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Effect of mycorrhizal inoculation and soil restoration on the growth of *Pinus halepensis* seedlings in a semiarid soil

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Abstract An experiment was carried out to evaluate the growth of mycorrhizal *Pinus halepensis* seedlings planted in a semiarid soil amended with urban refuse in southeast Spain. Three fungal species were used: *Pisolithus tinctorius*, *Rhizopogon roseolus*, and *Suillus collinitus*. After 8 months, inoculated seedlings grown under controlled conditions did not differ significantly from controls with regard to plant height and nutrient assimilation. Other features such as root development and stem dry weight showed that the plants grew better in the absence of mycorrhizal inoculation. The mycorrhizal seedlings and the controls were planted in three experimental plots treated with urban refuse (0, 6, and 12 Kg m^{-2}). After 1 year of growth under field conditions the results showed that the type of fungus inoculated significantly influenced *P. halepensis* development. This effect varied with the dose of urban refuse. Plant growth was encouraged by the application of refuse but only at the lowest dose. Under these conditions *P. tinctorius* was the most effective fungus and *R. roseolus* yielded poorer plant development. The highest application of urban refuse led to notably worse results and a significant decrease in seedling growth compared to controls. In the control plot (without refuse) *S. collinitus* was the most effective fungus in plant growth improvement. The smallest application of urban refuse had a positive effect on the assimilation of N, P, and K in seedlings inoculated with *P. tinctorius* and *S. collinitus*.

Key words: *Pinus halepensis* · Ectomycorrhizae · Arid soil restoration · Urban refuse amendments

Introduction

The semiarid areas surrounding the Mediterranean sea are seriously affected by soil degradation and desertification. Since the principal cause of this environmental degradation is the destruction of plant cover (Francis and Thornes 1990) a programme of revegetation seems the most logical way to control these processes. However, it is difficult to determine how best to encourage vegetal cover in areas of severe hydric stress, in which the physical properties and fertility of the soil have been badly damaged. In these hostile conditions pretreatment of both plants and soil may be necessary in order to increase plant resistance to the inhospitable environmental conditions, and to improve the quality and productivity of the soil (Albaladejo and Díaz 1990).

There are few species of tree capable of resisting the frequent droughts of arid and semiarid zones. *Pinus halepensis* is perhaps the best adapted to conditions of hydric stress and is, as a consequence, the most commonly used tree in reafforestation programmes in southeast Spain. Like other members of the Pinaceae, *P. halepensis* is necessarily mycorrhizal (Trappe 1981).

Mycorrhizal symbiosis is an important factor in the establishment of seedlings in semiarid and/or degraded areas. Besides other beneficial effects, it increases nutrient uptake (Harley and Smith 1983), facilitates the transport of water to plant roots (Duddridge et al. 1980; Parke et al. 1983) and acts as a defence mechanism against pathogenic organisms. The use of mycorrhizal symbiosis in the reafforestation of degraded zones has been widely studied and its effectiveness has been demonstrated (Marx 1975; Valdes 1985). However, mycorrhizal symbiosis in conjunction with *P. halepensis* is not so well understood and there are relatively few studies concerning it (Ruehle et al. 1981; Chevalier and Detolle 1984).

In contrast, organic amendment is a well tried and proven method of improving soil quality (Bastian and Ryan 1986). Soil physical (structure, permeability, water-holding capacity), chemical (nutrients, cation-exchange capacity, pH), and biological (organic C, microflora, microfauna) properties can be improved by the addition of organic compounds (Sauerbeck 1987). Among these materials, urban wastes have proved effective as soil fertilizers and conditioners (Bortlisz and Malz 1983) and have the

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advantages of low cost and wide availability. The use of these wastes on soils also helps to solve the environmental problem of waste disposal.

The aim of the present work was to evaluate the benefits of combined treatment with mycorrhizal inoculation of seedlings and soil improvement with urban refuse in the establishment of *P. halepensis* in a highly eroded soil of the Mediterranean semiarid area.

Materials and methods

Mycorrhizal inoculation of seedlings

Three species of basidiomycotina were used, *Pisolithus tinctorius* (Pers.) Coker and Couch from Cocentaina (Alicante), *Rhizopogon roseolus* (Corda) Th. M. Fr. from El Valle (Murcia), and *Suillus collinitus* (Fr.) O. Kuntze also from El Valle (Murcia). Specimens were collected from *P. halepensis* plantations in southern Spain. All had been shown to form ectomycorrhizae with *P. halepensis* (Torres et al. 1991).

To prepare the spore inoculum, fresh mature fruiting bodies were cut into pieces (3–5 cm^3) and blended with tap water at high speed for 2–3 min. Spore concentrations within the resulting suspension were determined with a haematocytometer and stored in the dark at 2 °C until used as inocula (Castellano and Molina 1990).

The substrate for this experiment consisted of a mixture of soil, sand, and vermiculite at 2:1:1 (by volume). This mixture was autoclaved at 120 °C for 20 min on 2 consecutive days and placed in 350-ml pots. Soil was collected from an established *P. halepensis* plantation in Santomera (Murcia). This soil is a Litic Haploxeroll, containing 16 mg g^{-1} available P, 0.64 mEq per 100 g available K, 0.20 mEq per 100 g Na, 3.89% organic C, and 0.068% total N.

One hundred replicates of each fungus used to inoculate the plants and controls were established and placed in a growth chamber at day and night temperatures of 25 and 20 °C, respectively, with a 16-h day length, 57% relative humidity, and 420 mmol m^{-2} s^{-1} photon flow.

Five to seven seeds per pot were sowed and thinned to one per pot inmediately after germination. Spores were applied three times, 2 weeks apart, 12 weeks after sowing, with a standard watering can. The amount of inoculum was calculated according to the spore concentration in the suspensions to supply each pot with 500000 spores per application.

The plants were grown for 8 months, and then 10 plants per treatment were harvested at random and fresh and dry (105 °C, 5 h) weights of shoots and roots, shoot height, and stem basal diameter were measured.

To assess mycorrhizal colonization, methods described in Grand and Harvey (1982) and Amaranthus and Perry (1989) were followed. 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 number of mycorrizhal tips per gram dry weight of root.

The P content in needles was determined according to Murphy and Riley (1962) and K was determined by flame photometry, both after digestion in nitric-perchloric acid (5:3). The total N content was determined in an automatic Nitrogen Analyzer.

The rest of the *P. halepensis* seedlings were grown under greenhouse conditions for 6 weeks before establishment in the field. During this time watering was reduced.

Field experiments

The experiments were located in Abanilla (30 km north of Murcia, southeast Spain). The annual rainfall averages 250–300 mm, mostly in the autumn and spring. The mean annual temperature is 19.2 °C, the potential evapotranspiration reaches 900 mm $year^{-1}$ and the drought period can extend for 10–12 months. The soil is a Xeric Torriorthent developed from marls, with a loamy clay texture. The vegetation is mainly slow-growing low shrubs with low proportion of plant cover and some *P. halepensis* spots.

The method used to improve soil quality was a single addition of urban refuse to the top 30 cm with a rotovator. Three experimental plots were established on a flat surface. One plot was left untreated (control) and the other two were amended with urban refuse at 6 and 12 kg m^{-2}. The refuse used in this experiment was solid fresh material, not composted or ground but allowed to mature naturally for 10–15 days. This material showed high biodegradability in an earlier experiment (García et al. 1992) through a comparative study of the structural-chemical composition of the soil organic matter 3 years after the addition. Inorganic and gross components of the urban refuse (plastic, glass, etc.) were removed before its addition to soil. Analytical characteristics of the refuse are shown in Table 1.

The application of urban refuse to the soil was in October 1990. During the 1st week of March 1991, the previously inoculated *P. halepensis* seedlings were planted in the experimental plots. Twenty-five seedlings per treatment, including the control, were planted 1 m apart in every experimental plots in a 5×5 array; at least 2 m separated the treatments.

At the time the seedlings were planted out the shoot height and basal stem diameter were measured. These parameters were also determined in the 1st week of June, September, and December 1991. In March 1992, 10 seedlings per treatment were dug up and transported to the laboratory. Then the number of mycorrhizal root-tips, fresh and dry (105 °C, 5 h) shoot weights, and nutrient (N, P, K) concentrations in the needles were assessed.

Soil analyses were conducted in October 1990 and April 1991 to characterize soil chemical changes between treatments. Total organic C was assessed by combustion at 1020 °C and determination in an automatic Carbon Analyzer, total carbonate was determined by means of Bernard's calcimeter, total N by Kjeldahl's method, total and extractable P with sodium bicarbonate by calorimetry according to Murphy and Riley (1962), total and extractable K with ammonium acetate, and total Na by flame photometry (Schollenberger and Simon 1954). Chloride was determined by potentiometric valuation with $AgNO_3$ (Stout and Johnson 1965), and sulphate by turbidimetric determination with $BaCl_2$ and arabic gum (Abrisqueta et al. 1962).

Table 1 Analytical characteristics of urban refuse [*total P* and *total Na* measured after perchloric acid digestion; *extractable P* extracted in sodium bicarbonate (Olsen et al. 1954), *exchangeable K* extracted in 0.05 *M* ammonium acetate]

Water content (%)	48.3
Ash (%)	42.5
pH (1:20 aqueous extract)	6.5
Conductivity (1:20; mS cm^{-1})	3.62
Organic C (%)	25.0
Total N (%)	1.5
Total P (%)	0.6
Extractable P (%)	0.05
Extractable K (%)	0.47
Total Na (%)	0.78
Cl^- (%)	1.10
SO_4^- (%)	1.71

Results

Effects of mycorrhizal inoculation under controlled growth conditions

Eight months after planting, the behaviour of the seedlings in relation to the treatments was very diverse, depending on the parameter considered. Table 2 shows the effects on growth, nutrient assimilation, and mycorrhizal colonization. Plant height was not affected by the type of fungus inoculated and there were no significant differences between the three types and the uninoculated plants. The diameter of the stem base was significantly smaller only in plants inoculated with *R. roseolus*. Root development was invariably significantly smaller in inoculated than in uninoculated seedlings and there was no difference between the three fungi tested. Stem development in uninoculated seedlings was significantly greater than that in seedlings inoculated with *P. tinctorius* and *R. roseolus*, with *S. collinitus* producing an intermediate degree of stem development.

Levels of N and P were similar in all treatments with the exception of seedlings inoculated with *R. roseolus*, which showed substantially lower values. The greatest degree of K uptake was reached by the plants inoculated with *P. tinctorius* followed by uninoculated seedlings and those inoculated with *S. collinitus*. By far the lowest K values were found in the plants inoculated with *R. roseolus*.

The greatest degree of mycorrhizal colonization was found in plants inoculated with *R. roseolus* (Table 2), five times higher than that found in *S. collinitus* and *P. tinctorius*. The uninoculated plants only showed occasional mycorrhizal colonization of roots, similar to that produced by fungi of the genus *Telephora* and also similar to that observed in the inoculated seedlings.

The method used to achieve mycorrhizal colonization under controlled growth conditions was very effective, especially in the case of *R. roseolus*. However, under these conditions, plants grew better in the absence of mycorrhizal inoculation (Table 2). An excessive proliferation of mycorrhizae (those plants inoculated with *R. roseolus*, for example) can negatively affect the growth and assimilation of nutrients by *P. halepensis* in laboratory conditions.

Plant growth under field conditions

Table 3 shows the growth of *P. halepensis* seedlings in the experimental plots. At time zero, there were no significant differences between treatments in height and base diameter. At 6 months, the seedlings in plot 1 (smaller dose of refuse) clearly showed a greater degree of development. In this plot, the plants inoculated with *P. tinctorius* showed the greatest development while those inoculated with *R. roseolus* were considerably inferior in both height and diameter, and the differences were even more pronounced after 9 months. *P. tinctorius* was also the most effective fungus in plot 2 (greater addition of refuse) although the differences noted were only significant when compared with the effects of *R. roseolus*.

Growth values in the control plot did not differ significantly from those obtained in plot 1. However, notable differences were found between the three fungi according to soil treatment. *P. tinctorius* was the most effective fungus in the two refuse-amended plots but not in the control plot; without refuse, the growth of seedlings inoculated with this fungus was even smaller than that of the uninoculated plants. *S. collinitus* alone produced significantly differences from uninoculated plants but *R. roseolus*-inoculated plants did not differ from uninoculated plants. In general, 9 months after planting, no great differences were seen between the treatments, although there was a tendency for the plants in plot 1 (smaller addition of refuse) to show better development and positive effects with mycorrhizal inoculation. This tendency was confirmed by data recorded at the end of the experiment.

Plant responses to refuse amendment and mycorrhizal inoculation

Mycorrhizal infection

Table 4 shows data on the effects of mycorrhizae in the plant root systems. One year after planting, the

Table 2 Effect of inoculation with several extomycorrhizal fungi on growth, nutrient assimilation, and root infection in *Pinus halepensis* under controlled conditions. Data are means of 10 measurements (*P. Pisolithus*, *S. Suillus*, *R. Rhizopogon*, *Mycorr.* mycorrhizal)

Inoculated fungus	Height (mm)	Basal diameter (mm)	Shoot weight		Root weight		Assimilated nutrients in shoot tissues (g kg^{-1})			Mycorr. root tips (no. g^{-1} dry weight)
			Fresh (g plant^{-1})	Dry (g plant^{-1})	Fresh (g plant^{-1})	Dry (g plant^{-1})	N	P	K	
None	132.3a	2.0a	2.05a	0.58a	2.69a	0.45a	11.8a	0.7a	7.5b	17c
P. tinctorius	119.6ab	1.8a	1.33b	0.38b	1.30b	0.23b	12.2a	0.7a	8.3a	235b
S. collinitus	111.6b	1.9a	1.66ab	0.46ab	1.83b	0.32b	12.8a	0.7a	7.2b	335b
R. roseolus	102.1b	1.5b	0.91b	0.27b	1.27b	0.24b	10.5b	0.5b	5.8c	1745a

Values in vertical columns followed by the same letter do not differ significantly ($P<0.05$) as determined by Tukey's test

Table 3 *Pinus halepensis* growth under field conditions. Values are means of 25 measurements ± SEM (for further explanations, see Table 2)

Urban refuse	Fungus	March 1991		June 1991		September 1991		December 1991	
		Height (cm)	Diameter (mm)	Height (cm)	Diameter (mm)	Height (cm)	Diameter (mm)	Height (cm)	Diameter (mm)
Control (none)	None	8.89 ± 0.54	2.06 ± 0.08	9.19 ± 0.77	2.15 ± 0.10	8.99 ± 0.64	2.15 ± 0.13	10.55 ± 0.58	2.54 ± 0.19
	P. tinctorius	8.0 ± 0.35	1.78 ± 0.21	8.33 ± 0.52	1.88 ± 0.17	8.20 ± 0.63	1.8 ± 0.21	8.86 ± 0.53	2.26 ± 0.10
	S. collinitus	8.32 ± 1.04	2.12 ± 0.12	9.88 ± 1.20	2.41 ± 0.12	10.63 ± 1.05	2.26 ± 0.18	12.80 ± 0.66	2.95 ± 0.26
	R. roseolus	8.43 ± 0.59	1.70 ± 0.06	9.81 ± 1.36	2.20 ± 0.08	9.14 ± 1.92	2.07 ± 0.14	10.51 ± 2.06	2.51 ± 0.10
6 kg m^{-2} (plot 1)	None	9.83 ± 0.81	1.96 ± 0.14	11.50 ± 1.04	2.25 ± 0.30	13.48 ± 1.29	2.84 ± 0.35	14.80 ± 1.46	3.83 ± 0.49
	P. tinctorius	8.97 ± 1.50	1.82 ± 0.09	12.27 ± 2.01	2.60 ± 0.40	16.21 ± 1.18	3.84 ± 0.62	19.70 ± 1.40	5.26 ± 0.64
	S. collinitus	8.33 ± 1.22	2.10 ± 0.21	8.83 ± 1.27	2.10 ± 0.30	11.85 ± 1.31	2.28 ± 0.19	13.31 ± 0.57	3.48 ± 0.60
	r. roseolus	9.11 ± 0.77	1.85 ± 0.17	8.70 ± 1.13	2.02 ± 0.37	10.80 ± 1.84	2.31 ± 0.28	12.14 ± 2.06	2.55 ± 0.47
12 kg m^{-2} (plot 2)	None	9.97 ± 1.10	1.88 ± 0.19	9.86 ± 1.08	1.93 ± 0.21	9.46 ± 1.13	2.36 ± 0.28	11.60 ± 1.74	2.68 ± 0.41
	P. tinctorius	9.92 ± 1.10	2.21 ± 0.16	11.64 ± 1.50	2.35 ± 0.15	13.27 ± 2.23	3.27 ± 0.60	15.84 ± 2.20	4.15 ± 0.59
	S. collinitus	8.32 ± 0.91	1.90 ± 0.18	8.37 ± 1.12	1.90 ± 0.20	10.47 ± 2.14	2.17 ± 0.26	12.68 ± 1.60	2.65 ± 0.24
	R. roseolus	8.82 ± 0.85	2.00 ± 0.22	7.91 ± 0.97	1.91 ± 0.33	6.86 ± 1.00	1.90 ± 0.19	9.37 ± 1.01	2.31 ± 0.21

uninoculated *P. halepensis* plants had significantly lower percentages of root colonization than the inoculated plants but not compared with the non-mycorrhizal plants of the control plot.

The mycorrhizae of *P. tinctorius* reached the greatest degree of development in plot 1 (smaller addition of refuse), while *S. collinitus* and *R. roseolus* reached their greatest development in plot 2 and the control plot, respectively. Taking the treatments as a whole, the highest percentage of root colonization was obtained in the plants inoculated with *S. collinitus*, although a significant difference from both *P. tinctorius* and *R. roseolus* was found only in the plot treated with the highest dose of refuse.

Seedling growth

All the growth parameters measured were directly correlated (Table 4). The greatest shoot dry weight was seen in the plants inoculated with *P. tinctorius* grown in plot 1 (least amount of refuse added); this value was significantly above the shoot dry weight of plants inoculated with *S. collinitus* in soil without refuse and with the smaller dose of refuse, of plants inoculated with *P. tinctorius* grown in soil treated with the larger dose of refuse, and of uninoculated plants grown in soil treated with the smaller dose of refuse. *R. roseolus* was not effective, being associated with a significant decrease in growth in comparison with the uninoculated plants in plot 1 (smaller dose of refuse).

These data show that the fungus used in the inoculation significantly influences plant development. This effect varied with the dose of refuse added. Thus, in the control plot, only *S. collinitus* produced a significant increase in the growth of *P. halepensis*. In plot 1, with the smaller application of refuse, *P. tinctorius* was the most effective fungus, the plants inoculated with *S. collinitus* and those not inoculated showing a similar development; in the same plot *R. roseolus* was responsible for notice-

Table 4 Effect of urban refuse amendments and mycorrhizal inoculation on growth, nutrient assimilation, and root infection in *Pinus halepensis* under field conditions. Data are means of 10 measurements (for further explanations, see Table 2)

Urban refuse (kg m^{-2})	Inoculated fungus	Height (mm)	Basal diameter (mm)	Shoot weight		Assimilated nutrients in shoot tissues (g kg^{-1})			Mycorr. root tips (no. g^{-1} dry weight)
				Fresh (g plant^{-1})	Dry (g plant^{-1})	N	P	K	
0	None	144.6bc	3.9c	6.96c	2.89c	9.9cd	0.5d	10.4abc	853cde
	P. tinctorius	128.6c	3.4c	4.98c	2.13c	8.3de	0.8bc	8.1c	770cde
	S. collinitus	186.8a	5.4b	13.38b	5.80b	11.2bc	0.7c	13.8a	1924ab
	R. roseolus	137.6c	3.7c	6.60c	2.70c	9.0de	0.5d	10.0bc	1200bcd
6	None	189.8a	5.3b	16.08b	6.67b	10.3cd	0.8bc	13.4ab	180e
	P. tinctorius	199.8a	6.6a	27.48a	12.83a	13.1a	1.0ab	13.7a	1476bc
	S. collinitus	177.0ab	5.0b	18.77b	7.32b	13.5a	1.1a	11.6abc	1520bc
	R. roseolus	177.2ab	3.4c	6.06c	2.53c	8.8de	0.5d	9.8bc	1097bcd
10	None	148.2bc	3.5c	5.61c	2.31c	9.7cde	0.6cd	10.8abc	227e
	P. tinctorius	191.8a	5.0b	13.39b	5.47b	12.2ab	0.6cd	12.6ab	478de
	S. collinitus	165.8abc	3.9c	7.61c	3.15c	10.3cd	1.0ab	10.3bc	2582a
	R. roseolus	153.4abc	3.2c	4.08c	1.68c	10.4cd	0.5d	8.5c	560de

Table 5 Soil chemical changes in response to different amounts of urban refuse applied to experimental plots (*C* control, *EC* electrical conductivity)

	Date and time after addition (months)					
	October 1990 (0.5)			April 1991 (7.5)		
	C	Plot 1	Plot 2	C	Plot 1	Plot 2
Urban refuse (kg m^{-2})	0	6	12	0	6	12
EC 1:5 (dS m^{-1})	1.66	2.35	2.20	1.32	1.96	1.89
Organic matter (%)	0.75	1.95	2.91	0.97	1.47	2.47
Total N (%)	0.06	0.16	0.15	0.04	0.23	0.27
$CaCO_3$ (%)	54.0	53.0	57.0	46.0	51.0	48.0
Available P (cmol kg^{-1})	0.16	0.42	0.74	0.22	0.39	0.88
Available K^+ (cmol kg^{-1})	1.02	1.48	1.69	0.92	1.68	2.04
Na^+ (cmol kg^{-1})	0.91	0.90	2.17	1.39	0.61	2.28
Cl^- (cmol kg^{-1})	0.30	0.38	0.80	0.50	0.38	1.98
SO_4^{2-} (cmol kg^{-1})	5.44	6.80	5.97	6.80	6.56	6.15

ably poorer development. *P. tinctorius* was again the most effective fungus in plot 2 (larger application of refuse) while no significant differences were observed between uninoculated plants and those inoculated with *S. collinitus* and *R. roseolus.*

Plant growth was encouraged by the application of refuse but only at the lower dose (6 kg m^{-2}). Higher doses led to notably worse results and there was even a significant decrease in growth in the seedlings inoculated with *S. collinitus* compared with uninoculated plants in soil treated with the same dose of refuse.

Nutrient assimilation

Unlike that observed for the growth parameters, the level of nutrients assimilated showed no direct correlation between each other in any of the treatments (Table 4). The highest N, P, and K contents were seen in the seedlings inoculated with *S. collinitus* and *P. tinctorius* in plot 1 (smaller application of refuse). As for the rest of the treatments, there seemed to be no clear pattern although, in general, *S. collinitus* and *P. tinctorius* produced a greater level of nutrient assimilation in all three experimental plots while lower values were obtained with *R. roseolus.*

Again, the smaller application of refuse had a positive effect on the assimilation of N, P, and K (this last to a lesser degree), although only in those seedlings inoculated with *P. tinctorius* and *S. collinitus.*

Soil chemical changes with refuse treatment

The results of chemical analyses of soil in the plots treated with different applications of refuse are shown in Table 5.

Discussion

The selection of effective ectomycorrhizal fungi is of great importance. Although most cases of ectotrophic symbiosis produce beneficial effects on plants (Alvarez 1991), it is also common to find no effects (France and Cline 1987), and even cases where mycorrhizal infection is responsible for significant reductions in plant growth (Hung 1983), perhaps due to competition for nutrients between the fungal symbiont and the host plant (Castellano and Molina 1989). It is clear that under the controlled growth conditions of the present experiment, mycorrhizal inoculation with the species tested produced no positive effect on *P. halepensis* seedlings. Indeed, the plants inoculated with *R. roseolus* showed poorer development and assimilated less nutrients than uninoculated plants. However, these results cannot necessarily be extrapolated to field conditions since the great diversity of microbiological and environmental factors which might affect the development of mycorrhizal symbiosis have not been studied in depth (Linderman 1988; Perry et al. 1987) and are difficult to reproduce in the laboratory.

The effectiveness of mycorrhizal inoculation in producing an increase in the growth of *P. halepensis* in the field has been demonstrated in the present study and, to a lesser extent, so has its ability to increase nutrient uptake. The results obtained in the control plot with no organic amendment demonstrate the beneficial effect of inoculation with *S. collinitus*, the improvements in plant development obtained being solely attributable to the symbiotic fungus. The other fungi used produced no differences from the uninoculated plants. *S. collinitus* is a very common species in reafforested pinewoods in southeast Spain (Torres et al. 1991) and one of the first to fructify after reafforestation. In this respect, it can be considered a naturally occurring pioneering fungus in the mycorrhizal colonization of *P. halepensis* and, as the first stages of growth are the most critical in reafforestation (Perry et al. 1987), inoculation with a pioneering fungus should favour the processes.

In the plots treated with refuse, it was the smaller dose that favoured the growth of *P. halepensis* seedlings, except those inoculated with *R. roseolus.* Danielson (1990) reported that the addition of sewage sludge had a positive effect on growth and mycorrhizal development in *Picea* and *Pinus* spp. although this effect could not be wholly attributed to the amendment. A similar type of effect occurred in the present experiment; in spite of the increase in nutrient levels brought about by application of the refuse (Table 5), no correlation was found between these and levels of mycorrhizal colonization, seedling growth, and nutrient uptake. However, there was a positive correlation between the overall levels of mycorrhizal colonization and P uptake by seedlings ($r = 0.3256$, $P < 0.01$) and, in the case of seedlings inoculated with *S. collinitus*, between the level of mycorrhizal colonization and N uptake ($r = 0.6452$, $P < 0.05$), but never with dry weight. It seems that the mycorrhizal effect was not quantitative and that a higher degree of root colonization was not matched by greater plant development although in some cases an increase in nutrient uptake was detected.

The higher application of refuse invariably led to a decrease in plant growth. The addition of refuse at a lower rate might have had a beneficial effect on some of the fungus/plant symbiosis, particularly with *P. tinctorius* and, to a lesser extent, with *S. collinitus*. When non-composted organic refuse is used as soil conditioner it can cause depressive effects on seed germination and plant growth (García et al. 1990), also on vesicular-arbuscular fungal populations (Roldán and Albaladejo 1993). The toxicity of urban refuse can be attributed to its content of phenolic and other aromatic substances with alcohol and aldehyde radicals. In fact, in other experiments with the same materials and environmental conditions, García et al. (1992) showed that the addition of refuse increased the percentage of cresols, phenols, 1–4, dimetoxibenzene, guaiacol, and furfural in the composition of the soil organic matter. After 3 years the phenols had increased still further with respect to the initial percentage.

In conclusion, the present has provided important information on how to regenerate vegetal cover on degraded land in a dry environment. The combination of soil amendment with urban refuse and mycorrhizal inoculation of individual seedlings can considerably improve the growth of *P. halepensis* in arid soils. However, tests must be carried out before planting since the correct choice of fungal species and the rate of refuse application to the soil are of prime importance if negative effects are to be avoided. It has been shown that with only one application of refuse, problems with heavy metal toxicity do not occur (Albaladejo and Stocking 1994). A full evaluation of other aspects such as off-site impacts, cost-benefit analyses, and the possible dissemination of pathogens will be necessary before large-scale refuse applications can be made. Further studies are required to address these problems.

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