EFFECT OF A COMPOSTED SEWAGE SLUDGE ON THE PERFORMANCE OF MYCORRHIZAL *RETAMA SPHAEROCARPA* L. SEEDLINGS AFFORESTED IN A DEGRADED SOIL UNDER TWO WATER REGIMES

M.M. Alguacil[1], F. Caravaca[1], G. Díaz[2], P. Marín[3], A. Roldán[1]

[1]CSIC-Centro de Edafología y Biología Aplicada del Segura. Department of Soil and Water Conservation. P.O. Box 164, Campus de Espinardo 30100-Murcia (Spain).

[2]Universidad Miguel Hernández de Elche. Department of Applied Biology. Avda. Ferrocarril, s/n. Edf. Laboratorios- 03202-Elche, Alicante (Spain).

[3]University of Murcia. Department of Agricultural Chemistry, Geology and Edaphology. Campus de Espinardo 30071-Murcia (Spain).

ABSTRACT

We studied the effect of inoculation with three arbuscular mycorrhizal (AM) fungi (*Glomus intraradices*, *Glomus deserticola* and *Glomus mosseae*) and addition of composted sewage sludge (SS) on root nitrate reductase activity, mycorrhizal colonisation, plant growth and nutrient uptake in *Retama sphaerocarpa* L. seedlings afforested in a semiarid Mediterranean degraded area, under well-watered and non-watered conditions. Six months after planting, the mycorrhizal inoculation and the irrigation of plants had had a strong effect on the growth parameters. There was a negative interaction between plant irrigation and mycorrhizal inoculation, with respect to increasing plant growth. The SS addition had a significant effect on the growth parameters but conferred no additional benefit when combined with mycorrhizal inoculation. Mycorrhizal inoculation, SS addition and plant irrigation had a significant effect on NR activity in roots and on foliar nutrients. The plant irrigation increased the positive effect of SS addition on NR activity and foliar N and K contents. The effect of mycorrhizal inoculation on NR activity did not depend on the water regime. The effectiveness of mycorrhizal inoculation on the establishment and growth of *R. sphaerocarpa* was independent of water regime. The composted SS addition was only effective when water availability in soil was high.

INTRODUCTION

Nitrogen-fixing shrubs, mainly leguminous species such as *Retama sphaerocarpa* (L.) Boissier, have been considered useful for revegetation of water deficient ecosystems that have low availability of nitrogen, phosphorus and other nutrients, because of their ability to develop symbiotic associations with both rhizobial bacteria and mycorrhizal fungi (Caravaca et al., 2003a). Inoculation with 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. Nitrate reductase activity, which catalyses the rate-limiting step in the nitrate assimilation pathway, has been proposed as an index for assessing the effectiveness of fungus-host plant combinations for mitigation of water-deficit stress (Caravaca et al., 2003b). The effect of AM fungi on nitrate assimilation is dependent on the fungus involved in the symbiosis and the host plant species. However, there are no reports in relation to the effect of mycorrhizal inoculation on the levels of nitrate reductase in *R. sphaerocarpa* plants under field conditions, nor on the effect of water regime.

The incorporation of certain organic amendments, such as sewage sludge, can favour seedling establishment under semiarid conditions. Such materials can improve the soil fertility by acting directly on its biological, physical and chemical properties, which, in turn, activates the microbial biomass, improves soil structure and increases water-holding capacity. However, little 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 different AM fungi and the addition of composted sewage sludge with respect to increasing and root nitrate reductase activity, mycorrhizal colonisation, plant growth and nutrient uptake in *R. sphaerocarpa* seedlings, afforested in a semiarid degraded soil, under well-watered and non-watered conditions.

MATERIALS AND METHODS

Study sites

The experimental area was located in Los Cuadros in the Province of Murcia (southeast Spain) (coordinates: 1º05'W and 38º10'N). The climate is semiarid Mediterranean with an average annual rainfall of 300 mm and a mean annual temperature of 19.2ºC; the potential evapotranspiration reaches 1000 mm y^{-1}.

Experimental design and layout

A factorial design in randomised blocks was established with three factors and five-fold replication. The first factor had two levels: addition or not of composted sewage sludge to the soil, the second had four levels: non-inoculation, inoculation of *R. sphaerocarpa* plants with three AM fungi (*Glomus intraradices*, *Glomus deserticola* and *Glomus mosseae*), and the third had two levels: well-watered or non-watered conditions. Each replication plot was 180 m^2. In early February 2003, composted sewage sludge was added to half of the holes (0-20 cm depth) and mixed manually with the soil, at a rate of 1%. The seedlings (inoculated and non-inoculated) were planted at least 1 m apart, between holes dug manually, with 3 m between blocks. At least 128 seedlings per block were planted. Half of the plants received no irrigation, except for the natural precipitation during the six months of the growth period (about 130 mm). The remaining plants were irrigated regularly (once a month) with tap water (30 mm each time) in order to reach a total precipitation of 310 mm.

Plant analyses

Six months after planting five plants of each treatment were harvested. Fresh and dry (105ºC, 5 hr) weights of shoots were recorded. The foliar concentrations of phosphorus and potassium were calculated after digestion in nitric-perchloric acid (5:3) for 6 h. The P concentration was determined by colorimetry, the N concentration was determined by Kjeldhal method and the K concentration was estimated by a flame photometer. NR 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 percentage of root length colonised by arbuscular mycorrhizal fungi was calculated by the gridline intersect method (Giovannetti and Mosse 1980) after staining with trypan blue.

RESULTS AND DISCUSSION

The inoculation with *G. intraradices*, *G. deserticola* or *G. mosseae* stimulated the growth of *R. sphaerocarpa* seedlings, *G. mosseae* being the most effective (Table 1). The greatest growth of *R. sphaerocarpa* seedlings inoculated with *G. mosseae* was related to the contents of foliar N and P (Table 1). The highest effectiveness of *G. mosseae* was not related to the extent of mycorrhizal infection, because all inoculated plants exhibited high infection rates in theirs roots, under both water regimes (Table 1). It is worth noting that the mycorrhizal inoculation treatments were very effective for increasing plant growth under non-irrigation conditions. These results confirm the effectiveness of mycorrhizal symbiosis in the successful establishment and growth of plants in a semiarid area, where water is by far the most limiting resource for plant growth.

***Table 1**. Shoot biomass, foliar nutrients and mycorrhizal infection in roots of R. sphaerocarpa in response to mycorrhizal inoculation treatments, composted sewage sludge (SS) addition and water regime previous planting and six months after planting (n=5). *Mean ± standard error.*

	0 months	6 months	
		non-watered	well-watered
Shoot (g dw)			
C	0.47±0.03	0.70±0.03	1.34±0.05
SS	0.47±0.03	0.84±0.07	2.01±0.06
G1	0.71±0.03	1.32±0.03	2.63±0.10
SSG1	0.71±0.03	1.50±0.12	2.44±0.13
G2	0.67±0.03	1.27±0.06	2.72±0.12
SSG2	0.67±0.03	1.23±0.07	3.24±0.24
G3	0.51±0.04	1.72±0.06	2.53±0.06
SSG3	0.51±0.04	1.46±0.04	3.95±0.32
Nitrogen (mg plant^{-1})			
C	8.1±0.3	9.9±0.6	13.8±0.3
SS	8.1±0.3	14.5±1.6	31.4±1.5
G1	12.9±0.5	21.9±0.6	31.6±1.2
SSG1	12.9±0.5	22.7±1.7	33.6±2.0
G2	10.1±0.6	24.9±1.4	29.9±1.3
SSG2	10.1±0.6	19.4±1.0	45.6±3.7
G3	8.5±0.4	31.6±1.8	28.5±1.3
SSG3	8.5±0.4	21.7±0.7	51.0±3.2
Phosphorus (mg plant^{-1})			
C	0.59±0.05	0.37±0.02	0.70±0.05
SS	0.59±0.05	0.48±0.03	3.26±0.11
G1	0.81±0.04	1.10±0.02	2.26±0.11
SSG1	0.81±0.04	1.25±0.08	6.47±0.11
G2	1.18±0.08	1.07±0.05	2.14±0.05
SSG2	1.18±0.08	1.61±0.10	8.88±0.65
G3	0.98±0.05	1.34±0.07	2.22±0.10
SSG3	0.98±0.05	1.73±0.06	9.52±0.40
Potassium (mg plant^{-1})			
C	7.1±0.5	10.7±0.9	20.0±0.8
SS	7.1±0.5	15.8±1.7	41.6±1.8
G1	8.6±0.5	28.5±1.4	50.7±1.3
SSG1	8.6±0.5	31.6±2.6	52.6±2.2
G2	8.9±0.4	29.3±2.1	42.3±2.2
SSG2	8.9±0.4	26.7±1.1	70.0±6.9
G3	8.1±0.7	30.7±0.8	36.9±2.6
SSG3	8.1±0.7	30.6±1.2	70.6±4.0
Colonisation (%)			
C	0.4±0.2	3.3±0.2	18.8±1.4
SS	0.4±0.2	15.7±2.2	37.3±1.3
G1	61.9±1.7	82.6±1.4	87.5±1.1
SSG1	61.9±1.7	82.8±1.9	83.0±1.0
G2	67.7±3.1	79.6±2.3	91.0±1.2
SSG2	67.7±3.1	81.8±2.7	80.3±0.9
G3	51.8±3.7	77.4±1.0	75.8±1.2
SSG3	51.8±3.7	63.6±1.5	76.0±2.5

C=control; SS=composted sewage sludge addition; G1=inoculation with G. intraradices; SSG1= composted SS addition and inoculation with G. intraradices; G2=inoculation with G. deserticola; SSG2=composted SS addition and inoculation with G. deserticola; G3=inoculation with G. mosseae; SSG3=composted SS addition and inoculation with G. mosseae.

The addition of composted sewage sludge to soil alone had little effect on increasing the growth of *R. sphaerocarpa* (Table 1). Thus, the effectiveness of composted sewage sludge was increased greatly by the irrigation of the plants. This could be due to an improvement in the available nutrient supply in soil, arising from the composted sewage sludge. The combined treatment increased the growth of the seedlings to an equal or even to a lesser extent than each mycorrhizal inoculation treatment alone, but it was more effective than soil amendment alone.

The mycorrhizal inoculation induced an increased NR activity in the roots of *R. sphaerocarpa* plants, independent of the irrigation of the plants (Table 2). The increase in nitrate reductase may be attributed to the contribution of hyphal transport of N. This could explain how the addition of composted sewage sludge alone to soil was not effective for increasing nitrate assimilation in non-irrigated plants, in spite of the input of nitrate from this residue. In contrast, the irrigation of the plants could have facilitated the mobility of the nitrate ions from the residue, consequently resulting in the increased nitrate uptake and assimilation seen in this study.

***Table 2**. Nitrate reductase (NR) activity in roots of R. sphaerocarpa in response to mycorrhizal inoculation treatments, composted sewage sludge (SS) addition and water regime six months after planting (n=5). *Mean ± standard error.*

	6 months	
	non-watered	well-watered
NR activity (nmol NO_2^- g FW^{-1} h^{-1})		
C	31±1	49±2
SS	36±2	58±5
G1	37±1	42±1
SSG1	38±1	119±14
G2	68±2	65±6
SSG2	61±1	136±7
G3	59±1	58±1
SSG3	76±3	153±14

C=control; SS=composted sewage sludge addition; G1=inoculation with G. intraradices; SSG1= composted SS addition and inoculation with G. intraradices; G2=inoculation with G. deserticola; SSG2=composted SS addition and inoculation with G. deserticola; G3=inoculation with G. mosseae; SSG3=composted SS addition and inoculation with G. mosseae.

In conclusion, the effectiveness of mycorrhizal inoculation on the establishment and growth of *R. sphaerocarpa* seedlings in a semiarid Mediterranean area was independent of the water regime. The capacity of AM fungi for increasing plant growth in water stress conditions may be related to nutrient uptake improvement and to an increase in N assimilation through NR activity. The addition of composted sewage sludge was only effective when the availability of water in the soil was high.

REFERENCES

Caravaca, F., Alguacil, M.M., Díaz, G., Roldán, A. 2003b. Use of nitrate reductase activity for assessing the effectiveness of mycorrhizal symbiosis in *Dorycnium pentaphyllum* under induced water deficit. Comm. Soil. Sci. Plant Anal., 34: 2291-2302.

Caravaca, F., Alguacil, M.M., Figueroa, D., Barea, J.M., Roldán, A. 2003a. Re-establishment of *Retama sphaerocarpa* as a target species for reclamation of soil physical and biological properties in a semiarid Mediterranean area. Forest Ecol. Manag., 182: 49-58.

Downs, M.R., Nadelhoffer, K.J., Melillo, J.M., Aber, J.D. 1993. Foliar and fine root nitrate reductase activity in seedlings of four forest tree species in relation to nitrogen availability. Trees (Berl), 7: 233-236.

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