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Effect of arbuscular mycorrhizae and induced drought stress on antioxidant enzyme and nitrate reductase activities in *Juniperus oxycedrus* L. grown in a composted sewage sludge-amended semi-arid soil

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Abstract

We studied the influence of inoculation with a mixture of three exotic arbuscular mycorrhizal (AM) fungi, *Glomus intraradices* Schenck & Smith, *Glomus deserticola* Trappe, Bloss. & Menge and *Glomus mosseae* (Nicol & Gerd.) Gerd. & Trappe, and the addition of composted sewage sludge (SS) on the activities of the antioxidant enzymes superoxide dismutase (SOD, EC 1.15.1.1) and total peroxidase (POX) and of shoot and root nitrate reductase (NR, EC 1.6.6.1) in *Juniperus oxycedrus* L. seedlings, an evergreen shrub, grown in a non-sterile soil under well-watered and drought-stress conditions. Both the inoculation with exotic AM fungi and the addition of composted SS stimulated significantly growth and the N and P contents in shoot tissues of *J. oxycedrus* with respect to the plants neither inoculated nor treated with composted SS that were either well-watered or droughted. Under drought-stress conditions, only inoculation with exotic AM fungi increased shoot and root NR activity (about 188% and 38%, respectively, with respect to the plants neither inoculated nor treated with composted SS). Drought increased the POX and SOD activities in both shoots of *J. oxycedrus* seedlings inoculated with exotic AM fungi and grown with composted SS, but the increase was less than in the plants neither inoculated nor treated with SS. Both the plants inoculated with exotic AM fungi and the plants grown with composted SS developed additional mechanisms to avoid oxidative damage produced under water-shortage conditions.

Introduction

Arbuscular mycorrhizal (AM) fungi are obligate symbiotic soil fungi that colonise the roots of the majority of plants and help to improve the performance of the plants in semi-arid conditions (Alguacil et al., 2004; Caravaca et al., 2003a) by increasing the supply of nutrients to the plant (Toro et al., 1997), improving soil aggregation and biochemical quality in eroded soils (Caravaca et al., 2002) and reducing water stress (Augé, 2001; Porcel and Ruíz-Lozano, 2004). In this way, the use of mycorrhizal fungi inoculation technologies is an effective method for carrying out successful reforestation programmes in semi-arid Mediterranean areas. Diverse studies have demonstrated the protection mechanisms that mycorrhizal plants have against the detrimental effects of drought stress: increased plant leaf gas exchange, photosynthetic rate and water use efficiency (Querejeta et al., 2003).

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Activated oxygen species (AOS), such as superoxide ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2) and hydroxyl radicals ($^{\bullet}OH$), are formed as by-products of normal metabolism in different cellular organelles (Scandalios, 1993). Under environmental stress conditions, such as drought stress, an increase in the generation of AOS has been described (Menconi et al., 1995; Munné-Bosch and Peñuelas, 2003) that may cause damage to cells. To mitigate and repair damage initiated by AOS, plants possess several mechanisms that detoxify $O_2^{\bullet-}$ and H_2O_2, called antioxidant systems. The primary components of these antioxidant systems include non-enzymatic antioxidants (carotenoids, ascorbate, glutathione and tocopherols) and enzymes such as superoxide dismutase (SOD, EC 1.15.1.1) and peroxidases (POX). The components of this antioxidant defence system can be found in different subcellular compartments (Hernández et al., 2001; Jiménez et al., 1997). Recently, it has been reported that inoculation with mycorrhizal fungi produced an increase in some antioxidant enzymes in shoots from mycorrhizal shrub species afforested in a degraded semi-arid soil (Alguacil et al., 2003) and in roots and nodules of mycorrhizal soybean plants (Porcel et al., 2003).

Another mechanism of protection against drought stress is nitrate reductase (NR, EC 1.6.6.1), which catalyses the rate-limiting step in the nitrate assimilation pathway. This enzyme is inducible by its substrate, nitrate ions (Kandlbinder et al., 2000). Under drought conditions, NR activity decreases in plants due to the lower uptake of nitrate from the soil by the root (Azcón et al., 1996). In such conditions, positive effects of inoculation with mycorrhizal fungi on nitrate acquisition and assimilation in plants have been reported in fast-growing herbaceous plants with high water requirements (Azcón et al., 2001), although these effects depend on the associated mycorrhizal fungus and the host plant species. However, it would be very interesting to study such mechanisms for slow-growing plants adapted to arid conditions, like *Juniperus oxycedrus* L., since no information is available about the effects of inoculation with mycorrhizal fungi on the levels of NR or on antioxidant enzymes in these plants under induced drought stress.

In the Mediterranean area of SE Spain, the application of certain organic amendments to soil, such as sewage sludge (SS), is an effective method for facilitating the recovery of the physical, chemical and microbiological properties of degraded soils, which favours the establishment and viability of a stable plant cover (Roldán et al., 1994). These carbon-rich materials have been used widely on agricultural lands, improving the soil fertility. However, little information is available on the use of such materials in revegetation programmes or on the influence of such materials on the performance of plants under stress conditions (Navas et al., 1999). Moreover, nothing is known about the interaction of such materials with mycorrhizal fungi inoculation and the physiological response of plants.

The objective of this study was to determine the influence of inoculation with a mixture of three exotic AM fungi and the addition of composted SS on the activities of some antioxidant enzymes (SOD and POX) and nitrate reductase in *J. oxycedrus* seedlings grown in a non-sterile soil, under well-watered and drought stress conditions.

Materials and methods

Materials

The soil was collected from Los Cuadros in the Province of Murcia (SE Spain) (coordinates: 1°05′ W and 38°10′ N). The climate is semi-arid 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 1).

The compost used in this experiment was produced from a mixture of wood shavings and an aerobically digested SS, at a rate of 1:1 (v:v). The SS was obtained from a water treatment plant in Murcia. The composting process involved a first stage lasting 2 months, during which the waste heaps were turned in open air nine times, and a second maturation stage, in which the products were allowed to stand on boards for 2 months so that they could stabilise. The composted SS was sieved, ground to 0.5 mm particles and air-dried for analysis. The pH and electrical conductivity were measured in a 1:10 (w/v) aqueous extract.

Table 1. Chemical, microbiological, biochemical and physical characteristics of the soil used in the experiment

pH (H_2O)	8.5 ± 0.0[a]
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

[a]Mean ± standard error ($n = 6$).

Table 2. Analytical characteristics of the composted SS used in the experiment

Ash (%)	18.6 ± 0.1[a]
pH (1:5)	6.1 ± 0.0
Electrical conductivity EC (1:5 μS cm^{-1})	3095 ± 48
Total organic C (g kg^{-1})	380 ± 4
Water-soluble C (μg g^{-1})	7245 ± 22
Water-soluble carbohydrates (μg g^{-1})	590 ± 53
Total N (g kg^{-1})	14.5 ± 0.1
N-NH_3 (μg g^{-1})	312 ± 13
N-NO_3 (μg g^{-1})	1967 ± 49
Total P (g kg^{-1})	4.5 ± 0.1
Total K (g kg^{-1})	2.3 ± 0.1
Fe (μg g^{-1})	6562 ± 165
Cu (μg g^{-1})	212 ± 8
Zn (μg g^{-1})	588 ± 30
Ni (μg g^{-1})	44 ± 3
B (μg g^{-1})	85 ± 2
Cd (μg g^{-1})	9 ± 1
Pb (μg g^{-1})	180 ± 28
Porosity (%)	78 ± 1

[a]Mean ± standard error ($n = 6$).

The organic matter content was determined by calcination at 750 °C for 4 h and the contents of total organic C and total N by dry combustion (Nelson and Sommers, 1982). The contents of heavy metals were determined by atomic absorption of the extract after nitric–perchloric digestion. The analytical characteristics of the composted SS are shown in Table 2.

Plants and mycorrhizal treatments

The plant used for the experiment, *Juniperus oxycedrus* L., one of 10 species in the genus *Juniperus* found throughout the world (Adams, 1998), is a low-growing tree reaching a height of 3–4 m, although often it grows as a shrub. This shrub has a typical Mediterranean distribution and is well-adapted to drought conditions because it can thrive with mean annual rainfall of less than 230 mm and a summer drought period which can extend for 4 months (Amaral Franco, 1986). However, knowledge of revegetation strategies involving *J. oxycedrus* is still very limited.

The mycorrhizal fungi used in the experiment, *Glomus intraradices* Schenck and Smith (EEZ 1), *Glomus deserticola* (Trappe, Bloss. and Menge) (EEZ 45) and *Glomus mosseae* (Nicol and Gerd.) Gerd. and Trappe (EEZ 43), were obtained from the collection of the experimental field station of Zaidín, Granada. The acronym EEZ refers to Estación Experimental del Zaidín.

AM fungal inoculum consisted of a mixture of rhizospheric soil from trap cultures (*Sorghum* sp.) containing spores, hyphae and mycorrhizal root fragments. Once germinated, the *J. oxycedrus* seedlings were transplanted into the temporary growth substrate, consisting of peat (*Sphagnum* type) and cocopeat from *Cocos nucifera* L. wastes (Projar S.A.) (1:1, v:v). The corresponding arbuscular mycorrhizal inoculum was applied at a rate of 5% (v/v) in 120-mL containers. The same amount of an autoclaved mixture of the inocula was added to non-inoculated plants, supplemented with a filtrate (< 20 μm) of the culture to provide the microbial populations accompanying the mycorrhizal fungi. Inoculated and non-inoculated seedlings were grown for 8 months under nursery conditions without any fertiliser treatment.

Experimental design

A factorial design was established with three factors and 8-fold replication. The first factor had two levels: addition or not of composted SS to

the soil. The second factor had two levels: non-inoculation or inoculation of *J. oxycedrus* plants with a mixture of three exotic AM fungi (*G. intraradices*, *G. deserticola* and *G. mosseae*). The third had two levels: well-watered or drought-stressed conditions.

In early February 2004, the experimental soil was placed in 1500-mL (13 cm diameter, 11.3 cm height) capacity pots, which had drainage holes. Composted SS residue was mixed manually with the soil at a rate of 5% (v/v) into half of the pots. Seedlings together with the temporary growth substrate (inoculated and non-inoculated) were transplanted to pots (one per pot). Eight replicates per treatment were established (64 seedlings in total). Plants were watered regularly with decalcified water until the initiation of the drought treatment (after 4 months of growth). At this time the plants were allowed to dry until soil water potential reached −0.6 MPa and maintained under such conditions for 15 days (from 13 June until 28 June). The experiment was conducted in a greenhouse, located in the Campus of Espinardo (Murcia, Spain). During the experiment, the temperature ranged from 11 to 34 °C and the relative humidity was between 40% and 80%. Mid-day photosynthetically active radiation (PAR) averaged 260 μE m^{-2} s^{-1}.

Soil water potential

Soil water potential was determined by a pressure plate apparatus and soil water content was measured by weighing the soil before and after drying at 110 °C for 24 h (Richards, 1941). A characteristic soil moisture curve was constructed and used to correlate soil water content and soil water potential (Ψ) by gravimetric measurement of soil water content in the pots.

Plant analyses

After the water stress period, basal stem diameters and heights of plants were measured with callipers and rulers, respectively. Plants were harvested and the roots were washed free from soil under a stream of cold tap water. Fresh and dry (105 °C, 5 h) weights of shoots and roots were recorded. Plant tissues were ground before chemical analysis. The foliar contents of phosphorus were determined, after digestion in nitric-perchloric acid (5:3) for 6 h, by colorimetry (Murphy and Riley, 1962) and the N concentration was determined by the Kjeldahl method.

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

Shoot enzyme extraction

All operations were performed at 4 °C. Shoots (2 g) were homogenised with a mortar and pestle in 4 mL of ice-cold 50 m*M* Tris–acetate buffer, pH 6.0, containing 0.1 m*M* ethylenediaminetetraacetic acid (EDTA), 5 m*M* cysteine, 2% (w/v) polyvinylpyrrolidone (PVP), 0.1 m*M* phenylmethylsulphonyl fluoride (PMSF) and 0.2% (v/v) Triton X-100. The homogenate was centrifuged at $14\,000 \times g$ for 20 min and the supernatant fraction was filtered through Sephadex G-25 columns (NAP, Pharmacia Biotech AB, Uppsala, Sweden), equilibrated with the same buffer used for the homogenisation.

Assays performed

Total SOD activity was assayed by the ferricytochrome *c* method using xanthine/xanthine oxidase as the source of O_2^{-} radicals (McCord and Fridovich, 1969). Total peroxidase was analysed according to Ros-Barceló (1998).

NR activity was assayed *in vivo* by measuring NO_2^- production in tissue that had been vacuum-infiltrated with buffered NO_3^- solutions (Downs et al., 1993). The leaves and roots from the non-stressed and stressed seedlings were collected in the morning, between 8:30 and 11:00 solar time. Leaves and roots of *J. oxycedrus* were cut into 5-mm sections. Approximately 300 mg of leaf punches and 300 mg of roots were placed in 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 aliquots with 1 mL of 1% sulphanilamide in 1 *M* HCl and 1 mL of 0.01% *N*-1-napthyl-ethylenediamine hydrochloride. After 15 min, the optical density was measured at 540 nm with a Beckman spectrophotometer.

Statistical analysis

Data were log-transformed to achieve normality. The effects of composted SS addition, inoculation with mycorrhizal fungi and water regime, and their interactions, on measured variables were tested by a three-way analysis of variance and comparisons among means were made using the Least Significant Difference (LSD) test calculated at $P < 0.05$. Statistical procedures were carried out with the software package SPSS (Statistical Package for the Social Science) 10.0 for Windows.

Results

Growth, nutrient assimilation and mycorrhizal fungi colonisation

Both the addition of composted SS and the inoculation with exotic mycorrhizal fungi increased significantly shoot and root dry weights, height and basal diameter in *J. oxycedrus* seedlings with respect to the non-inoculated and non-treated with composted SS plants, under either well-watered or drought-stressed conditions (Table 3). The addition of composted SS increased root biomass and height of the plants to a very significant degree ($P < 0.001$, Table 4). Under drought-stress conditions, inoculation with exotic mycorrhizal fungi was more effective with respect to increasing shoot biomass than the addition of the composted SS alone (about 44% higher with respect to non-inoculated plants grown in the amended soil). Fifteen days of water shortage did not have significant effects on the growth parameters determined (Table 4).

Both the exotic mycorrhizal fungi and the addition of composted SS increased foliar nutrients with respect to the non-inoculated and non-treated with composted SS plants in both water regimes (Table 3), the inoculation with exotic mycorrhizal fungi being the most effective treatment for increasing foliar N and P contents. As observed for the growth parameters, the water regime did not have an effect on foliar nutrients in shoots of *J. oxycedrus* seedlings (Table 4).

Juniperus oxycedrus seedlings inoculated with exotic AM fungi had significantly higher percentages of root colonisation than the plants colonised by native AM fungi (Table 3). Before water stress, the addition of composted SS to soil decreased both the natural colonisation and the percentages of root colonisation in plants inoculated with exotic AM fungi. Percentages of mycorrhizal roots varied with the water regime, particularly in the inoculated plants with exotic AM fungi (Table 4). There was a positive interaction, with respect to increasing the AM fungi colonisation, between the water regime, inoculation with exotic mycorrhizal fungi and the addition of composted SS.

Nitrate reductase activity

Composted SS addition had no significant effect on NR activity in shoots of *J. oxycedrus* seedlings, under either well-watered or drought-stressed conditions (Tables 4 and 5). The water regime affected shoot NR activity to a very significant degree ($P < 0.001$). There was a positive interaction, with respect to increasing the shoot NR activity, between the water regime and inoculation with exotic mycorrhizal fungi. The seedlings inoculated with exotic AM fungi showed the highest values of shoot NR activity in drought stress conditions. Compost addition and exotic AM fungi inoculation increased significantly the NR activity in roots (Table 4). The water stress decreased NR activity in roots of *J. oxycedrus* plants grown in the soil amended with composted SS (Table 5).

Antioxidant enzyme activities

Inoculation with exotic mycorrhizal fungi, composted SS addition and water regime had very significant effects on SOD and POX activities in shoots of *J. oxycedrus* (Table 4). Drought significantly increased the POX and SOD activities in shoots of *J. oxycedrus* plants, except for POX activity in plants treated with composted SS (Table 5). However, both plants inoculated with the mixture of exotic AM fungi and plants treated with composted SS reached values higher than non-inoculated and non-treated with composted SS plants, in both well-watered and drought-stress conditions.

Table 3. Growth parameters, foliar nutrients and root infection of *J. oxycedrus* seedlings in response to inoculation with mycorrhizal fungi, composted SS addition and water regime ($n = 8$)

	Well watered	Drought stressed
Shoot (*g dw*)		
No SS, no AM inoculum	2.64 ± 0.07[A]a	2.55 ± 0.05a
SS	3.92 ± 0.30b	3.26 ± 0.02b
AM inoculum	4.30 ± 0.24b	4.70 ± 0.08c
SS and AM inoculum	4.10 ± 0.22b	4.73 ± 0.13c
Root (*g dw*)		
No SS, no AM inoculum	1.46 ± 0.13a	1.25 ± 0.10a
SS	3.03 ± 0.24c	2.26 ± 0.04b
AM inoculum	2.30 ± 0.12b	2.70 ± 0.05c
SS and AM inoculum	2.41 ± 0.15b	2.90 ± 0.06c
Height (*cm*)		
No SS, no AM inoculum	18.75 ± 0.11a	17.17 ± 0.29a
SS	26.10 ± 0.72b	22.25 ± 0.04b
AM inoculum	24.00 ± 0.78b	24.83 ± 0.73b
SS and AM inoculum	29.00 ± 0.21b	27.63 ± 0.45b
Basal diameter (*mm*)		
No SS, no AM inoculum	2.86 ± 0.05a	2.89 ± 0.04a
SS	3.47 ± 0.01b	2.96 ± 0.07ab
AM inoculum	3.40 ± 0.06b	3.49 ± 0.05b
SS and AM inoculum	3.10 ± 0.06b	3.22 ± 0.05b
Nitrogen (*mg plant*$^{-1}$)		
No SS, no AM inoculum	15.4 ± 0.9a	14.2 ± 0.2a
SS	30.1 ± 1.0b	28.2 ± 1.1b
AM inoculum	34.1 ± 1.2b	35.4 ± 1.3c
SS and AM inoculum	32.4 ± 1.3b	30.9 ± 1.0bc
Phosphorus (*mg plant*$^{-1}$)		
No SS, no AM inoculum	0.90 ± 0.11a	0.88 ± 0.02a
SS	2.80 ± 0.25b	2.77 ± 0.03b
AM inoculum	3.20 ± 0.23b	3.37 ± 0.08c
SS and AM inoculum	2.95 ± 0.17b	3.05 ± 0.05bc
Colonisation (*%*)		
No SS, no AM inoculum	17.50 ± 0.49b	13.80 ± 0.83a
SS	4.50 ± 0.07a	10.70 ± 0.65a
AM inoculum	62.50 ± 0.49d	41.67 ± 1.19b
SS and AM inoculum	32.50 ± 0.21c	64.00 ± 1.57c

Values in columns followed by the same letter are not significantly different (LSD, $P < 0.05$).
[A]Mean ± standard error.

Discussion

Both the inoculation of seedlings with a mixture of three exotic AM fungi and the addition of composted SS to soil alone stimulated the growth of *J. oxycedrus*. The extent of mycorrhizal infection is of importance when studying the influence of AM fungi on the host plant. Large differences in AM percentage colonisation between non-inoculated and inoculated with exotic AM fungi seedlings persisted throughout the drought-stress experiment, as the local indigenous AM fungi from the soil and/or composted SS showed little capacity to colonise shrub roots. The local AM fungi community from the experimental soil was much less effective than the added *Glomus* inoculum at stimulating host plant growth. Remarkably, *Juniperus* shrubs inoculated with the exotic AM fungi species had comparable size to those treated with composted SS, even though it decreased AM colonisation in the well-watered plants. This could be due to an improvement in the available nutrient supply in soil, arising from the composted SS. Cox et al. (2001) showed that the use of soil amendments can improve soil productivity, increasing the soil nutrient status for some limiting nutrients such as N and P. Thus, we report here that plants grown in the amended soil had higher nutrient (N and P) contents in their tissues than plants grown in the non-amended soil. Likewise, differential improvement of host plant nutrient status by the exotic AM fungi inoculum over native AM fungi from the soil was also recorded under both water regimes.

The effect of drought stress on plant acquisition of mineral nutrients may be ascertained by measuring nitrate reductase activity, which is involved in nitrate assimilation. So, NR activity may be an indicator of the metabolic and physiological status of plants and has been used to quantify changes in plant physiology under stress conditions (Caravaca et al., 2003b; Ruíz-Lozano and Azcón, 1996). In this study, we have found increased NR activity only in roots and shoots of *J. oxycedrus* plants inoculated with the mixture of three exotic AM fungi, thus leading to much higher shoot N content than in plants neither inoculated nor treated with composted SS under water-stressed conditions. In contrast, the addition of composted SS to soil alone had any effect on the NR activity under such conditions. This finding indicates the active contribution of the exotic fungi compared to the native fungi from the soil and/or composted SS to nitrate uptake and assimilation in such conditions, even in shrubs adapted to arid conditions, such as *J. oxycedrus*. This could be a consequence of a better access of plants inoculated with exotic AM fungi,

Table 4. Three factor ANOVA (inoculation with mycorrhizal fungi, composted SS addition and water regime) for all parameters studied in the *J. oxycedrus* seedlings

				Interactions			
Source of variation	Sewage sludge (SS)	Mycorrhiza (M)	Water regime (WR)	SS*M	SS*WR	M*WR	SS*M*WR
Shoot biomass	0.014	< 0.001	0.404	0.045	0.518	0.036	0.867
Root biomass	< 0.001	0.001	0.907	0.002	0.864	0.054	0.704
Height	< 0.001	< 0.001	0.051	0.038	0.248	0.080	0.845
Basal diameter	0.672	0.003	0.387	0.001	0.114	0.038	0.076
Colonisation	0.001	< 0.001	0.026	0.185	0.060	0.132	< 0.001
Foliar N	< 0.001	< 0.001	0.562	0.322	0.415	0.052	0.080
Foliar P	0.001	< 0.001	0.468	0.185	0.283	0.080	0.102
Shoot NR activity	0.955	0.044	< 0.001	0.679	0.516	< 0.001	0.016
Root NR activity	< 0.001	< 0.001	0.012	0.036	0.181	0.028	0.001
POX	< 0.001	< 0.001	< 0.001	0.005	0.160	< 0.001	< 0.001
SOD	< 0.001	< 0.001	< 0.001	0.185	0.799	0.939	0.690

P significance values.

Table 5. Shoot and root nitrate reductase (NR), shoot total peroxidase (POX) and superoxide dismutase (SOD) activities of *J. oxycedrus* seedlings in response to inoculation with mycorrhizal fungi, composted SS addition and water regime ($n = 8$)

	Well watered	Drought stressed
Shoot NR activity (nmol NO_2^- g FW^{-1} h^{-1})		
No SS, no AM inoculum	3.58 ± 0.12[A]b	8.29 ± 0.46a
SS	2.77 ± 0.07b	9.38 ± 0.33a
AM inoculum	1.98 ± 0.07a	23.85 ± 3.18c
SS and AM inoculum	3.01 ± 0.21b	15.64 ± 1.10b
Root NR activity (nmol NO_2^- g FW^{-1} h^{-1})		
No SS, no AM inoculum	6.54 ± 0.13a	6.62 ± 0.21a
SS	12.93 ± 0.52b	7.22 ± 0.16ab
AM inoculum	10.65 ± 0.19b	9.12 ± 0.07b
SS and AM inoculum	11.10 ± 0.71b	12.01 ± 0.42b
POX (Units mg^{-1} protein)		
No SS, no AM inoculum	34 ± 0.1c	154 ± 0.42d
SS	16 ± 1.56b	12 ± 0.1a
AM inoculum	11 ± 0.36b	72 ± 6.65c
SS and AM inoculum	3 ± 0.36a	43 ± 4.12b
SOD (Units mg^{-1} protein)		
No SS, no AM inoculum	374 ± 41d	1017 ± 64.7d
SS	169 ± 20.44b	399 ± 26b
AM inoculum	200 ± 2.40c	643 ± 85c
SS and AM inoculum	44 ± 0.50a	155 ± 12a

Values in columns followed by the same letter are not significantly different (LSD, $P < 0.05$).
[A]Mean ± standard error.

through the extraradical mycelium, to the forms of N which are usually unavailable when water availability is limited (Subramanian and Charest, 1999). The present results are also consistent with the finding (Ruíz-Lozano and Azcón, 1996) that inoculation with exotic mycorrhizal fungi induced an increased NR activity in lettuce plants in water-limited environments, although in this case the plant tested was a vegetable with high watering requirements. 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). In this sense, the mycorrhizal effect could be interpreted as an indirect response to the improved nutrient status, particularly of phosphorus. The fact that the higher foliar P contents in shoots occurred in plants inoculated with the mixture of exotic AM fungi could explain how these plants had higher values of NR activity. Likewise, a positive correlation ($P < 0.05$) was observed between the foliar P levels and the values of shoot NR activity in drought-stressed plants inoculated with the mixture of three exotic AM fungi. Furthermore, the mycorrhizal effect on the NR activity of the host plants could be also a consequence of fungal NR activity. In fact, it has been shown, using molecular approaches, that some AM fungi produce such enzymatic activity (Kaldorf et al., 1998).

The effectiveness of AM fungi can be measured in terms of host plant growth under different environmental conditions (Ruíz-Lozano et al., 1995). In this respect, the inoculation with the mixture of exotic AM fungi used in this study was the most effective treatment for improving the growth of plants under both well-watered and drought-stress conditions, compared to plants neither inoculated nor treated with composted SS. The effect of the exotic mycorrhizal fungi was greater for root biomass than for aerial biomass when grown under stress conditions. It has been hypothesised that mycorrhizae can alter the morphology of the root system, yielding a more extensive absorbing area, which may be considered a mechanism of drought tolerance (Ibrahim et al., 1990). Likewise, mycorrhizae are known to increase the xylem pressure potential, by increasing root biomass and therefore improving water uptake (Augé, 2001). In general, it is worth noting that the water regime had no effect on growth and nutrient uptake of *J. oxycedrus* seedlings, probably due to the short duration of the drought treatment and because these plants are well-adapted to drought conditions.

It is well known that the antioxidant systems of plants act as important tolerance mechanisms against drought stress. Enhanced SOD and POX activities have been associated with induced resistance of plants to drought stress (Ruíz-Lozano, 2003). Both activities are involved in superoxide radical and hydrogen peroxide scavenging. In previous works, where several antioxidant enzymes have been studied under the same stress conditions, differential responses in mycorrhizal plants have frequently been observed. Under induced water stress, SOD specific activity was higher in shoots and roots of inoculated plants than in P-fertilised, non-inoculated *Lactuca sativa* plants, showing increases of 99% and 150%, respectively (Ruíz-Lozano et al., 1996). An increase in several antioxidant enzymes has been observed also in shoots of mycorrhizal shrubs in semi-arid Mediterranean conditions (Alguacil et al., 2003). In our study, drought considerably enhanced oxidative damage in shoots of *J. oxycedrus* plants, which was confirmed by the increases in SOD and POX activities observed in such plants. The levels of SOD and POX activities in shoots of *J. oxycedrus* plants and the increases produced by drought in such enzymatic activities were higher than those recorded by Zhang and Kirkham (1996) in leaves of sorghum and sunflower. The addition of composted SS and the inoculation with exotic mycorrhizal fungi, as well as the combined treatment, produced a decrease in SOD and POX activities compared to plants neither inoculated nor treated with composted SS, under both water regimes. In general, this decrease was more pronounced in plants treated with composted SS and inoculated with the exotic mycorrhizal fungi. A similar response was observed in mycorrhizal soybean plants, that showed lower ascorbate peroxidase (APX) activity values relative to the corresponding non-mycorrhizal plants (Porcel et al., 2003). Similarly, in nodules, SOD, catalase and APX activities were lower in droughted, mycorrhizal plants than in non-mycorrhizal plants (Porcel et al., 2003). The decrease in antioxidant enzymes observed in both plants inoculated with exotic mycorrhizal fungi and the plants grown with composted SS could be explained partially by the fact that these plants may be submitted to a lower oxidative stress under both control and drought-stress conditions. This could be attributed to primary drought-avoidance mechanisms, such as the active water transfer from AM fungi to the host (Porcel and Ruíz-Lozano, 2004) or increased water uptake related to mycorrhizal changes in root morphology (Ibrahim et al., 1990) or the greater water-retention properties of

an amended soil (Caravaca et al., 2002). These observations agree with the proposal by Bartels (2001) that both the prevention of oxidative stress and the elimination of reactive oxygen species are the most effective approaches used by plants to gain tolerance against several abiotic stresses, including drought.

In conclusion, the most effective treatment, with respect to improving the growth of *J. oxycedrus* plants under greenhouse conditions, was the inoculation with a mixture of three exotic AM fungi of seedlings, independent of the water regime. The capacity of AM fungi for increasing plant tolerance to the drought stress imposed may have been related to nutrient uptake improvement and to an increase in N assimilation through NR activity. Drought increased the POX and SOD activities in both shoots of *J. oxycedrus* seedlings inoculated with exotic AM fungi and grown with composted SS, but the increase was less than in the non-inoculated and non-treated with SS plants. This might indicate that both the plants inoculated with exotic AM fungi and the plants grown with composted SS also developed mechanisms to avoid oxidative damage produced under water-shortage conditions.

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