

## Growth response of *Pinus halepensis* to inoculation with *Pisolithus arhizus* in a terraced rangeland amended with urban refuse

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### Abstract

A field experiment was carried out to evaluate the growth response of *Pinus halepensis* seedlings inoculated with *Pisolithus arhizus* and planted in a terraced rangeland amended with urban solid refuse. The application of the organic amendment mediated a significant increase in soil fertility and soil water content. Twenty seven months after planting seedling survival rates did not differ significantly among treatments and were above 95% in all cases. Growth of *P. halepensis* was significantly ( $p < 0.01$ ) enhanced by the refuse application independently of their mycorrhizal status at the beginning of the experiment. A multiple regression analysis including available soil P concentration and sorptivity as independent variables explained up to 60% of the variance in pine growth observed across treatments. Inoculation with *P. arhizus* also significantly ( $p < 0.01$ ) enhanced pine growth with respect to the controls grown in both amended and nonamended plots. It was concluded that the combination of soil terracing, refuse amendment and *P. arhizus* inoculation significantly improved the performance of *Pinus halepensis*, and this methodology could be successfully applied in afforestation programmes in semiarid and degraded sites.

### Introduction

The semiarid areas surrounding the Mediterranean coast are seriously affected by excessive human pressure leading to soil degradation and desertification processes, mainly due to the destruction of plant layer. In these areas the revegetation programmes are limited by the severe hydric stress and the lack of suitable physical properties and fertility of soils. *Pinus halepensis* Mill. is perhaps the best adapted forest-tree species to these hostile conditions and is considered a good ecological alternative for afforestation in meso-mediterranean areas (Elena-Roselló et al., 1990), therefore it is the most commonly used tree in afforestation programmes in Southeast Spain.

Like other members of the Pinaceae, *P. halepensis* is mycorrhizal (Torres and Honrubia, 1993; Trappe, 1981). The importance of mycorrhizal symbiosis in forest plants establishment has been stressed by many authors (Allen, 1991; Amaranthus and Trappe, 1993;

Perry et al., 1987). This symbiotic relationship profoundly affects plant growth by improving nutrient uptake (Cumming, 1993; Harley and Smith, 1983; Rousseau and Reid, 1991) and reducing water stress (Boyle and Hellenbrand, 1991). However, mycorrhizal symbiosis in conjunction with *P. halepensis* is not so well understood and there are relatively few studies concerning it (Chevalier and Detolle, 1984; Lapeyrie, 1990; Ruehle et al., 1981; Torres and Honrubia, 1994). Moreover, the knowledge of the effect of organic amendments to the soil on mycorrhizal symbiosis is even more scarce (Aniwat and Somboon, 1990; Danielson, 1991).

Organic additions, especially urban wastes, is a proven method to enhance soil physical and chemical properties (Albaladejo et al., 1994; Diaz et al., 1994). Roldán and Albaladejo (1994) demonstrated the beneficial effect of *Pisolithus tinctorius* inoculation and urban refuse addition on the growth of *P. halepensis* in a Xeric Torriorthent. In the latter experiment the soil was not mechanically prepared, although this is a com-

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mon practice in afforestation in Southeast Spain, mainly soil terracing. Terraces can improve infiltration and soil water-holding capacity, reduce runoff and increase water availability (Serrada, 1990). On the other hand, the soil profile is disturbed, the most fertile epipedons are eliminated (Finkel, 1986) and these affect the reservoir of fungal spores and other propagules of organisms important for decomposition, nutrient cycling and mycorrhiza formation (Allen, 1989; Aziz and Habte, 1989; Habte, 1989). All these processes are poorly understood. Our study therefore was designed to determine the combined effect of urban refuse amendment and *Pisolithus arhizus* inoculation on the growth of *Pinus halepensis* seedlings grown in a semiarid rangeland subjected to mechanical terracing.

## Materials and methods

### *The field study site*

The study site was located in El Aguilucho experimental area (UTM: 30S XG5395) on the Carrascoy range in Murcia Province (Southeast Spain) and at a mean elevation of 180 m. The climate is semiarid mediterranean, with extremely hot and dry summers. The average annual rainfall is 300 mm occurring mostly in autumn and spring. The mean annual temperature is 18 °C, and the potential evapotranspiration reaches 900-1000 mm yr<sup>-1</sup>. The predominant soil is a Xerollic Calciorthid developed from limestones (Soil Survey Staff, 1975) with a sandy loam texture.

In June 1992, an area of 2500 m<sup>2</sup> on an east-facing hillslope ranging from 25 to 30% was terraced. The mechanical terraces (4 m wide) were excavated by a bulldozer and the petrocalcic layer present in these terraces was broken by deep ripping with a subsoiler along the planting line.

### *Materials*

The urban refuse used in the experiment was a solid fresh material, neither composted nor ground but allowed to mature for 15 days. The refuse came from the Murcia Municipal Treatment Plant. Analytical characteristics of the urban solid refuse (USR) determined by standard methods (Page et al., 1982) are shown in Table 1.

The mycorrhizal fungus used in the experiment was *Pisolithus arhizus* (Pers.) Rauschert (= *P. tinctorius* (Pers.) Coker and Couch). The fruiting bodies were

Table 1. Analytical characteristics of the urban solid refuse used in the experiment

Ash (%)	40.6
pH (1:10 aqueous extract)	6.8
Conductivity (1:10; mS cm <sup>-1</sup> )	4.4
Organic C (%)	25.3
Total N (%)	1.19
Total P (%)	0.55
Extractable C (%)	4.81
C fulvic acids (%)	3.17
C humic acids (%)	1.64
Carbohydrates (%)	4.95

collected from a *P. halepensis* plantation in Cocentaina (Alicante, Southeast Spain).

### *Mycorrhizal inoculation of seedlings*

To prepare the spore inoculum, the basidiospores were suspended in sterile boiled tap water with Tween 80 and stored at 2 °C until use. The *P. halepensis* Mill. seeds came from the El Valle nursery (Murcia). They underwent no prior scarification or stratification treatment before sowed in 300 cc bags containing a mixture of 3/4 of soil and 1/4 of peat (v/v).

Spores were applied three times, 1 month apart, 12 weeks after sowing. The amount of inoculum was calculated according to the spore concentration in the suspensions to supply each plant with 5 × 10<sup>5</sup> spores per application. Five hundred seedlings were mycorrhized and grown for one year under nursery conditions without any fertilization treatment. A similar amount of *P. halepensis* seedlings were grown under the same conditions and were not treated with inoculum.

### *Field procedures*

In October 1992, two 600 m<sup>2</sup> experimental plots were established in the previously terraced hillslope. USR was applied in a single addition to one of the plots. The dose used was 10 kg m<sup>-2</sup> (fresh weight) and it was incorporated into the top 30 cm of the soil by means of a rotovator.

In November 1992, 80 inoculated *P. halepensis* seedlings and 80 noninoculated plants were planted in the plots following a randomized design. The seedlings were planted at least 1 m apart, one in each hole, in a single row per terrace. A distance of 3 m or more

separated the pines inoculated with *P. arhizus* from the noninoculated plants.

### *Measurements*

Basal stem diameters and heights of the seedlings were measured with calipers and rules at the time of planting and every three months afterwards.

Gravimetric determination of soil moisture (105 °C, 24 hr) was carried out every 15 days. Ten sampling spots per plot were randomly chosen. Root-zone (10-20 cm) 50 g soil samples were obtained at each spot with hand driven probes.

Water infiltration rates in the soils were assessed during March 1995 using a single ring infiltrometer which was 30 cm both in diameter and height. Ten infiltration runs were carried out at each plot. The infiltrations were performed with ponding condition and maintained at a constant head of 5 cm from the soil surface by manual topping up. All runs were made during a dry period. Sorptivity values were determined from infiltration data using Philip's two term infiltration model (Philip, 1957; Sutikto and Chikamori, 1993).

Soil microbial populations were assessed in February and May 1993. Culturable heterotrophs were quantified by the plate dilution method. Culturable bacteria were determined in PCA (Oxoid) and culturable fungal propagules in Rose Bengal agar (Oxoid) supplemented with chloramphenicol (Merck, 0.05%).

In December 1993, soil samples were taken at 20 randomly selected points per plot. Soil analyses were conducted to characterize the soil chemical properties. Total N and total C were assessed by pretreatment with HCl to eliminate carbonates (Colombo and Baccanti, 1989) followed by combustion at 1020 °C and determination in an Automatic Nitrogen and Carbon Analyzer. Available P was extracted with sodium bicarbonate (Olsen et al., 1954) and determined by colorimetry according to Murphy and Riley (1962). Extractable K with ammonium acetate was determined by photometry (Schollemberger and Simon, 1954). Electrical conductivity was potentiometrically evaluated from the 1:1 saturation extract. The carbohydrates content values were obtained by the anthrone colorimetric method (Brink et al., 1960) after hydrolysis with concentrated H<sub>2</sub>SO<sub>4</sub> using glucose standards.

In December 1993, five plants per treatment were harvested at random. To assess mycorrhizal infection the methods described in Grand and Harvey (1982) and Amaranthus and Perry (1989) were followed. The

roots were subsampled in three 2-cm cross-sections of the upper, middle and lower root system. Root tips in these sections appearing mycorrhizal and active were counted, and the results were expressed as the percentage of mycorrhizal root tips.

### *Statistical analysis*

All data were subjected to analysis of variance or t-Student test. Height and basal stem diameter measurements were log-transformed to compensate for log-normally distributed values. Significant differences ( $p < 0.05$ ) were determined by the Newman Keuls test. Soil water content data were analysed using the Wilcoxon signed rank test for comparison of two series. Multiple regression analysis between soil properties and seedlings growth were carried out.

## **Results and discussion**

### *Changes in soil properties*

The application of USR produced a significant increase in soil fertility, as shown in Table 2. Fourteen months after the refuse incorporation, the concentration of available P recorded in the amended plot was seven times greater than in the control plot. Levels of total N, exchangeable K, organic C and carbohydrates also increased between two and three times. The effect of the addition was highly significant ( $p < 0.01$ ) in all cases. Similar soil fertility increments after USR amendment were recorded by Albaladejo et al. (1994) in a previous experiment, although the P increase they reported was not so high.

An excess in soil electrical conductivity levels can negatively affect plant development. In this sense, we found values twice as high as in the control plot fourteen months after the amendment. This is in agreement with the results of Albaladejo and Diaz (1990) and Albaladejo et al. (1994), who found no negative effects on soil biological productivity at the same refuse application rate of our experiment. Moreover, salinity usually diminishes rapidly in the root zone after the first rainstorms (Guidi et al., 1983). The nonamended plot also showed a high salinity level, which indicates that soil terracing unearthed a salts-rich stratum. Hence, USR was only partly responsible for the high electrical conductivity values reached in the fertilized plot.

The evolution of the mean soil moisture recorded in the two plots during a 25 months period is shown in

Table 2. Effect of USR application on soil characteristics. Data were assessed 14 months after *Pinus halepensis* planting. Each value is mean of 20 replicates

	Amended plot	Control plot
Sorptivity ( $\text{cm min}^{-1/2}$ )	6.501b	1.240a
Available P ( $\text{mg g}^{-1}$ )	0.022b	0.003a
Total N ( $\text{mg g}^{-1}$ )	1.46b	0.50a
Exchangeable K ( $\text{cM kg}^{-1}$ )	6.83b	1.73a
Organic C ( $\text{mg g}^{-1}$ )	14.6b	6.5a
Electrical Conductivity ( $\text{dS m}^{-1}$ )	4.72b	2.32a
Carbohydrates ( $\text{mg glucose g}^{-1}$ )	3.88b	1.30a

Values in rows sharing a letter do not differ significantly ( $p < 0.05$ ) as determined by Duncan's test.

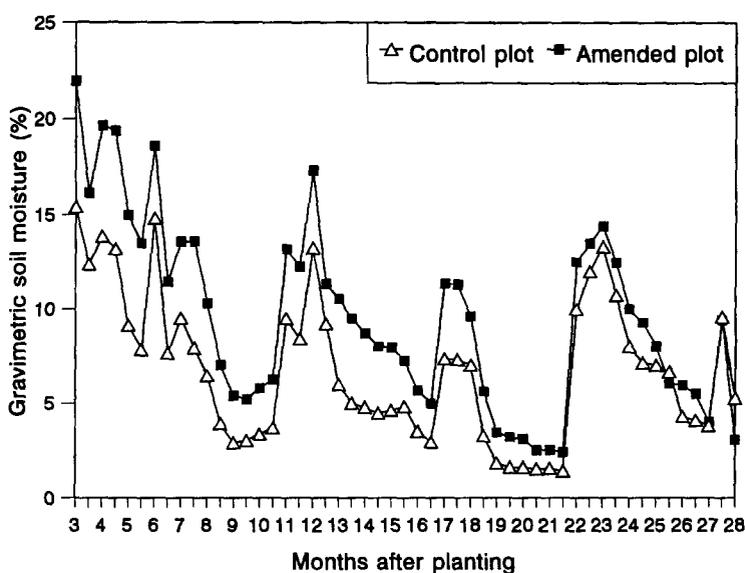


Fig. 1. Effect of USR application on the evolution of the gravimetric soil moisture in the experimental plots where *Pinus halepensis* seedlings were grown. Month 3 = Feb 1992. Each value is mean of 10 replicates.

Figure 1. Soil water content was always significantly higher in the amended plot ( $p < 0.01$ ). The combination of increased soil moisture and high fertility levels enhanced soil microbial populations and favoured plant recolonization of the terraces from adjacent areas. The total number of culturable bacteria and viable fungal propagules increased in the amended plot, as shown in Table 3. These microorganisms participate mechanically in soil aggregation, acting as binding agents between particles and excreting polysaccharides into the medium (Reinersten et al., 1984; Roldán et al., 1994; Tisdall and Oades, 1982). In the amended plot, the improvement in soil structure enhanced water retention and increased the infiltration capaci-

ty of the soil (see sorptivity data in Table 2), which explains the higher soil moisture content recorded in the amended plot with respect to the control. Similar results were obtained by Lax et al. (1994). Moreover, the percentage of plant cover recorded in the plot with USR addition was considerably higher than in the non-amended plot ever since the first stages of the experiment (data not shown in this paper). Diaz et al. (1994) reported that after refuse application the spontaneous process of plant colonization and the development of the corresponding rhizosphere stimulate soil microflora through root exudates and litter production, improving soil structure and water retention.

Table 3. Effect of USR amendment on culturable heterotrophic populations in soil (colony forming units). Data are means of 3 measurements  $\pm$  SE

	February 1993		May 1993	
	Culturable bacteria (c.f.u. g <sup>-1</sup> d.w.)	Fungal propagules (c.f.u. g <sup>-1</sup> d.w.)	Culturable bacteria (c.f.u. g <sup>-1</sup> d.w.)	Fungal propagules (c.f.u. g <sup>-1</sup> d.w.)
Control	4.27 $\pm$ 0.26 $\times$ 10 <sup>5</sup>	7.2 $\pm$ 1.2 $\times$ 10 <sup>3</sup>	3.8 $\pm$ 0.23 $\times$ 10 <sup>6</sup>	2 $\pm$ 0.57 $\times$ 10 <sup>5</sup>
Amended plot	2.14 $\pm$ 0.11 $\times$ 10 <sup>8</sup>	1.62 $\pm$ 0.16 $\times$ 10 <sup>7</sup>	8.23 $\pm$ 0.41 $\times$ 10 <sup>7</sup>	9.66 $\pm$ 1.2 $\times$ 10 <sup>6</sup>

The differences in soil water content between the two plots tended to decrease towards the end of the period considered. This tendency can be partly explained by the severe drought that affected the area during the last 12 months of the experiment (only 190 mm of rainfall recorded in this time). A persistent deficiency in soil moisture can strongly depress microbial activity (Kushner, 1980), therefore deteriorating the soil structure and hiding the positive effect of the USR on the soil water holding capacity. On the other hand, the more dense herbaceous layer developed in the amended plot might have caused an earlier consumption of the available soil water.

#### Growth of *Pinus halepensis*

Seedling survival rates did not differ significantly among treatments twenty seven months after planting, and were above 95% in all cases. Figure 2 shows the evolution of the pines height. At time of planting, the *Pinus halepensis* seedlings inoculated with *Pisolithus arhizus* had significantly ( $p<0.05$ ) greater values than the two sets of non-inoculated plants. However, nine months after planting the pines grown in the amended plot showed significantly ( $p<0.01$ ) greater heights than the pines of the non-amended plot, independently of their mycorrhizal status at the beginning of the experiment. This difference tended to increase with time.

A similar pattern can be observed with basal diameter growth (Fig. 3). Six months after planting, basal diameter values reached by the seedlings grown in the amended plot were significantly higher ( $p<0.01$ ) than those corresponding to the pines of the control plot, and this gap increased subsequently.

These results are in agreement with those reported by Danielson (1990), who found that sewage sludge addition to the soil enhanced *Pinus* spp. growth. Roldán and Albaladejo (1994) demonstrated that USR incorporation to the soil improves the performance

of *Pinus halepensis* seedlings under dry conditions. The significantly ( $p<0.01$ ) higher pine growth rate that we recorded in the amended plot is attributable to improved fertility levels and increased amount of water availability. A multiple regression analysis including soil P concentration and sorptivity as independent variables was able to explain up to 60% of the variance in pine growth observed across treatments.

The percentages of root mycorrhizal colonization corresponding to the pines randomly sampled 15 months after planting are shown in Table 4. By that time, most of the non-inoculated seedlings had become mycorrhizal as a result of natural infection, although the degree of mycorrhizal colonization of the pines inoculated in the nursery was still significantly ( $p<0.01$ ) higher. Overall, the pines grown in the amended plot showed a lower degree of mycorrhization than the pines of the control plot, which can be attributed to the application of the refuse. As pointed out by several authors (García et al., 1992; Linderman, 1989; Roldán and Albaladejo, 1993), chemicals with a toxic effect on plant roots (phenolic substances, organic acids of low molecular weight, etc.) can be released to the soil in the early stages of the organic residues decomposition. Pera et al. (1983) and Roldán and Albaladejo (1993) reported negative effects of USR application on mycorrhizal fungi populations. On the contrary, Danielson (1990) found that sewage sludge addition stimulated mycorrhizal development in *Picea* and *Pinus*, although this effect could not be wholly attributed to the amendments. Marx et al. (1977) found that N and P levels in the medium influenced ectomycorrhizal development, and that low fertility enhanced it. This is consistent with the higher degree of mycorrhizal colonization that we recorded in the nonamended plot, where fertility level was very low.

Inoculation with *P. arhizus* significantly ( $p<0.01$ ) enhanced pine growth with respect to the corresponding controls in the two plots. As regards shoot length,

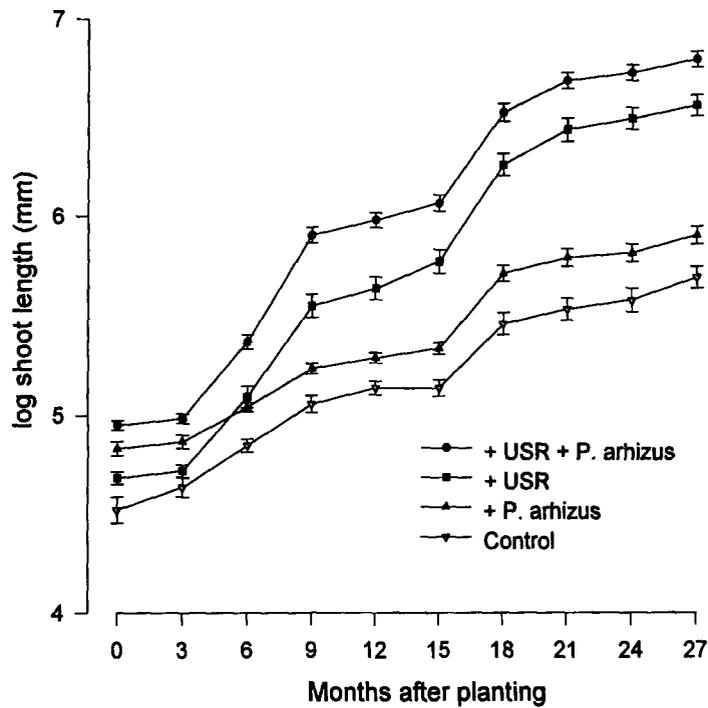


Fig. 2. Shoot length evolution of *Pinus halepensis* seedlings grown in field conditions after inoculation with *Pisolithus arhizus* and application of USR to the soil. Bars represent standard error ( $n = 40$ ). Month 0 = Nov 1992.

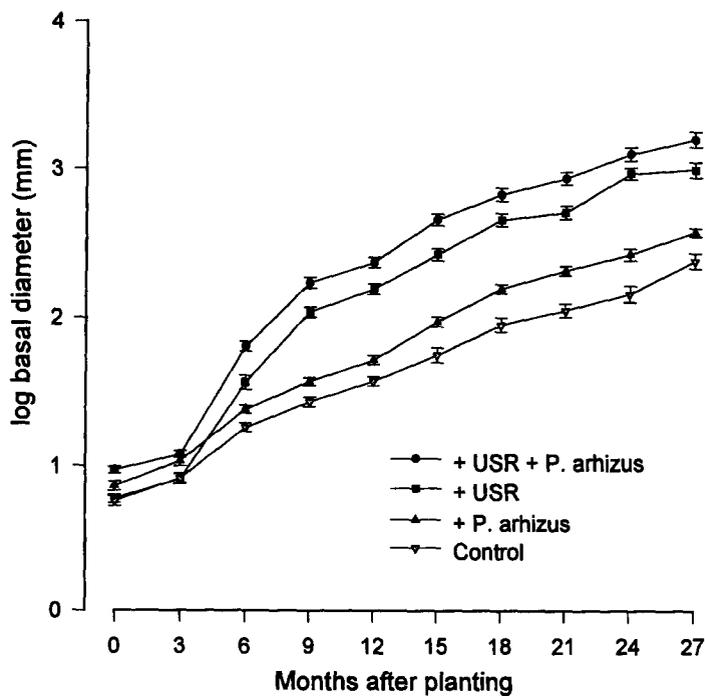


Fig. 3. Basal diameter evolution of *Pinus halepensis* seedlings grown in field conditions after inoculation with *Pisolithus arhizus* and application of USR to the soil. Bars represent standard error ( $n = 40$ ). Month 0 = Nov 1992.

**Table 4.** Effect of USR application to soil and *P. arhizus* inoculation on short lateral roots development and root infection in *Pinus halepensis* after 14 months growing under field conditions. Data are means of five replicates

	Short lateral roots (tips g <sup>-1</sup> d.w.)	Mycorrhized Short lateral roots (tips g <sup>-1</sup> d.w.)	% Mycorrhized Short lateral roots
+ USR + <i>P. arhizus</i>	277.19b	195.70b	70.84c
+USR	117.04a	35.19a	30.64a
+ <i>P. arhizus</i>	321.62b	261.88c	81.86d
Control	138.12a	54.88a	39.92b

Values in columns sharing a letter do not differ significantly ( $p < 0.05$ ) as determined by Duncan's test.

the amount of growth stimulation by *P. arhizus* after 27 months under field conditions was 22% in the non-amended plot and 24% in the fertilized plot. Roldán and Albaladejo (1994) demonstrated that mycorrhizal infection may increase nutrient uptake in *Pinus halepensis* seedlings. This might partly explain the better performance of the inoculated seedlings as compared with the control pines in the non-amended plot, where nutrient concentration was low. However, fertility levels reached after USR addition were well above those considered for limiting pine growth (Cumming, 1993; Marschner, 1986). Therefore, the ability of *P. arhizus* to enhance *Pinus halepensis* growth at the amended plot might be more related to the capacity of the fungus to stimulate water uptake or to produce growth promoting substances than to increase nutrient uptake. Ectomycorrhizal fungi can produce a wide range of hormones and vitamins of the B group (Alvarez, 1991). Ek et al. (1983) proved the capacity of *P. tinctorius* for producing IAA, while Tyminska et al. (1986) attributed improved growth of *P. tinctorius* inoculated seedlings to the fungal production of IAA. Accordingly, Guehl et al. (1990) found that infected *Pinus pinea* seedlings tended to exhibit higher CO<sub>2</sub> assimilation rates than the controls. David et al. (1983) demonstrated that mycorrhizal fungi could promote root growth in *Pinus pinaster*. Greater root systems may lead to better soil water exploitation in dry environments. On the other hand, mycorrhizal fungi can improve the performance of conifer seedlings during water stress, enabling the plant to take up water against steeper water potentials (Boyle and Hellenbrand, 1991) or increasing water use efficiency (Guehl et al., 1990). Since water is the major limiting factor for plant growth under the semiarid conditions of the experimental area, we believe that it was increased

tolerance to water stress what determined the higher growth rate of *P. arhizus* inoculated seedlings as compared with control seedlings in the amended plot.

It can be concluded that the combination of soil terracing, USR amendment and mycorrhizal inoculation with *Pisolithus arhizus* considerably improved the performance of *Pinus halepensis* seedlings under our experimental conditions. This methodology could be successfully applied in afforestation programmes in semiarid areas.

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