PCR was performed with the eukaryote specific primers LSU0061 (LR1) and LSU0599 (NDL22) followed by nested PCR with the primer combinations LSURK4f and LSURK7r (van Tuinen et al. 1998, Kjøller & Rosendahl 2000) and FLR3 and FLR4 (Gollotte et al. 2004). PCR were performed as described by Kjøller & Rosendahl (2000). All positive PCR were

sequenced using RK4f or FLR3 as sequencing primer. Parsimony analyses were conducted in PAUP.

Urban open space re-vegetation

The small urban space is located in Badalona (Barcelona Spain, 41º26'N 2º13'E), it is a small layout, in a slope, between blocks of apartments, underutilised and unattractive. The soil was heavily degraded and contained building debris from nearby construction sites. The plants used for this experiment were *Rosmarinus officinalis* L. inoculated with AMF or not in the nursery.

Hydroseeding experiment

The hydroseeding system was used to improve soil coverage and erosion control in the quarry where no topsoil was added and in a small waste land in an urban location. The plants used were the legumes *Medicago lupulina* and *Lotus corniculatus* and the grasses *Lolium perenne*, *Festuca ovina* and *Brachypodium. phoenicoides*. The fungus, *G. intraradices*, was also chosen according to the results of a previous experiment (Estaún et al, 2006). The results of the hydroseeding procedure were evaluated in the field and in greenhouse conditions. Three months after the hydroseeding procedure, the number of grasses and legumes in five squares of 20cm x 20cm in each of the replicated treatments in the field locations was recorded. Plants under greenhouse conditions were harvested and the shoot weight of legumes and grasses was recorded separately, the root weight/cm^2 and the root colonisation were determined.

Results and Discussion

Quarry re-vegetation

All plants established the mycorrhizal symbiosis at the nursery. At transplant inoculated and non-inoculated plants were similar in size.

After 8 months growth in the quarry with added top soil all control plants sampled presented the symbiosis and there were no differences in plant growth (Photo 3) Molecular probes show that the most widespread fungi were *G. intraradices*, and *Glomus microaggregatum* although other fungi were present in the roots of the control plants(Figure1a).

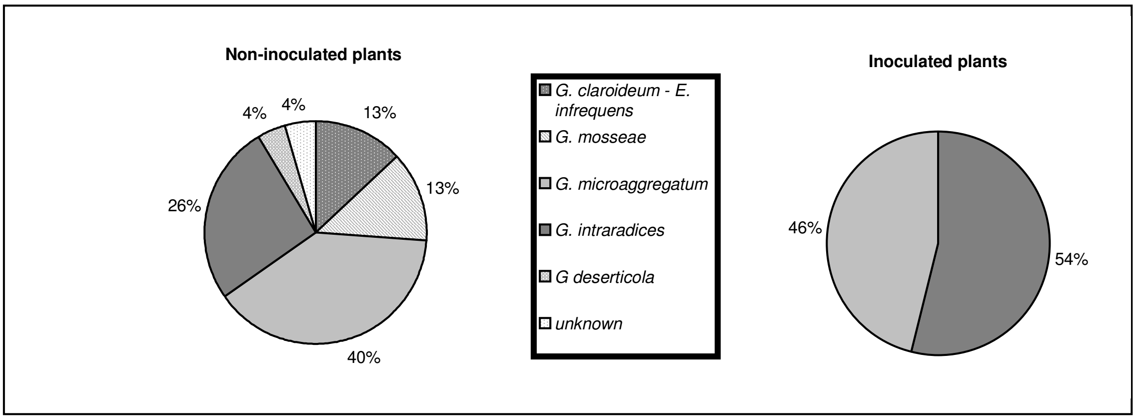

Figure 1a: Diversity of AM fungi detected in mycorrhizal roots of non-inoculated and inoculated shrubs 23 months after transplant in the quarry with added topsoil

In the quarry where no top soil was added all inoculated plants grew better, although 60% of the non inoculated plants sampled presented the symbiosis. *Glomus intraradices* was the only fungus detected in the roots of the inoculated plants whilst in the roots of the control plants other fungi were found (Figure 1b).

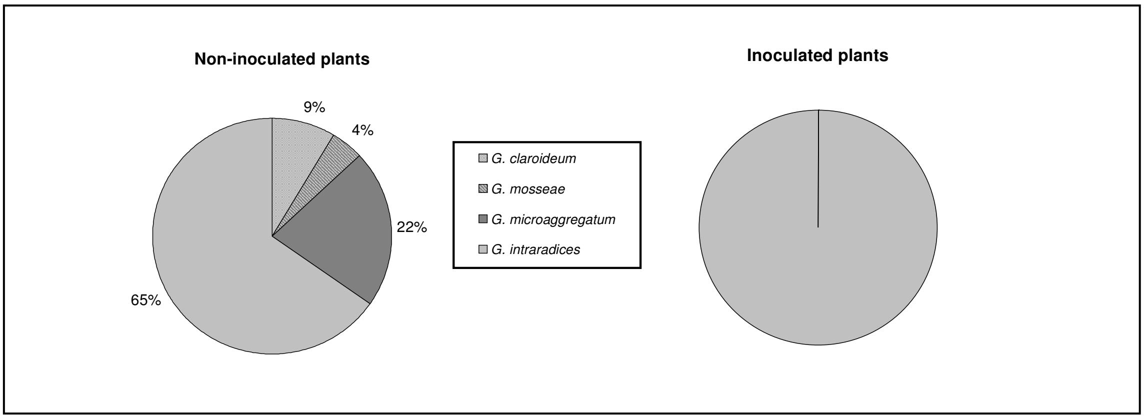

Figure 1b: Diversity of AM fungi detected in mycorrhizal roots of non-inoculated and inoculated shrubs 23 months after transplant in the quarry with no added topsoil

Our results show that adding stored top soil is a good system to enhance mycorrhizal colonisation in quarry restoration; otherwise it is important to inoculate the plants used with an effective fungus. Molecular probes show that in the Mediterranean ecosystems studied, with eroded soils and high pH, the AMF species diversity is very low and *G. intraradices* is present in a high percentage of the samples, indicating the resilience and adaptation to these conditions of this species.

Urban open space re-vegetation

As many of the areas that need rehabilitation often are in pronounced slopes, the growth and development of *Rosmarinus officinalis* plants inoculated and non-inoculated with *G. intraradices* was evaluated in different gradient slopes. The inoculation at the nursery level was found to increase plant growth and coverage in the two gradients of slope considered (Figure 2).

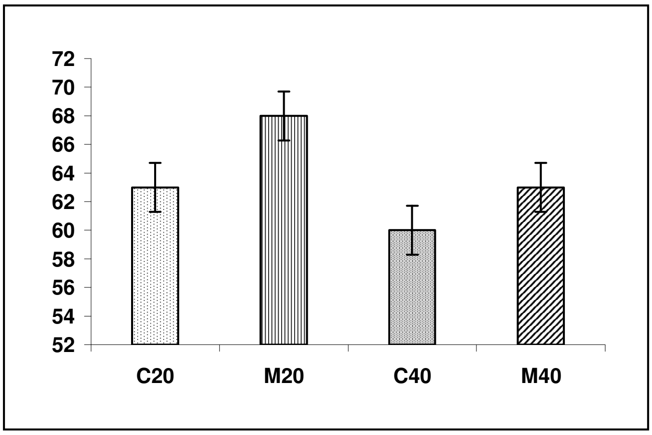

Figure 2: Height of *R. officinalis* plants grown in 20º and 40º slopes inoculated with G. intraradices (M) or non-inoculated (C) 12 months after trasplant.

For the rehabilitation of the urban layout, *R. officinalis* was considered to be the most adequate plant due to its soil and climate stress resistance and its aromatic and ornamental properties. Nursery inoculated plants survived transplant and the summer drought stress (Figures 3a and 3b) (Photo 4). The inoculation of plants under these circumstances is cost effective due to the high costs of labour if plants need to be replaced.

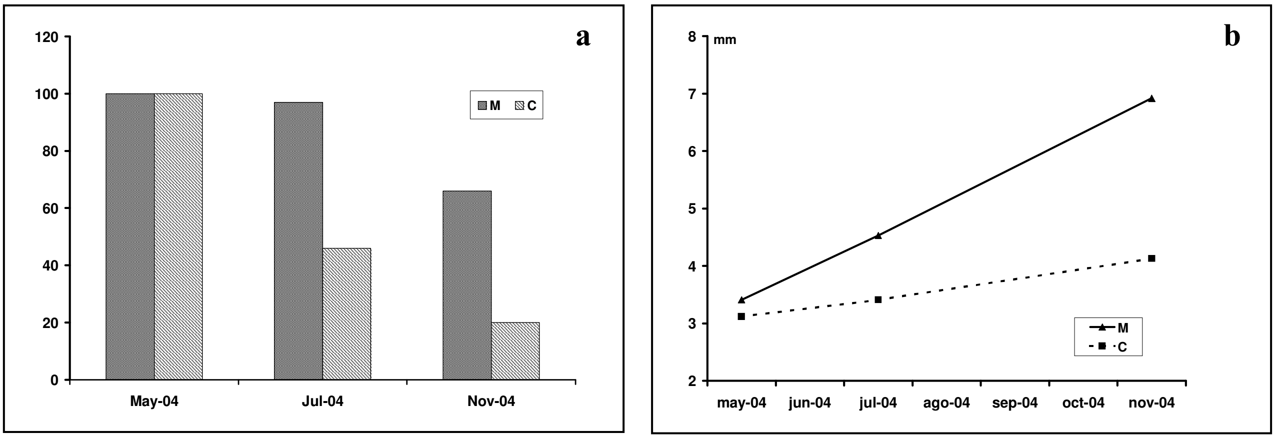

Figure 3: Survival **(a)** and growth **(b)** of *R. officinalis* plants grown in a urban layout inoculated with G. intraradices (M) or non-inoculated (C) 8 months after trasplant.

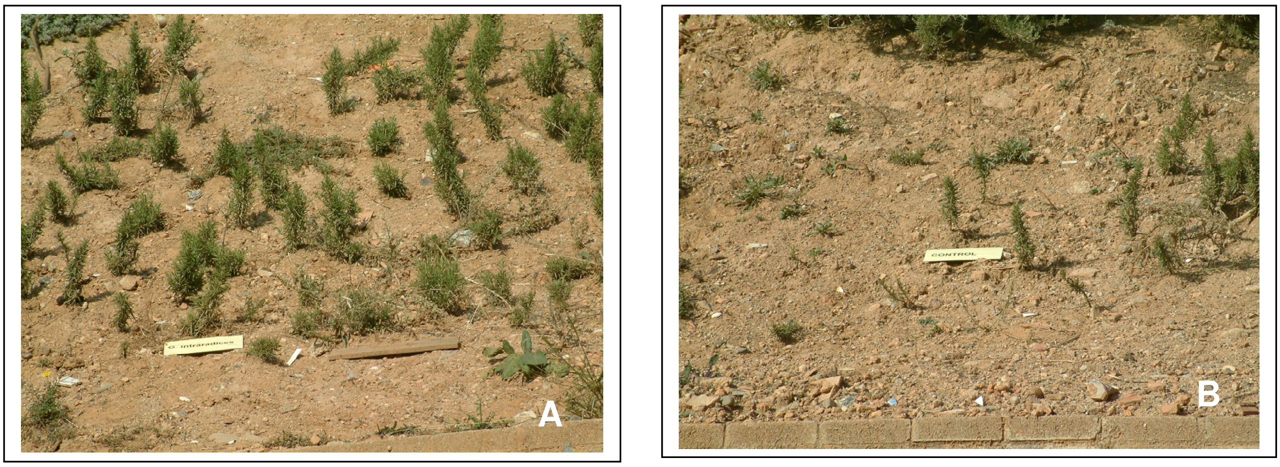

Photo 4: Re-vegetation of a urban lay-out with R. officinalis 8 months after plant establishment (A. Plants inoculated with G. intraradices, B: plants non-inoculated)

Hydroseeding experiment

The addition of inocula increased the total weight of plants recovered both in the greenhouse and in the field experiments (Figure 6) (Photo5). The same results apply for the analysis of the legumes dry weight, where the weight of legumes in the mycorrhizal treatments was increased by tenfold respect to the non-mycorrhizal treatments. Considering the dry weight of grasses there is no significant effect of the inoculation with *G. intraradices* although there is a significant effect of the inoculation in the legumes/grasses ratio, which is higher in all mycorrhizal treatments.

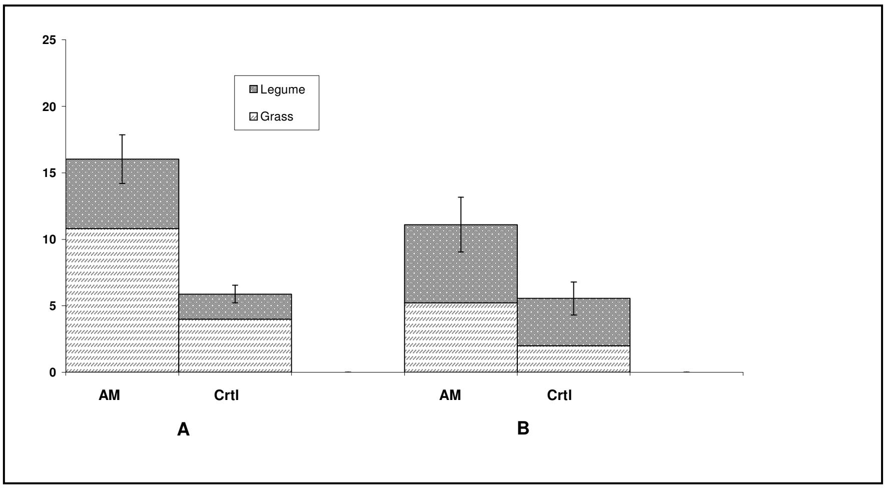

Figure 4: Total number of plants and numbers of legumes and grasses in two locations (A and B) three months after the hydroseed application. Data are means of the plants observed in five 20cm x 20 cm squares in each of the three replicates per treatments per location ± 1.98SE of total number of plants

Photo 5: Hydroseeding of a urban layout 3 months after application (A: non-inoculated, B: inoculated with *G. intraradices*)

Conclusions

Transplanting native plants inoculated with AMF is a good system to establish shrubs and trees in eroded semi-arid lands (Requena *et al*,2001; Caravaca *et al*, 2003; Alguacil *et al*, 2005) but it is impracticable when considering large areas (Greipsson and El-Mayas 1999). The integration of the AMF inoculation with the hydroseeding technique might permit the use of mixtures of native grasses and legumes, and can facilitate the establishment of other mycorrhizal plants (Enkhuya *et al*, 2005) in large or inaccessible areas where transplanting cannot be considered as an option for rehabilitation.

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