ELSEVIER

Ecological Engineering 19 (2002) 289–295

ECOLOGICAL ENGINEERING

www.elsevier.com/locate/ecoleng

Short communication

Microsite and mycorrhizal inoculum effects on the establishment of *Quercus coccifera* in a semi-arid degraded steppe

Fernando T. Maestre[a,*], Susana Bautista[a,b], Jordi Cortina[a], Gisela Díaz[c], Mario Honrubia[c], Ramón Vallejo[b]

[a] *Departamento de Ecología, Universidad de Alicante, Apartado de correos 99, 03080 Alicante, Spain*
[b] *Centro de Estudios Ambientales del Mediterráneo, C/Charles Darwin 14, 46980 Paterna, Spain*
[c] *Departamento de Biología Vegetal, Universidad de Murcia, Campus de Espinardo, 30100, Murcia, Spain*

Received 18 February 2002; received in revised form 7 June 2002; accepted 19 June 2002

Abstract

Alpha grass (*Stipa tenacissima*) steppes are widely distributed within the semi-arid areas of the Mediterranean Basin, and represent a degraded stage of vegetation. We evaluated the effect of *S. tenacissima* tussocks and nursery inoculation with mycorrhizae on the survival of the native shrub *Quercus coccifera*. Experiments were carried out in three steppes located in semi-arid southeastern Spain. Survival during the first months after plantation was significantly higher in the surroundings of *S. tenacissima* tussocks than in open areas between the tussocks. We did not find any effect of nursery inoculation on seedling survival. This effect may be related to the fact that the used fungal strain did not survive the drought summer conditions in the study area. Our results support the idea of a facilitative effect of *S. tenacissima* on introduced shrubs, which may be related to improved environmental conditions in tussock microsites. However, this effect may not be enough for the establishment of seedlings in years with below-average rainfall and a strong summer drought. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Facilitation; Mycorrhizal inoculation; *Quercus coccifera*; Restoration; Semi-arid zones; Steppes; *Stipa tenacissima*

1. Introduction

Land restoration in semi-arid areas faces a number of constraints related to soil degradation and water shortage (Whisenant, 1999; Vallejo et al., 2000a,b). Current approaches to improving woody seedling establishment in these areas include the amelioration of water stress using efficient water management practices (Whisenant et al., 1995; Wilson and Witkowski, 1998; Shachak et al., 1998; Yohannes, 1999), the improvement of soil conditions using organic amendments (García et al., 1998), and the utilization of facilitation by existing vegetation (Maestre et al., 2001). It is well

* Corresponding author. Tel.: +34-96-590-9564; fax: +34-96-590-3565
E-mail address: ft.maestre@ua.es (F.T. Maestre).

0925-8574/02/$ - see front matter © 2002 Elsevier Science B.V. All rights reserved.
PII: S0925-8574(02)00097-6

known that mycorrhizae enhance the ability of the plant to become established and to cope with stress situations such as nutrient deficiency, drought, and soil disturbance (Cumming, 1993; Schreiner et al., 1997; Wu et al., 1999; Morte et al., 2001). Thus, mycorrhizal inoculation with suitable fungi has been also proposed as a promising tool for improving restoration success in semi-arid degraded areas (Pigott, 1982; Roldán and Albaladejo, 1994; Garbaye, 2000). However, field testing of mycorrhizal inoculation effects on seedling performance in these areas is still scarce (Roldán et al., 1996; Querejeta et al., 1998).

In the Mediterranean Basin, steppes of the tussock grass *Stipa tenacissima* L. dominate the landscape of large semi-arid regions (Le Houérou, 2001). These steppes resulted from the disturbance of woody shrublands by human activities, as livestock grazing, fires and agriculture have been occurring in these ecosystems during centuries (Puigdefábregas and Mendizábal, 1998). Previous studies have evaluated the effects of *S. tenacissima* tussocks on water infiltration and availability, soil properties, and microclimate (Puigdefábregas and Sánchez, 1996; Cerdà, 1997; Maestre et al., 2001, 2002). These works suggest that microsites close to *S. tenacissima* tussocks are favorable for plant establishment as compared with between-tussocks open microsites. Maestre et al. (2001) have shown the potential use of facilitation by *S. tenacissima* for establishing woody shrubs in steppe areas. In this paper we report the results of field experiments on the interaction of mycorrhizal inoculation and microsite–tussock and open–treatments on the establishment of introduced seedlings of the native woody shrub *Quercus coccifera* L. in *S. tenacissima* steppes. We expected that both tussock microsite and mycorrhizal inoculation would increase seedling survival. However, since effectiveness of mycorrhizal symbioses is highly affected by the availability of water and other soil resources (Eltrop and Marschner, 1996; Morte et al., 2001), we also expected that seedling response to mycorrhizal inoculation would vary depending on the planting microsite.

2. Methods

2.1. Study area

This study was conducted at three *S. tenacissima* steppes located at the province of Alicante, in southeastern Spain (Aguas, 38° 31′N 0° 21′W, 450 m a.s.l., 12° slope, 160° SE aspect; Campello, 38° 30′N 0° 23′W, 380 m a.s.l., 18° slope, 140° SE aspect; Ballestera, 38° 28′N 0° 22′W, 140 m a.s.l., 21° slope, 170° S aspect). Climate is Mediterranean semi-arid, with a 30-year average annual precipitation ranging from 358 to 388 mm in the studied sites (Pérez Cueva, 1994). The vegetation is sparse, with bare ground areas covering between 40 and 55% of total surface. Vegetated patches are dominated by *S. tenacissima* and the sprouting grass *Brachypodium retusum* (Pers.) P. Beauv. Woody patches are scarce and mainly formed by the Mediterranean bushes and shrubs *Globularia alypum* L., *Ephedra fragilis* Desf., and *Rhamnus lycioides* L. subsp. *lycioides*.

2.2. Experimental design

To investigate the effect of *S. tenacissima* tussocks and mycorrhizal inoculation we conducted experimental field plantations in December 1999. We arranged the experiments in a fully replicated factorial design, with all possible combinations between microsite and mycorrhizal status at each of the sites. We selected two planting microsites: the ‘tussock’ microsite was located upslope and adjacent to *S. tenacissima* tussocks; the ‘open’ microsite was located in the inter-tussocks areas (see Maestre et al., 2001 for details). Site preparation was carried out manually during autumn 1999. It consisted in digging a minimum hole (25 cm × 25 cm × 25 cm) to avoid soil disturbance as much as possible. At each site (hereafter plot), we planted 35 seedlings for each combination of microsite (tussock/open) and mycorrhizal inoculation treatments (control/inoculated), resulting in a total of 140 seedlings per plot.

Quercus coccifera seedlings were cultivated in 350 cm^3 Poliforest® containers, using as growing medium a mixture of sphagnum peat, black peat and the combination of perlite and vermiculite

(50:25:25 v/v). Inoculation procedure was performed according to Honrubia et al. (1995). We used solid mycelial inoculum and sporal inoculum of *Pisolithus tinctorius* (Pt 42AM) collected in a mixed forest of *Quercus ilex* L. subsp. *rotundifolia* and *Pinus halepensis* Miller located in a dry area of the Murcia region (SE Spain). Solid mycelial inoculum was applied once to the root surface when seedlings started to develop the roots, in a dose of 1/10 (v/v). In addition, spore slurries were applied several times at monthly intervals by watering in a dose of 0.1 g/plant. All seedlings of the inoculated treatment were checked morpho- and anatomically prior to plantation to ensure that they had mycorrhizal short roots.

We used seedling survival as response variable to evaluate seedling performance to both microsite and mycorrhizal inoculation. We assessed seedling survival in January, May, October and December of 2000. The first summer in the field is a key stage in plant establishment in semi-arid Mediterranean areas (Maestre, 2002; Vilagrosa, 2002), and thus seedling response during the first year after transplantation can be used to evaluate the performance of management techniques such as those used here. We also measured the soil moisture monthly in tussock and open microsites by using time-domain reflectometry (TDR, Topp and Davis, 1985). In randomly selected planting holes, we installed five 20 cm length TDR probes per microsite and plot. We employed a Tektronix 1502C metallic TDR cable tester (Tektronix, Beauverton, OR) for measurements.

2.3. Statistical analyses

Analyses of survival frequency data were based on hierarchical log-linear models (Agresti, 1990). We analyzed seedling survival separately for each sampling period, testing for independence in a four-way table (Survival, Inoculation, Microsite, and Plot). TDR measurements were analyzed separately for each plot with repeated measures ANOVA. All the statistical analyses were performed using the SPSS 9.0 package (SPSS, Chicago, IL).

3. Results

During 2000, the precipitation at the study sites ranged between 150 (Ballestera) and 264 (Aguas) mm, a 42–68% below the 30-year average (Fig. 1). These drought years are not uncommon in the study area, since more than 60% of all years have below-average annual precipitation values (Pérez Cueva, 1994). It is interesting to remark that 42–

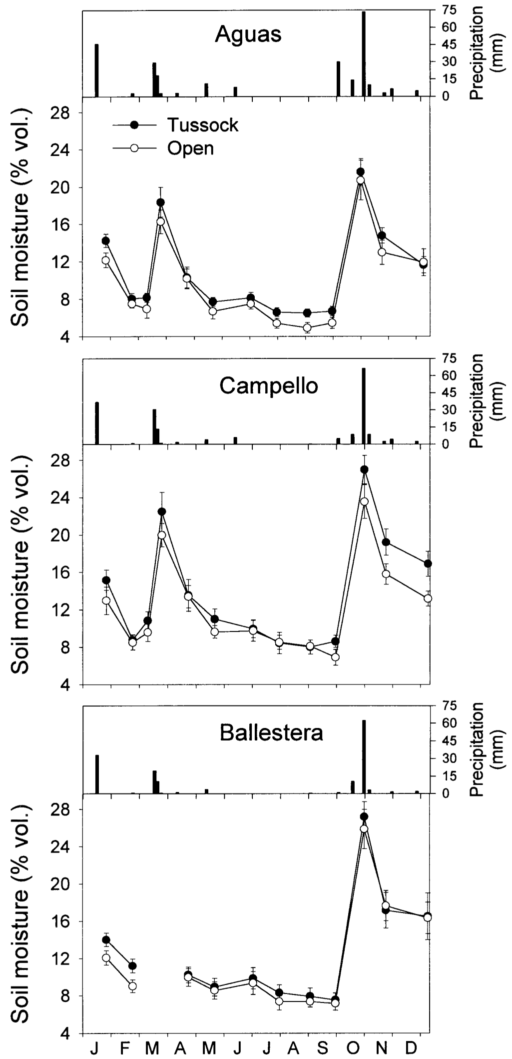

Fig. 1. Dynamics of soil moisture in the tussock and open microsites on the experimental plots during 2000. Data represent mean ± 1 S.E. ($n = 5$). Bar graphs show rainfall events as measured by an on-site meteorological station.

52% of total rainfall was received during the last three months of the year, and that before-summer precipitation (January–May) was 18–54% below the 30-year average. The sites experienced a strong summer drought period, with 110 days without rainfall. Soil moisture, as measured with TDR probes, remained below 8% during summer, and reached maximum values with autumn rainfalls. For most of the sampling dates, tussock and open microsites showed similar values, except in some cases in winter and summer with higher soil water content in tussock microsites (Fig. 1). However, we did not find any overall microsite effect on soil moisture in any plot (repeated measures ANOVA, $F_{Aguas} = 1.59$, df = 1,8, $P = 0.24$; $F_{Campello} = 1.23$, df = 1,8, $P = 0.30$; $F_{Ballestera} = 0.03$, df = 1,7, $P = 0.87$).

During the first months after planting we found a strong microsite effect, with significantly higher survival in tussock microsites in both January and May 2000 (Fig. 2, Table 1). We did not find any effect of micorrhizal inoculation on seedling survival. The significant plot × survival dependence found in both January and May reflected the differences in initial mortality between the sites, as survival in Aguas was higher as compared with that of Campello and Ballestera (Fig. 2). We did not find any significant three-way or higher order effect for any sampling date (Table 1). One year after transplantation there were no alive seedlings for any combination of treatment, microsite and plot. Mortality occurred in all plots mainly during summer.

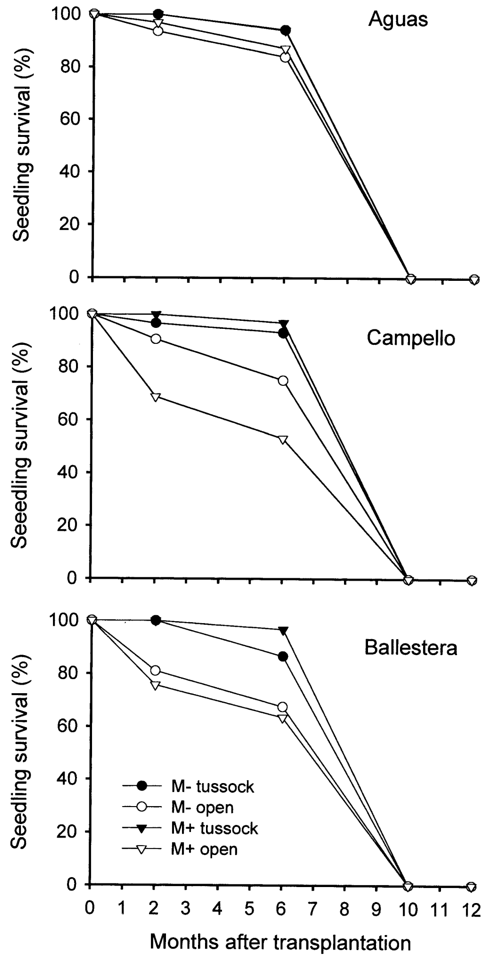

Fig. 2. Survival of planted *Quercus coccifera* seedlings at the three experimental plots. M^- = control seedlings; M^+ = inoculated seedlings.

4. Discussion

Accordingly with previous observations (Maestre et al., 2001), the microsite provided by *S. tenacissima* tussocks was able to facilitate the establishment of shrubs during the first months after plantation, regardless of the inoculation treatment. This facilitation seems to be mediated by changes in soil properties and microclimate induced by *S. tenacissima*, most of them potentially leading to higher water availability in tussock microsites (Puigdefábregas and Sánchez, 1996; Cerdà, 1997; Maestre et al., 2001, 2002). Our results did not show a significant overall effect of microsite in soil moisture, but clear differences were observed in some periods. Further manipulative experiments are needed to define the conditions that may promote differences in water availability between tussock and open microsites and to elucidate the mechanisms involved in the facilitation observed.

Mycorrhizal inoculation did not improve seedling survival in the field. One possible explanation for this result is that native fungi, well-adapted to the target soil–plant system, could have colonized the introduced seedlings, masking the potential

Table 1
Summary of the hierarchical log linear analysis describing microsite, mycorrhizal inoculation, and plot effects on *Quercus coccifera* survival

Effects	d.f.	January 2000		May 2000	
		G^2	P	G^2	P
[a]3-way or higher order	9	8.684	0.467	7.361	0.600
[b]P × S	2	7.687	0.021	10.721	0.005
[b]M × S	1	34.504	< 0.001	36.465	< 0.001
[b]I × S	1	0.167	0.683	1.575	0.210

$n = 35$ cases per each plot × microsite × inoculation treatment combination. P = plot (Aguas, Campello and Ballestera), M = microsite (Tussock and Open), I = inoculation (Control and Inoculated) and S = survival (%). No results for the measurements of November and December 2000 are showen because all seedlings were dead.

[a] Test the significance of three-way or higher order effects for the saturated models.

[b] Results for two-way models after elimination of higher order non-significant effects.

benefits of nursery inoculation. It has been shown that natural mycorrhizal potential in semi-arid ecosystems may be effective enough to guarantee spontaneous plant mycorrhization, even in highly degraded areas (Perry et al., 1987; Roldán and Albaladejo, 1994; Díaz and Honrubia, 1995; Requena et al., 1996). However, all the natural plant species in the study area are arbuscular-mycorrhizal (AM) simbionts, and probably none or very few propagules of ectomycorrhizal fungal species may persist there. *Quercus coccifera*, as most of *Quercus* species, is considered an ectomycorrhizal species (Rothwell et al., 1983; Watson et al., 1990; Dickie et al., 2001), and no previous AM-infection in *Q. coccifera* has been reported. On the other hand, it is also likely that the fungal strain used, which came from a less stressing area, did not survive the summer drought in the study sites, resulting in the lack of positive effects by nursery mycorrhization observed. The successful application of mycorrhizal treatments in restoration programs could be improved by exploiting the natural mycorrhizal potential of the target sites, after testing for nursery production the most favorable and compatible mycorrhizal fungus–host seedling species combination.

In spite of the differences in survival observed among the experimental sites, we did not find any interaction effect between treatments and plots, remarking the consistence of the seedling response to the treatments applied. The low precipitation registered during 2000 may explain the high mortality found in this experiment, as seedling survival in semi-arid environments is strongly limited by soil moisture availability (Harrington, 1991). In agreement with other studies (Grantz et al., 1998; Alloza and Vallejo, 1999; Maestre et al. 2001), transplant shock appeared to have a moderate effect on seedling survival, as compared with summer stress, under the less stressing conditions (tussock microsite in Campello and Ballestera, both microsites in Aguas). However, under particularly stressful conditions mortality rate can be high even before the summer.

Introduction of woody plants in degraded semi-arid areas has several limitations related with the low levels of resources and the heterogeneity in their distribution (Whisenant, 1999). Thus, new developments are required to ensure its successful restoration (Vallejo et al., 2000b). Our results highlight the potential of facilitation by existing vegetation to optimize the chance of successful revegetation using transplants. However, benefits provided by vegetated microsites may be not enough to ensure seedling establishment when the climatic conditions after planting are extremely dry.

Acknowledgements

We wish to thank Felipe Gil, VAERSA workers and the regional forest services (Conselleria de Medio Ambiente) for technical assistance. Manuel

Ruiz, José García, Antonio Soriano and José Huesca helped during the fieldwork. The research for this paper was funded by a FPU fellowship of the Ministerio de Educación, Cultura y Deporte awarded to F. T. Maestre, and by the European Commission funded REDMED project (contract number ENV4-CT97-0682). CEAM Foundation is supported by Generalitat Valenciana and Bancaixa.

References

Agresti, A., 1990. Categorical Data Analysis. Wiley, New York, 576 pp.

Alloza, J.A., Vallejo, V.R., 1999. Relación entre las características meteorológicas del año de plantación y los resultados de las repoblaciones. Ecología 13, 173–187.

Cerdà, A., 1997. The effect of patchy distribution of *Stipa tenacissima* L. on runoff and erosion. J. Arid Environ. 36, 37–51.

Cumming, J.R., 1993. Growth and nutrition of nonmycorrhizal and mycorhizal pitch pine (*Pinus rigida*) seedlings under phosphorus limitation. Tree Physiol. 13, 173–187.

Díaz, G., Honrubia, M., 1995. Effect of native and introduced arbuscular mycorrhizal fungi on growth and nutrient uptake of *Lygeum spartum* and *Anthyllis cytisoides*. Biol. Plantarum. 37, 121–129.

Dickie, I.A., Koide, R.T., Fayish, A.C., 2001. Vesicular-arbuscular mycorrhizal infection of *Quercus rubra* seedlings. New Phytol. 151, 257–264.

Eltrop, L., Marschner, H., 1996. Growth and mineral nutrition of non-mycorrhizal and mycorrhizal Norway spruce (*Picea abies*) seedlings grown in semi-hydroponic sand culture. I. Growth and mineral nutrient uptake in plants supplied with different forms of nitrogen. New Phytol. 133, 469–478.

Garbaye, J., 2000. The role of ectomycorrhizal symbiosis in the resistance of forests to water stress. Agriculture 29, 63–69.

García, C., Hernández, T., Albaladejo, J., Castillo, V., Roldán, A., 1998. Revegetation in semiarid zones: influence of terracing and organic refuse on microbial activity. Soil Sci. Soc. Am. J. 62, 670–676.

Grantz, D.A., Vaughn, D.L., Farber, R.J., Kim, B., Ashbaugh, L., Van Curen, T., Campbell, R., Bainbridge, D., Zinck, T., 1998. Transplanting native plants to revegetate abandoned farmland in the Western Mojave Desert. J. Environ. Qual. 27, 960–967.

Harrington, G.N., 1991. Effects of soil moisture on shrub seedling survival in a semi-arid grassland. Ecology 72, 1138–1149.

Honrubia, M., Torres, P., Díaz, G., Morte, A., 1995. Biotecnología Forestal: Micorrización y micropropagación de plantas. Universidad de Murcia, Murcia, 86 pp.

Le Houérou, H.N., 2001. Biogeography of the arid steppeland north of the Sahara. J. Arid Environ. 48, 103–128.

Maestre, F.T., 2002. La restauración de la cubierta vegetal en zonas semiáridas en función del patrón espacial de factores bióticos y abióticos. Ph.D. dissertation. University of Alicante, Alicante.

Maestre, F.T., Bautista, S., Cortina, J., Bellot, J., 2001. Potential of using facilitation by grasses to establish shrubs on a semiarid degraded steppe. Ecol. Appl. 11, 1641–1655.

Maestre, F.T., Huesca, M.T., Zaady, E., Bautista, S., Cortina, J., 2002. Infiltration, penetration resistance and microphytic crust composition in contrasted microsites within a Mediterranean semi-arid steppe. Soil Biol. Biochem. 34, 895–898.

Morte, A., Díaz, G., Rodríguez, P., Alarcón, J.J., Sánchez-Blanco, M.J., 2001. Growth and water relations in mycorrhizal and nonmycorrhizal *Pinus halepensis* plants in response to drought. Biol. Plantarum. 44, 263–267.

Pérez Cuvea, J.A., 1994. Atlas climático de la Comunidad Valenciana. Conselleria de Obras Públicas, Urbanismo y Transportes, Valencia, 205 pp.

Perry, D.A., Molina, R., Amaranthus, M.P., 1987. Mycorrhizae, mycorrrhizospheres and reforestation: current knoledge and research needs. Can. J. For. Res. 17, 929–940.

Pigott, C.D., 1982. Survival of mycorrhizas formed by *Cenococcum geophilum* in dry soils. New Phytol. 92, 513–517.

Puigdefábregas, J., Mendizábal, T., 1998. Perspectives on desertification: western Mediterranean. J. Arid Environ. 39, 209–224.

Puigdefábregas, J., Sánchez, G., 1996. Geomorphological implications of vegetation patchiness on semiarid slopes. In: Anderson, M.G., Brooks, S.M. (Eds.), Advances in Hillslope Processes, vol. 2. Wiley, London, pp. 1027–1060.

Querejeta, J.I., Roldán, A., Albaladejo, J., Castillo, V., 1998. The role of mycorrhizae, site preparation, and organic amendment in the afforestation of a semi-arid Mediterranean site with *Pinus halepensis*. Forest Sci. 44, 203–211.

Requena, N., Jeffries, P., Barea, J.M., 1996. Assessment of natural mycorrhizal potential in a desertified semi-arid ecosystem. Appl. Environ. Microbiol. 62, 842–847.

Roldán, A., Albaladejo, J., 1994. Effect of mycorrhizal inoculation on the growth of *Pinus halepensis* seedlings in a semiarid soil. Biol. Fertil. Soils 18, 143–149.

Roldán, A., Querejeta, I., Albaladejo, J., Castillo, V., 1996. Growth response of *Pinus halepensis* to incoculation with *Pisolithus arhizus* in a terraced rangeland amended with urban refuse. Plant Soil 179, 35–45.

Rothwell, F.M., Hacskaylo, E., Fisher, D., 1983. Ecto and endomycorrhizal fungus associations with *Quercus imbricaria* L. Plant Soil 71, 309–312.

Schreiner, R.P., Mihara, K.L., McDaniel, H., Bethlenfalvay, G.J., 1997. Mycorrhizal fungi influence plant and soil functions and interactions. Plant Soil 188, 199–209.

Shachak, M., Sachs, M., Moshe, I., 1998. Ecosystem management of desertified shrublands in Israel. Ecosystems 1, 475–483.

Topp, G.C., Davis, J.L., 1985. Measurement of soil water content using time-domain reflectometry (TDR): a field evaluation. Soil Sci. Soc. Am. J. 49, 19–24.

Vallejo, V.R., Bautista, S., Cortina, J., 2000a. Restoration for soil protection after disturbances. In: Trabaud, L. (Ed.), Life and Ecosystems in the Mediterranean. WIT Press, Southampton, pp. 301–344.

Vallejo, V.R., Serrasolses, I., Cortina, J., Seva, J.P., Valdecantos, A., Vilagrosa, A., 2000b. Restoration strategies and actions in Mediterranean degraded lands. In: Enne, G., Zanolla, Ch., Peter, D. (Eds.), Desertification in Europe: Mitigation Strategies, Land Use Planning. Office for Official Publications of the European Communities, Luxembourg, pp. 221–233.

Vilagrosa, A., 2002. Estrategias de resistencia al déficit hídrico en *Pistacia lentiscus* L. y *Quercus coccifera* L. Implicaciones en la repoblación forestal. Ph.D. dissertation. University of Alicante, Alicante.

Watson, G.W., Von der Heide-Spavka, K.G., Howe, V.K., 1990. Ecological significance of endo/ectomycorrhiza in the oak sub-genus *Erythrobalanus*. Arboriculture J. 14, 107–116.

Whisenant, S.G., 1999. Repairing Damaged Wildlands. Cambridge University Press, Cambridge, 312 pp.

Whisenant, S.G., Thurow, T.L., Maranz, S.J., 1995. Initiating autogenic restoration on shallow semiarid sites. Restor. Ecol. 3, 61–67.

Wilson, T.B., Witkowski, E.T.F., 1998. Water requirements for germination and early seedling establishment in four African savanna woody plant species. J. Arid Environ. 37, 541–550.

Wu, B., Watanabe, I., Hayatsu, M., Nioh, I., 1999. Effect of ectomycorhizae on the growth and uptake and transport of ^{15}N-labeled compounds by *Pinus tabulaeformis* seedlings under water-stressed conditions. Biol. Fert. Soils 28, 136–138.

Yohannes, F., 1999. Improvement of water application efficiency by moisture conservation methods for establishment of planted tree seedlings. Commun. Soil Sci. Plant Anal. 30, 1119–1125.