LAND DEGRADATION & DEVEDLOPMENT

Land Degrad. Develop. **15**: 153–161 (2004)

Published online in Wiley InterScience (www.interscience.wiley.com). **DOI**: 10.1002/ldr.600

AM FUNGI INOCULATION AND ADDITION OF MICROBIALLY-TREATED DRY OLIVE CAKE-ENHANCED AFFORESTATION OF A DESERTIFIED MEDITERRANEAN SITE

F. CARAVACA,[1]* M. M. ALGUACIL,[1] M. VASSILEVA,[2] G. DÍAZ[3] AND A. ROLDÁN[1]

[1]*CSIC-Centro de Edafología y Biología Aplicada del Segura, Department of Soil and Water Conservation, PO Box 164, Campus de Espinardo, 30100-Murcia, Spain*

[2]*CSIC-Estación Experimental del Zaidín, Microbiology Department, Profesor Albareda, 1, 18008-Granada, Spain*

[3]*SACE-University of Murcia, Campus de Espinardo, 30100-Murcia, Spain*

Received 2 September 2003; Revised 28 October 2003; Accepted 5 November 2003

ABSTRACT

A field experiment was carried out to compare the effectiveness of inoculation with three arbuscular mycorrhizal (AM) fungi, namely *Glomus intraradices* Schenck & Smith, *Glomus deserticola* (Trappe, Bloss. & Menge) and *Glomus mosseae* (Nicol & Gerd.) Gerd. & Trappe, and the addition of *Aspergillus niger*-treated dry olive cake (DOC) in the presence of rock phosphate, in increasing root nitrate reductase (NR) and acid phosphatase activities, mycorrhizal colonization, plant growth and nutrient uptake in *Dorycnium pentaphyllum* L. seedlings afforested in a semiarid degraded soil. Three months after planting, both the addition of fermented DOC and the mycorrhizal inoculation treatments had increased root NR activity significantly, particularly the inoculation with *G. deserticola* (by 75 per cent with respect to non-inoculated plants), but they had no effect on root acid phosphatase. Mycorrhizal inoculation treatments with *G. deserticola* or *G. mosseae* on their own were even more effective than the addition of fermented DOC alone in improving the growth and (NPK) foliar nutrients of *D. pentaphyllum* plants. The combined treatment involving the application of microbially-treated agrowastes and mycorrhizal inoculation with AM fungi, particularly with *G. mosseae*, can be proposed as a successful revegetation strategy for *D. pentaphyllum* in P-deficient soils under semiarid Mediterranean conditions. Copyright © 2004 John Wiley & Sons, Ltd.

KEY WORDS: *Glomus deserticola*; *Glomus intraradices*; *Glomus mosseae*; semiarid areas; fungi inoculation

INTRODUCTION

The establishment of a plant cover based on the use of seedlings with an optimized microbiological and physiological status is paramount in order to carry out successful reafforestation programmes in desertified ecosystems, particularly those developed under Mediterranean environments (Requena *et al.*, 2001). Drought-tolerant, native shrub species have been recommended for re-establishment of functional shrublands and recovery of desertified Mediterranean ecosystems. Previous studies have demonstrated that woody legumes, which form symbioses with arbuscular mycorrhizal (AM) fungi, are useful for such nutrient-deficient, arid ecosystems because their associated rhizobial symbioses constitute a source of N input for the ecosystem (Requena *et al.*, 2001). *Dorycnium pentaphyllum* L. is a leguminous, low-growing shrub, which belongs to the natural succession in certain plant communities of semiarid Mediterranean ecosystems in the southeast of Spain. It is also well-adapted to water stress conditions and, therefore, potentially could be used in the revegetation of semiarid disturbed lands. However, knowledge of reafforestation strategies involving *D. pentaphyllum* is still very scarce.

*Correspondence to: Dr F. Caravaca, CSIC-Centro de Edafología, Department of Soil and Water Conservation, PO Box 164, Campus Espinardo, 30100-Murcia, Spain.
E-mail: fcb@cebas.csic.es

Inoculation with symbiotic microorganisms, especially arbuscular-mycorrhizal (AM) fungi, is an effective method of enhancing the ability of the host plants to become established and to cope with stress situations such as nutrient deficiency, drought and soil disturbance. In fact, several authors have indicated that mycorrhizal fungi may improve the performance of seedlings, either by stimulating water uptake (Augé, 2001) or by increasing nutrient uptake by the plant, particularly N and P (Jeffries *et al.*, 2003). Nitrate has been suggested as being the preferential N source for AM fungi associated with plants grown in neutral to alkaline soils (Azcón *et al.*, 2001). Uptake and transport of nitrate occur through the extraradical mycelium of AM fungi (Tobar *et al.*, 1994). An increase in activity of nitrate reductase (NR), which catalyses the rate-limiting step in the nitrate assimilation pathway, has been shown for both roots and shoots of AM plants (Azcón and Tobar, 1998). NR activity has been proposed as an index for assessing the effectiveness of fungus–host plant combinations for mitigation of water-deficit stress (Caravaca *et al.*, 2003a). The presence of such an enzymatic activity in AM fungi (Kaldorf *et al.*, 1998) has also been shown. However, this effect depends on the associated mycorrhizal fungus.

The quality and productivity of forested soils can be improved by the addition of organic amendments to the soil (Caravaca *et al.*, 2003b). The effectiveness of such amendments depends greatly on their stability. The use of agrowastes of lignocellulosic nature, such as dry olive cake (DOC), has been seen to have a detrimental effect on seed germination, plant growth and microbial activity. In fact, several studies have reported the phytotoxic and antimicrobial effects of olive-mill residues due to their phenol, organic acid and fatty acid contents (Linares *et al.*, 2003). Efforts to decrease the environmental impact of olive-mill wastes include biological fermentation with filamentous fungi, such as *Aspergillus niger* (Vassilev *et al.*, 1995), or white-rot fungi (Linares *et al.*, 2003). Such bio-systems, involving agrowastes and microorganisms, have been used for rock-phosphate (RP) solubilization and improvement of crop plant growth and nutrition in agricultural soils (Vassilev *et al.*, 1996). However, no information is available on the use of such materials in revegetation programmes.

The objective of this study was to compare the effectiveness of inoculation with three AM fungi and the addition of fermented DOC residue with respect to increasing nitrate reductase and acid phosphatase activities in roots, mycorrhizal colonization, plant growth and nutrient uptake in *D. pentaphyllum* seedlings afforested in a semiarid degraded soil.

MATERIALS AND METHODS

Study Sites

The experimental area was located in Los Cuadros in the Province of Murcia (southeast Spain) (coordinates: 1° 05′W and 38° 10′N). The climate is semiarid Mediterranean with an average annual rainfall of 300 mm and a mean annual temperature of 19·2°C; the potential evapo-transpiration reaches 1000 mm y^{-1}. The loam soil used was a Typic Haplocalcid (Soil Survey Staff, 1999) developed from Quaternary sediments (Table I).

Materials

The DOC obtained from an olive-mill located in Granada (Spain), was dried at 60°C and then ground to pass a 2 mm-pore screen. Portions of 5 g of DOC were mixed with 50 ml of Czapek solution (agar 15·0 g L^{-1}; di-potassium hydrogen phosphate 1·0 g L^{-1}; iron(II) sulfate heptahydrate 0·01 g L^{-1}; potassium chloride 0·5 g L^{-1}; magnesium sulfate heptahydrate 0·5 g L^{-1}; sodium nitrate 3·0 g L^{-1}; sucrose 30·0 g L^{-1}; pH = 7·3) for static fermentation in 250 ml Erlenmeyer flasks. Rock phosphate (Morocco fluorapatite, 12·8 per cent P, 1 mm mesh), was added at a rate of 0·75 g per flask. Media were sterilized by autoclaving at 120°C for 30 minutes. A spore suspension of *Aspergillus niger* NB2 ($1{\cdot}2 \times 10^7$) was spread carefully over the surface of the media. The mixture was allowed to ferment at 30°C for 20 days without shaking. The characteristics of the DOC after fermentation were: pH, 4·0; electrical conductivity (1:10), 1231 μS cm^{-1}; total P, 0·38 per cent; total N, 0·62 per cent; total organic C, 22·2 per cent and water soluble C, 1146 μg g^{-1}.

One representative leguminous shrub from this area, found generally in semiarid shrublands in southeastern Spain, namely *D. pentaphyllum*, was used for the reafforestation experiment.

Table I. Chemical, biochemical, microbiological and physical characteristics of the soil used in the experiment

pH (H_2O)	8·5 ± 0·0*
EC (1:5, μS cm^{-1})	225 ± 2
Texture	loam
Total organic C (g kg^{-1})	10·3 ± 0·3
Total carbohydrates (μg g^{-1})	552 ± 20
Water soluble C (μg g^{-1})	100 ± 1
Water soluble carbohydrates (μg g^{-1})	8 ± 0
Total N (g kg^{-1})	0·95 ± 0·02
Available P (μg g^{-1})	7 ± 0
Extractable K (μg g^{-1})	222 ± 4
Microbial biomass C (μg g^{-1})	396 ± 11
Dehydrogenase (μg INTF g^{-1})	51 ± 1
Urease (μmol NH_3 g^{-1} h^{-1})	0·31 ± 0·03
Protease-BAA (μmol NH_3 g^{-1} h^{-1})	0·60 ± 0·04
Phosphatase (μmol PNP g^{-1} h^{-1})	0·28 ± 0·02
β-Glucosidase (μmol PNP g^{-1} h^{-1})	0·46 ± 0·01
Aggregate stability (%)	11·5 ± 0·4
Bulk density (g cm^{-3})	1·10 ± 0·02

*Mean ± standard error ($N = 6$).

Mycorrhizal Inoculation of Seedlings

The mycorrhizal fungi used in the experiment, *Glomus intraradices* Schenck & Smith, *Glomus deserticola* (Trappe, Bloss. & Menge) and *Glomus mosseae* (Nicol & Gerd.) Gerd. & Trappe, were obtained from the collection of the experimental field station of Zaidín, Granada.

AM fungal inoculum consisted of a mixture of rhizospheric soil from trap cultures (*Sorghum* sp.) containing spores, hyphae and mycorrhizal root fragments. Once germinated from seeds of *D. pentaphyllum* collected from the experimental area, seedlings were transplanted into the growth substrate, consisting of peat and cocopeat (1:1, v:v). The corresponding arbuscular mycorrhizal inoculum was applied at a rate of 5 per cent (v/v). The same amount of an autoclaved mixture of the inocula was added to control plants, supplemented with a filtrate (<20 μm) of culture to provide the microbial populations accompanying the mycorrhizal fungi. Inoculated and non-inoculated seedlings were grown for eight months under nursery conditions without any fertilizer treatment.

Experimental Design and Layout

A factorial design in randomized blocks was established with two factors and five-fold replication. The first factor was the addition of fermented DOC residue to the soil, and the second was the inoculation of *D. pentaphyllum* plants with three AM fungi (*G. intraradices*, *G. deserticola* or *G. mosseae*) in the nursery.

Each replication plot (five plots in total) was 180 m^2. Planting holes 40 × 40 cm long and 30 cm deep were dug manually. In early February 2003, fermented DOC residue was added to half of the holes (0–20 cm depth) and manually mixed with the soil at a rate of 5 per cent. The seedlings (inoculated and non-inoculated) were planted at least 1 m apart between holes, with 3 m between blocks. At least 64 seedlings per block were planted (eight plants × eight treatments in each block).

Measurements

Three months after planting, coinciding with the end of flowering stage, five plants of each treatment were harvested. Basal stem diameters and heights of plants were measured, fresh and dry (105°C, 5 h) mass of shoots and roots were recorded. Plant tissues were ground prior to chemical analysis. Foliar concentrations of P and K were determined after digestion in nitric–perchloric acid (5:3) for 6 h. Foliar P was determined by colorimetry (Murphy and Riley, 1962), foliar N was colorimetrically measured after Kjeldhal digestion and foliar K was estimated by flame photometry (Schollemberger and Simon, 1954).

The percentage of root length colonized by AM fungi was calculated by the gridline intersect method (Giovannetti and Mosse, 1980) after staining with trypan blue (Phillips and Hayman, 1970).

Nitrate reductase activity was assayed *in vivo* by measuring NO_2^- production in tissue that has been vacuum infiltrated with buffered NO_3^- solutions (Downs *et al.*, 1993). The roots and shoots from the seedlings were collected in the morning between 08:30 and 11:00 solar time. Roots and shoots of *D. pentaphyllum* were cut into 5 mm sections. Approximately 300 mg of tissue was placed into tubes containing 2 mL of an incubation medium consisting of 0·05 M tris-HCl pH 7·8 and 0·25 M KNO_3. The tubes were sealed and kept in the dark at 30°C for 1 h. The nitrite released into the medium was determined after incubation by treating 1 ml of the aliquots with 1 ml of 1 per cent sulphanilamide in 1 M HCl and 1 ml of 0·01 per cent *N*-1-napthyl-ethylenediamine hydrochloride (NNEDA). After 15 min, the optical density was measured at 540 nm with a Beckman spectrophotometer (Keeney and Nelson, 1982).

Acid phosphatase activity was determined using *p*-nitrophenyl phosphate disodium (PNPP, 0·115 M) as substrate. Two mL of 0·5 M sodium acetate buffer at pH 5·5 using acetic acid and 0·5 ml of substrate were added to 100 mg of fresh root tissue and incubated at 37°C for 90 minutes. The reaction was stopped by cooling at 2°C for 15 min. Then, 0·5 ml of 0·5 M $CaCl_2$ and 2 ml of 0·5 M NaOH were added, and the mixture was centrifuged at 4000 rpm for 5 min. The *p*-nitrophenol (PNP) formed was determined by spectrophotometry at 398 nm (Tabatabai and Bremner, 1969). Controls were made in the same way, although the substrate was added before the $CaCl_2$ and NaOH.

Statistical Analysis

Data were log transformed to achieve normality. Fermented DOC addition, mycorrhizal inoculation and their interaction effects on measured variables were tested by a two-way analysis of variance and comparisons among means were made using the least significant difference (LSD) test, calculated at $p < 0{\cdot}05$. Statistical procedures were carried out with the software package SPSS 10·0 for Windows.

RESULTS

Growth Parameters of D. pentaphyllum

The percentage of plant survival oscillated between about 80 and 95 per cent and there were no significant differences between treatments. At the time of planting, the shoot and root dry weights, height and basal diameter of inoculated *D. pentaphyllum* plants were slightly greater than for non-inoculated plants but the differences were not significant (Table II). Three months after planting, both the addition of fermented DOC and mycorrhizal inoculation treatments had significantly stimulated the production of shoot biomass, height and basal diameter of *D. pentaphyllum* with respect to the control plants (Tables II and III). Only mycorrhizal inoculation treatments had a significant effect on the root biomass of the plants (Table III). The combined treatment of fermented DOC addition and inoculation with *G. mosseae* had the strongest effect on shoot biomass of *D. pentaphyllum* (on average 328 per cent greater than the control plants).

Foliar Nutrients and Mycorrhizal Infection of D. pentaphyllum

Foliar N, P and K contents in inoculated *D. pentaphyllum* seedlings were significantly higher than in non-inoculated plants previous to planting in the field (Table IV). The inoculated plants had an average of 77, 60 and 61 per cent of their short lateral roots colonized by *G. intraradices*, *G. deserticola* and *G. mosseae*, respectively (Table IV). The roots of the non-inoculated plants were not colonized by mycorrhizal fungi.

With the exception of *G. intraradices*, mycorrhizal inoculation treatments were more effective than fermented DOC addition at increasing foliar nutrients in *D. pentaphyllum* seedlings three months after planting (Tables III and IV). The highest values for foliar N, P and K contents were recorded in the plants inoculated with *G. mosseae* and grown in the amended soil. Inoculation with *G. intraradices*, *G. deserticola* or *G. mosseae* had significantly enhanced AM colonization after three months in the field, similar levels of root colonization being reached in all the mycorrhizal inoculation treatments (Tables III and IV).

Table II. Growth parameters of *D. pentaphyllum* seedlings in response to mycorrhizal inoculation treatments and fermented DOC addition previous planting and three months after planting ($n = 5$)

	Months	
	0	3
Shoot (g dw)		
C	0·78 ± 0·02*	1·25 ± 0·05
A	0·78 ± 0·02	2·66 ± 0·10
G1	0·92 ± 0·07	2·77 ± 0·05
AG1	0·92 ± 0·07	4·18 ± 0·02
G2	1·02 ± 0·08	3·51 ± 0·05
AG2	1·02 ± 0·08	4·54 ± 0·10
G3	0·83 ± 0·03	4·30 ± 0·02
AG3	0·83 ± 0·03	5·35 ± 0·10
Root (g dw)		
C	0·71 ± 0·02	1·11 ± 0·03
A	0·71 ± 0·02	1·89 ± 0·06
G1	0·94 ± 0·07	2·08 ± 0·06
AG1	0·94 ± 0·07	1·48 ± 0·04
G2	1·00 ± 0·06	2·61 ± 0·06
AG2	1·00 ± 0·06	1·79 ± 0·07
G3	0·82 ± 0·03	1·70 ± 0·03
AG3	0·82 ± 0·03	1·89 ± 0·06
Height (cm)		
C	38 ± 1	31 ± 1
A	38 ± 1	36 ± 2
G1	40 ± 1	44 ± 1
AG1	40 ± 1	53 ± 2
G2	46 ± 1	49 ± 2
AG2	46 ± 1	51 ± 1
G3	45 ± 2	42 ± 1
AG3	45 ± 2	55 ± 1
Basal diameter (mm)		
C	1·8 ± 0·0	2·4 ± 0·0
A	1·8 ± 0·0	2·9 ± 0·1
G1	2·0 ± 0·0	3·0 ± 0·1
AG1	2·0 ± 0·0	3·6 ± 0·1
G2	2·1 ± 0·1	3·3 ± 0·1
AG2	2·1 ± 0·1	3·5 ± 0·1
G3	1·9 ± 0·0	3·2 ± 0·1
AG3	1·9 ± 0·0	3·9 ± 0·1

C = control; A = fermented DOC addition; G1 = inoculation with *G. intraradices*; AG1 = fermented DOC addition and inoculation with *G. intraradices*; G2 = inoculation with *G. deserticola*; AG2 = fermented DOC addition and inoculation with *G. deserticola*; G3 = inoculation with *G. mosseae*; AG3 = fermented DOC addition and inoculation with *G. mosseae*.
*Mean ± standard error.

Nitrate Reductase and Acid Phosphatase Activities in Roots of D. pentaphyllum

Most of the NR activity was in the roots of *D. pentaphyllum*, since activity was not detected in the shoots of these seedlings. Both the addition of fermented DOC and mycorrhizal inoculation treatments increased significantly root NR activity (Tables III and V), particularly the inoculation with *G. deserticola* (by 75 per cent with respect to

Table III. Two factor ANOVA (mycorrhizal inoculation treatments and fermented DOC addition) for all parameters studied in the rhizosphere soil of *D. pentaphyllum* seedlings three months after planting—*p* significance values

	Amendment (A)	Mycorrhiza (M)	Interaction (A × M)	Comparison between means					
				C vs. G1	C vs. G2	C vs. G3	G2 vs. G1	G3 vs. G1	G3 vs. G2
Shoot	<0·001	<0·001	0·002	<0·001	<0·001	<0·001	<0·001	<0·001	<0·001
Root	0·299	<0·001	<0·001	0·070	<0·001	0·056	0·007	0·919	0·009
Height	<0·001	<0·001	0·002	<0·001	<0·001	<0·001	0·223	0·923	0·189
Basal diameter	<0·001	<0·001	0·027	<0·001	<0·001	<0·001	0·253	0·003	0·050
Foliar N	<0·001	<0·001	0·001	<0·001	<0·001	<0·001	0·901	<0·001	<0·001
Foliar P	<0·001	<0·001	<0·001	<0·001	<0·001	<0·001	<0·001	<0·001	<0·001
Foliar K	<0·001	<0·001	0·001	<0·001	<0·001	<0·001	0·982	<0·001	<0·001
Colonization	0·649	<0·001	0·853	<0·001	<0·001	<0·001	0·102	0·078	0·895
Nitrate reductase	0·001	<0·001	0·480	0·003	<0·001	0·066	0·031	0·204	0·001
Acid phosphatase	0·654	0·481	0·905	0·312	0·457	0·301	0·789	0·487	0·356

C = control with and without fermented DOC addition; G1 = inoculation with *G. intraradices* with and without fermented DOC addition; G2 = inoculation with *G. deserticola* with and without fermented DOC addition; G3 = inoculation with *G. mosseae* with and without fermented DOC addition.

non-inoculated plants). The highest NR activity was recorded in the roots of seedlings inoculated with *G. deserticola* and grown in the amended soil.

Mycorrhizal inoculation treatments and fermented DOC addition had no significant effect on acid phosphatase in roots of *D. pentaphyllum* seedlings (Tables III and V).

DISCUSSION

The role of AM fungi in the stimulation of growth and nutrient uptake of many host plants is well documented (Jeffries *et al.*, 2003). In this study, the inoculation of seedlings with *G. intraradices*, *G. deserticola* or *G. mosseae* significantly stimulated the production of shoot biomass. It is important to emphasize that mycorrhizal inoculation with *G. deserticola* or *G. mosseae* alone was even more effective than the addition of fermented DOC alone in improving the performance of *D. pentaphyllum* plants. The fact that the foliar N, P and K contents of plants inoculated with AM fungi were similar to (in *G. intraradices*-inoculated plants) or even higher than (*G. deserticola*- and *G. mosseae*-inoculated plants) those of non-inoculated plants grown in the amended soil reaffirms the key role of mycorrhizae in sustaining the plant cover in P-deficient soils. The mycorrhizal inoculation treatments showed different levels of effectiveness with respect to improving the performance of *D. pentaphyllum* seedlings. *G. mosseae* was the most effective at increasing plant growth. Assuming that the shoot/root ratio reflects the degree of effectiveness of AM fungi (Tobar *et al.*, 1994), *G. mosseae* achieved the highest shoot/root ratio (2·53). Total plant nutrient contents can also be taken as a representative indicator of mycorrhizal effectiveness, because they take into account the well-balanced effects of nutrient acquisition and biomass production (Jeffries *et al.*, 2003). Mycorrhizal inoculation treatments appeared effective in improving nutrient content. The highest contents of N, P and K in shoot tissue were observed in the seedlings inoculated with *G. mosseae*, which might explain why the growth of *D. pentaphyllum* was greatest in this treatment. However, the higher effectiveness of *G. mosseae* was not related to the extent of mycorrhizal infection, because all inoculated plants exhibited high infection rates in their roots and there were no differences between mycorrhizal treatments.

The results of this study demonstrate the viability of applying the fermented DOC residue in the presence of rock phosphate in order to improve the growth of *D. pentaphyllum*. This could be due to an improvement in the available nutrient supply in the soil, arising from the fermented DOC. During the course of *A. niger* fermentation, the rock phosphate solubilizes, increasing the level of bio-available P in the DOC (Vassilev *et al.*, 1998). Thus, plants grown in the amended soil had higher (NPK) nutrient contents in their tissues than non-amended control plants.

Table IV. Foliar nutrients and root infection of *D. pentaphyllum* seedlings in response to mycorrhizal inoculation treatments and fermented DOC addition previous planting and three months after planting ($n = 5$)

	Months	
	0	3
Nitrogen (mg plant^{-1})		
C	6·3 ± 0·4*	19·0 ± 0·6
A	6·3 ± 0·4	50·6 ± 2·4
G1	13·1 ± 1·2	51·4 ± 1·7
AG1	13·1 ± 1·2	88·7 ± 1·9
G2	20·6 ± 0·9	56·5 ± 1·2
AG2	20·6 ± 0·9	84·1 ± 3·4
G3	9·2 ± 0·4	87·8 ± 2·0
AG3	9·2 ± 0·4	109·5 ± 2·0
Phosphorus (mg plant^{-1})		
C	0·41 ± 0·01	1·33 ± 0·12
A	0·41 ± 0·01	2·26 ± 0·09
G1	0·64 ± 0·03	2·09 ± 0·05
AG1	0·64 ± 0·03	7·79 ± 0·17
G2	0·88 ± 0·03	2·37 ± 0·01
AG2	0·88 ± 0·03	5·70 ± 0·09
G3	0·95 ± 0·05	3·75 ± 0·06
AG3	0·95 ± 0·05	9·86 ± 0·56
Potassium (mg plant^{-1})		
C	5·3 ± 0·4	15·5 ± 0·7
A	5·3 ± 0·4	44·9 ± 0·8
G1	12·7 ± 0·8	45·8 ± 0·8
AG1	12·7 ± 0·8	69·1 ± 3·0
G2	17·9 ± 0·4	48·8 ± 1·1
AG2	17·9 ± 0·4	66·2 ± 2·5
G3	8·8 ± 0·4	72·5 ± 1·3
AG3	8·8 ± 0·4	88·9 ± 1·5
Colonization (%)		
C	0 ± 0	22 ± 3
A	0 ± 0	27 ± 2
G1	77 ± 3	92 ± 1
AG1	77 ± 3	89 ± 1
G2	60 ± 4	82 ± 1
AG2	60 ± 4	83 ± 1
G3	61 ± 5	80 ± 1
AG3	61 ± 5	83 ± 1

C = control; A = fermented DOC addition; G1 = inoculation with *G. intraradices*; AG1 = fermented DOC addition and inoculation with *G. intraradices*; G2 = inoculation with *G. deserticola*; AG2 = fermented DOC addition and inoculation with *G. deserticola*; G3 = inoculation with *G. mosseae*; AG3 = fermented DOC addition and inoculation with *G. mosseae*.
*Mean ± standard error.

The combination of fermented DOC addition and mycorrhizal inoculation treatments can considerably improve the performance of *D. pentaphyllum*. This result contrasts with the widely accepted idea that mycorrhizae provide little advantage to seedlings grown in fertilized soils (Yanai *et al.*, 1995). The rapid growth of inoculated seedlings as compared with the uninoculated seedlings, in the amended soil might be related to the capacity of the fungus to increase available P uptake from fermented DOC (Nadian *et al.*, 1996). The fact that the highest contents of P in

Table V. Nitrate reductase (NR) and acid phosphatase activities in roots of *D. pentaphyllum* seedlings in response to mycorrhizal inoculation treatments and fermented DOC addition three months after planting ($n = 5$)

Treatment	NR activity (root) (nmol NO_2^- g FW^{-1} h^{-1})	Acid phosphatase (μmol PNP g^{-1} h^{-1})
C	169 ± 10*	104·5 ± 0·7
A	248 ± 12	97·2 ± 1·5
G1	250 ± 11	94·3 ± 1·0
AG1	314 ± 14	95·1 ± 1·3
G2	296 ± 8	100·1 ± 1·2
AG2	372 ± 13	96·2 ± 1·0
G3	245 ± 9	102·0 ± 1·1
AG3	259 ± 9	98·0 ± 1·4

C = control; A = fermented DOC addition; G1 = inoculation with *G. intraradices*; AG1 = fermented DOC addition and inoculation with *G. intraradices*; G2 = inoculation with *G. deserticola*; AG2 = fermented DOC addition and inoculation with *G. deserticola*; G3 = inoculation with *G. mosseae*; AG3 = fermented DOC addition and inoculation with *G. mosseae*.
*Mean ± standard error.

leaves occurred for seedlings grown in amended soil and inoculated with AM fungi demonstrated a higher accumulation of P as a consequence of mycorrhizal inoculation. These results reaffirm the compatibility of these biosystems with the AM fungi–*D. pentaphyllum* symbiosis.

Nitrate reductase activity is responsible for nitrate assimilation and is inducible by its substrate (Kandlbinder *et al.*, 2000). Thus, NR activity is highly sensitive to the metabolic and physiological plant status. The increased NR activity found in AM plants is an indication of the mycorrhizal ability to promote plant adaptation to drought resistance (Azcón and Tobar, 1998). Some authors have indicated that the increase in NR activity of mycorrhizal plants with respect to non-mycorrhizal ones can be related to the phosphate requirements of this enzyme (Ruíz-Lozano and Azcón, 1996). This could explain how non-inoculated plants growing in the amended soil had similar values of NR activity to those of plants inoculated with *G. intraradices* or *G. mosseae*. In this study, *G. deserticola* was the most effective fungus in increasing the root NR activity of *D. pentaphyllum*. Recently, we have reported the effectiveness of *G. deserticola* with respect to the increasing of root NR activity and N uptake in shoot tissues of *D. pentaphyllum* seedlings under water-stressed conditions (Caravaca *et al.*, 2003a). This means that NR activity was regulated not only according to the P content in the host plant, but also by the colonizing AM fungus, indicating specific physiological behaviours of the different AM fungi. The present results are also consistent with the finding by Ruíz-Lozano and Azcón (1996) that NR activity differs in various AM fungal species colonizing the roots of lettuce plants. Likewise, the differing effects of AM fungi on this enzymatic activity could be a consequence of fungal NR activity. In fact, Kaldorf *et al.* (1998) described assimilatory NR activity in mycorrhizal fungi.

Phosphatase is a hydrolase related to the P-cycle in which organic P is transformed into plant-available P. Phosphatases are inhibited by the final product of the enzymatic reaction, inorganic phosphorus, representing a feed-back inhibition. Fermented DOC and mycorrhizal inoculation treatments had no effect on the acid phosphatase activity in roots of *D. pentaphyllum*. Khalil *et al*. (1994) found a positive correlation between root phosphatase and AM formation, but the level of the correlation depended on the plant species tested. The phosphatase activity in roots of *D. pentaphyllum* was not related to plant growth. This agrees with the finding of Azcón and Barea (1997), suggesting that a high phosphatase activity does not compensate for an inadequate supply of assimilable P to the plant. In this regard, high values of phosphatase activity were recorded in control plants, which grew in the soil having a low concentration of available inorganic P.

In conclusion, the combined treatment involving the application of microbially-treated agrowastes and mycorrhizal inoculation with AM fungi, particularly with *G. mosseae*, can be proposed as a successful revegetation strategy for *D. pentaphyllum* in P-deficient soils under semiarid Mediterranean conditions.

ACKNOWLEDGEMENTS

This research was supported by the EC + CICYT co-financed FEDER programme (REN 2000-1724-CO3-01).

REFERENCES

Augé RM. 2001. Water relations, drought and vesicular-arbuscular mycorrhizal symbiosis. *Mycorrhiza* **11**: 3–42.

Azcón R, Barea JM. 1997. Mycorrhizal dependency of a representative plant species in mediterranean shrublands (*Lavandula spica* L.) as a key factor to its use for revegetation strategies in desertification-threatened areas. *Applied Soil Ecology* **7**: 83–92.

Azcón R, Tobar RM. 1998. Activity of nitrate reductase and glutamine synthetase in shoot and root of mycorrhizal *Allium cepa*. Effect of drought stress. *Plant Science* **133**: 1–8.

Azcón R, Ruíz-Lozano JM, Rodríguez R. 2001. Differential contribution of arbuscular mycorrhizal fungi to plant nitrate uptake (^{15}N) under increasing N supply to the soil. *Canadian Journal of Botany* **79**: 1175–1180.

Caravaca F, Alguacil MM, Díaz G, Roldán A. 2003a. Use of nitrate reductase activity for assessing the effectiveness of mycorrhizal symbiosis in *Dorycnium pentaphyllum* under induced water deficit. *Communications in Soil Science and Plant Analysis* **34**: 2291–2302.

Caravaca F, Figueroa D, Alguacil MM, Roldán A. 2003b. Application of composted urban residue enhanced the performance of afforested shrub species in a degraded semiarid land. *Bioresource Technology* **90**: 65–70.

Downs MR, Nadelhoffer KJ, Melillo JM, Aber JD. 1993. Foliar and fine root nitrate reductase activity in seedlings of four forest tree species in relation to nitrogen availability. *Trees (Berlin)* **7**: 233–236.

Giovannetti M, Mosse B. 1980. An evaluation of techniques for measuring vesicular-arbuscular mycorrhizal infection in roots. *New Phytologist* **84**: 489–499.

Jeffries P, Gianinazzi S, Perotto S, Turnau K, Barea JM. 2003. The contribution of arbuscular mycorrhizal fungi in sustainable maintenance of plant health and soil fertility. *Biology and Fertility of Soils* **37**: 1–16.

Kaldorf M, Schmelzer E, Bothe H. 1998. Expression of maize and fungal nitrate reductase genes in arbuscular mycorrhiza. *Molecular Plant Microbe Interactions* **11**: 439–448.

Kandlbinder A, Weiner H, Kaiser WM. 2000. Nitrate reductase from leaves of Ricinus (*Ricinus communis* L.) and spinach (*Spinacia oleracea* L.) have different regulatory properties. *Journal of Experimental Botany* **51**: 1099–1105.

Keeney DR, Nelson DW. 1982. Nitrogen-inorganic forms. In *Methods of Soil Analysis. II. Chemical and Microbiological Properties*, Page AL, Miller RH, Keeney DR (eds). American Society of Agronomy: Madison, Iowa; 643–698.

Khalil S, Loynachan TE, Tabatabai MA. 1994. Mycorrhizal dependency and nutrient uptake by improved and unimproved corn and soybean cultivars. *Agronomy Journal* **86**: 949–958.

Linares A, Caba JM, Ligero F, de la Rubia T, Martínez J. 2003. Detoxification of semisolid olive-mill wastes and pine-chip mixtures using *Phanerochaete flavido-alba*. *Chemosphere* **51**: 887–891.

Murphy J, Riley JP. 1962. A modified single solution method for determination of phosphate in natural waters. *Analytica Chimica Acta* **27**: 31–36.

Nadian H, Smith SE, Alston AM, Murray RS. 1996. The effect of soil compaction on growth and P uptake by *Trifolium subterraneum*: interactions with mycorrhizal colonization. *Plant and Soil* **182**: 39–49.

Phillips JM, Hayman DS. 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Transactions of the British Mycological Society* **55**: 158–161.

Requena N, Pérez-Solis E, Azcón-Aguilar C, Jeffries P, Barea JM. 2001. Management of indigenous plant-microbe symbioses aids restoration of desertified ecosystems. *Applied and Environmental Microbiology* **67**: 495–498.

Ruíz-Lozano JM, Azcón R. 1996. Mycorrhizal colonization and drought stress as factors affecting nitrate reductase activity in lettuce plants. *Agriculture, Ecosystems and Environment* **60**: 175–181.

Schollemberger CJ, Simon RH. 1954. Determination of exchange capacity and exchangeable bases in soils. *Soil Science* **59**: 13–24.

Soil Survey Staff. 1999. *Soil Taxonomy: A Basic System of Soil Classification for Making and Interpreting Soil Surveys*. USDA Natural Resources Conservation Service. Agricultural Handbook No. 436. US Government Printing Office: Washington DC.

Tabatabai MA, Bremner JM. 1969. Use of *p*-nitrophenol phosphate in assay of soil phosphatase activity. *Soil Biology and Biochemistry* **1**: 301–307.

Tobar RM, Azcón R, Barea JM. 1994. Improved nitrogen uptake and transport from ^{15}N-labelled nitrate by external hyphae of arbuscular mycorrhiza under water-stressed conditions. *New Phytologist* **126**: 119–122.

Vassilev N, Baca MT, Vassileva M, Franco I, Azcón R. 1995. Rock phosphate solubilization by *Aspergillus niger* grown on sugar-beet waste medium. *Applied Microbiology and Biotechnology* **44**: 546–549.

Vassilev N, Franco I, Vassileva M, Azcón R. 1996. Improved plant growth with rock phosphate solubilized by *Aspergillus niger* grown on sugar beet waste. *Bioresource Technology* **55**: 237–241.

Vassilev N, Baca MT, Vassileva M, Franco I, De Nobili M. 1998. Mineralization of three agro-industrial wastes by an acid-producing strain of *Aspergillus niger*. In *The Science of Composting*, De Bertoldi M, Sequi P, Lenunes B, Papi T (eds). Chapman and Hall: London; 1375–1379.

Yanai RD, Fahey TJ, Miller SL. 1995. Efficiency of nutrient acquisition by fine roots and mycorrhizae. In *Resource Physiology of Conifers: Acquisition, Allocation and Utilization*, Smith WK, Hinckley TM (eds). Academic Press: London; 75–103.

