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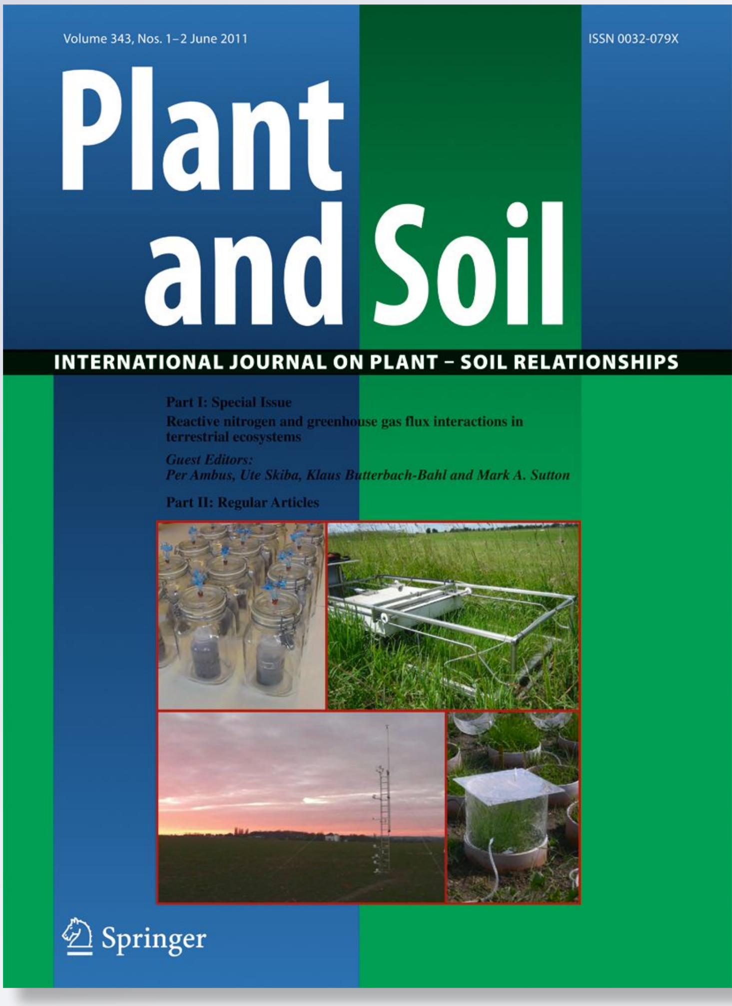

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REGULAR ARTICLE

Using hydrogel and clay to improve the water status of seedlings for dryland restoration

Esteban Chirino · Alberto Vilagrosa · V. Ramón Vallejo

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Abstract In dryland ecosystems, post-transplant water stress produces high seedling mortality after the first summer following outplanting. Our aim was to assess the effects of clay and hydrogel, both on the water holding capacity of the growing media and on various morphological and physiological characteristics of *Quercus suber* seedlings in the nursery and, subsequently, during the first 2 years in the field. *Quercus suber* L. seedlings were grown in four types of growing media: CS (Control growing media, standard mixture of limed peat and coconut peat, 1:1 v/v ratio), SC-10 (CS mixed with sepiolite clay at 10% v/v) and HS (CS mixed with hydrogel Stockosorb® K-400 at two doses, 0.7 and 1.5% w/w). HS-1.5 showed the best results, increasing the water holding capacity of the root plug, improving seedling water status and increasing seedling survival in the field. SC-10 showed an intermediate effect on seedling response in the field. Mixing hydrogel with a peat-based growing medium to form root plugs is a suitable technique for cultivating species to be planted in areas with a strong water deficit. This technique reduces post-transplant water stress in seedlings during their first months in the field and contributes to improve forest-restoration methods in dryland ecosystems.

Keywords *Quercus suber* · Plant stock quality · Substrate · Water availability · Carbon isotope composition

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E. Chirino (✉) · A. Vilagrosa · V. R. Vallejo
CEAM - Departament of Ecology, University of Alicante,
Apdo. 99, 03080 Alicante, Spain
e-mail: Esteban.Chirino@ua.es

E. Chirino
Forest Restoration Group, Mediterranean Center for Environmental Studies (Foundation CEAM),
Apdo. 99, 03080 Alicante, Spain

A. Vilagrosa · V. R. Vallejo
Mediterranean Center for Environmental Studies (Foundation CEAM),
Alicante, Spain

Abbreviations

CS	Control growing media
SC-10	CS growing media mixed with sepiolite clay at 10% v/v
HS-0.7	CS growing media mixed with hydrogel at 0.7 w/w
HS-1.5	CS growing media mixed with hydrogel at 1.5% w/w
H_s	Shoot height
RCD	Root collar diameter
SI	Slenderness index
DW_S	Shoot dry weight
DW_R	Root dry weight
DW_R/DW_S	Root/shoot ratio
SDW	Seedling dry weight
DW_R/SDW	Root dry weight/seedling dry weight ratio

Ψ_{pd}	Predawn xylem water potential
VWC	Volumetric water content
$\delta^{13}C$	Carbon isotope composition
Δ_{leaf}	Carbon isotope discrimination
PDB	Pee Dee Belemnite Standard
gs	Stomatal conductance
GLM	General Lineal Model

Introduction

Water is a limited resource in dry and semiarid ecosystems, and plants are very often subjected to water stress conditions (Di Castri 1973; Kramer and Boyer 1995). Under such unfavourable conditions, seedling mortality rates are generally high after the first summer following outplanting (Vilagrosa et al. 1997; Vallejo and Alloza 1998). This situation is expected to worsen in the future according to Global Climate Change projections for the Mediterranean region, which point to an increase in temperatures and in the frequency and intensity of droughts (IPCC 2007). There is thus a real need to improve forest restoration techniques in these areas, beginning with the application of innovative technologies during nursery culture, with the aim of improving plant stock quality, seedling field performance and abiotic conditions on the plantation site (Cortina et al. 2006; Chirino et al. 2009). In this context, an important challenge for forest restoration is to increase the water holding capacity of the growing media used in the nursery. This would allow seedlings to maintain adequate hydration, reduce their post-transplant stress and improve their water status during the first months after outplanting.

Currently, a wide variety of organic and inorganic materials, e.g., peat, coconut fiber, vermiculite, perlite, sand, clay, and composting of forest waste, cattle manure and sewage sludge, etc., are available for producing the different types of growing media used in nursery cultures. In this sense, several studies have highlighted the importance of these mixtures in the quality of the plant produced (Chong and Lumis 2000; Marfà et al. 2002; Yu and Zinati 2006; Marianthi 2006; Owen et al. 2008; Mañas et al. 2008). Nevertheless, with respect to the nursery culture of forestry species, only rarely have certain types of clay like sepiolite and/or water-absorbent polymers such as Stockosorb® been mixed with a peat-based growing medium to form root plugs.

Sepiolite (hydrated magnesium silicate) is a natural clay mineral. It presents a fibrous structure with interior channels that allow the penetration of organic and inorganic ions (Donat 2009). Although it has mainly been used in industry, it has also been used in livestock, in gardening, in the remediation of contaminated soils and as an enzyme-clay complex for soil application (Carrasco et al. 1995; Yang et al. 2006). Sepiolite clay shows an exceptional capacity to absorb water; it can retain 250% of its weight in water (Alvarez 1984; Francis et al. 2007). The water-absorbent polymer Stockosorb® is a potassium ammonium polyacrylate/polyacrylamide copolymer. It is a cross-linked polymer with the ability to absorb a high volume of water due to network spaces created by its cross-linked structure. The term hydrogel is sometimes used to describe cross-linked copolymers because when the dry crystals absorb water they take on the consistency of a gel (American Soil Technologies Inc. 2010). Hydrogels can absorb a volume of water 400 times their own weight (Bouranis et al. 1995), and due to this high water-holding capacity (Chatzoudis and Rigas 1999) they have been used successfully in agriculture and forest restoration as soil amendments (Viero et al. 2000; 2002; Günes 2007). Nevertheless, an overdose of hydrogels can produce negative effects in some cases (Sarvaš et al. 2007).

Cork oak (*Quercus suber* L.) is a typical Mediterranean resprouting species, which presents great interest for restoration in fire-prone ecosystems (Vallejo et al. 2006) and great socioeconomic importance in the Mediterranean region (Aronson et al. 2009). The present work was carried out in the framework of a project to recover degraded cork oak woodlands where seedling mortality in restoration projects has shown high values after the first summer, i.e. nearly 50% (Pérez-Devesa et al. 2008; Trubat et al. 2010). In this context, our study proposes cultivating cork oak seedlings in a peat-based growing medium mixed with sepiolite clay and Stockosorb® hydrogel in order to reduce transplant shock. The hypothesis of this work was that mixing both clay and hydrogel into a peat-based growing medium would help to increase the water holding capacity of the root plug, reduce seedling post-transplant water stress, and improve seedling water status in the field. To test this hypothesis, our objective was to assess the effects of clay and hydrogel both on the water holding capacity of the growing media and on various morphological and physiological characteristics of

Quercus suber seedlings in the nursery and, subsequently, during the first 2 years in the field.

Materials and methods

Nursery experiments

Plant material and growing media

Cork oak (*Quercus suber* L.) acorns were seeded in four growing-media types. The control growing media (CS) was a standard mixture of limed peat and coconut peat (1:1 v/v). The CS growing media was mixed with sepiolite clay (granulometry: 2–6 mm) at 10% v/v (SC-10) or with a water-absorbent polymer hydrogel Stockosorb® K-400 (HS, granulometry: 0.2–0.8 mm) at two doses (0.7 and 1.5% w/w). The nursery culture was carried out under outdoor conditions at the Santa Faz public nursery, Alicante, Spain (38° 23′N, 0° 26′W, elevation: 80 ma.s.l., 240° SW facing) with a mean annual rainfall of 353 mm and a mean temperature of 18°C (Pérez Cueva 1994). Cork oak acorns from Serra d'Espadà (Castellón, Spain) were supplied by the Regional Government Forest Service (Banc de Llavors, Quart de Poblet), and they were seeded in March 2004. For this, 300 cm^3 Forespot® containers were used. The substrate used in all growing media was fertilized in the factory with 57 mg NO3, 69 mg NH4, 60 mg P and 344 mg K per litre of substrate. An additional slow-release fertilizer (Osmocote plus_, N-P-K: 14-8-14; approximates longevity of 12 months at a mean temperature of 21°C) was mixed with the substrate at a dose of 1 g/L of substrate. The watering regime was moderated according to seedling water demand and was the same for all treatments.

Seedling morphology at the end of the nursery culture

At the end of the nursery culture, after 11 months of nursery cultivation, seedling morphological characterization was carried out. Ten seedlings per treatment were randomly sampled. Shoot height (H_s) and root collar diameter (*RCD*) were measured. Afterwards, seedlings were cut at the cotyledon insertion point and separated into four fractions: leaves, stem, fine roots (diameter <2 mm) and tap root (diameter >2 mm). Dry weight of each fraction was determined after oven drying at 70°C for 48 h. Slenderness index (*SI*) was determined as the relation between shoot height and root collar diameter [$SI = H_s/RCD$]. Others variables such as shoot dry weight (DW_S), root dry weight (DW_R), seedling dry weight (*SDW*), root/shoot ratio (DW_R/DW_S) and root dry weight/seedling dry weight ratio (DW_R/SDW) were determined.

Controlled drought period in the nursery

Another set of ten seedlings per treatment was randomly selected for an imposed drought period. The aims were to determine the water holding capacity of the root plug and the degree of water stress experienced by the seedlings after a controlled period without rain and without watering. For this, we determined the volumetric water content in the root plug growing media and the predawn xylem water potential in the same set of seedlings. The night before the beginning of the drought period, the seedlings were watered to field capacity and placed under outdoor conditions. Early the next morning (1st day, optimum moisture conditions), predawn xylem water potential (Ψ_{pd}) was determined in five seedlings per treatment using a Sholander pressure chamber (Soil Moisture 3005, Soil moisture Equipment Corp., Santa Barbara, CA, USA). In the same set of seedlings we sampled 40 cm^3 of the growing media from the root plug to determine the volumetric water content (*VWC*). Dry weight of the growing media sampled was determined after oven drying at 70°C for 72 h. After seven days under outdoor conditions, at full sunlight, without rain and without watering (drought conditions), *VWC* in the root plug and Ψ_{pd} were determined in another set of five seedlings using the same methods previously described. During the drought period the environmental conditions were monitored. Mean daily (± standard error) temperature and relative humidity were 27±0.4°C and 89±3% respectively. Diurnal values (6:00 AM to 6:00 PM) of PAR varied between 515 and 1,003 mmol $m^{-2}.s^{-1}$.

Field experiments

Seedling survival and growth after outplanting in the field

After the nursery period, two experimental plots were established in March 2005 in a degraded shrubland in Serra d'Espadà (Eslida, Castellón, Spain, 39°51′ 56.44″ N, 0°19′25.22″ W; 687 ma.s.l.; 20° slope;

south-facing slope 210° SW; loam soil over rock acidic sandstone). The selected plantation site had been affected by a wildfire in 1991. Twenty-five randomly selected seedlings per treatment were planted in each experimental plot in 40×40×40-cm planting holes. In the field, seedling survival and growth (height and root collar diameter) were monitored for two consecutive years (2005 and 2006).

Precipitation on field plots

The annual rainfall in 2005 (415.5 mm) and 2006 (466.4 mm) was lower than the average annual rainfall (485.3 mm), representing 86% and 96% of the average annual value respectively. In contrast with 2005, the summer of 2006 was very dry (Fig. 1).

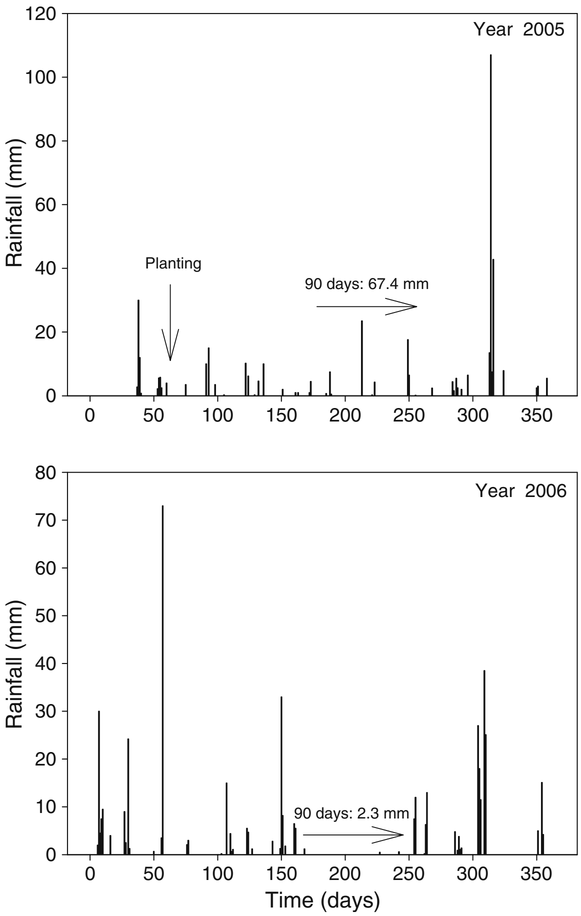

Fig. 1 Daily rainfall during the outplanting monitoring period (2005–2006). Very dry period from June 11 to September 10, 2006 (90 days, rainfall accumulated: 2.3 mm). Average pluviometry from June 11 to September 10, 2005 (90 days, rainfall accumulated: 67.4 mm)

From June 11 to September 10, 2006 (90 days) only 2.3 mm of rainfall were registered (3 rain events); in contrast, during the same period in 2005, 67.4 mm were registered, which could be considered a normal pluviometry for this period. These values show the variability of the rainfall distribution on the plantation site during the year and the severe drought conditions during the second year after outplanting.

Root system growth of seedlings in the field

To analyze how the different growing media could influence the capacity of new root growth to colonize the surrounding soil, the root systems of the seedlings were studied by means of excavations carried out on one of the experimental plots. This test was implemented 17 months after planting. Five seedlings per treatment were randomly selected and excavated, and the new root growth colonizing the plantation hole was studied. New roots growing outside the root plug were counted, cut and their dry weight determined. Maximum rooting depth was also determined. Moreover, morphological characteristics of the seedlings and biomass were determined, as previously described.

Carbon isotope composition ($\delta^{13}C$) in the tap root

In order to study the stress suffered by the seedlings, we determined the carbon isotope composition ($\delta^{13}C$) of the seedlings excavated in the field when the new root growth was studied. Unlike other studies where the $\delta^{13}C$ determination is carried out in leaf samples (Damesin et al. 1997; Buchmann et al. 1997), in our study we used tap root samples. Previous experiments in dryland ecosystems have shown that aboveground parts of seedlings experience very low growth during the first years after outplanting. In contrast, belowground seedling parts experience an important increase in biomass to colonize the new soil volume and, consequently, a higher carbon accumulation (Vilagrosa et al. 1997; Chirino et al. 2008). Thus, changes in $\delta^{13}C$ due to stress would be more easily detected in the tap root ($\delta^{13}C_{root}$) than in shoots or leaves ($\delta^{13}C_{leaf}$). In this context, from each seedling excavation (five seedlings per treatment), one tap root sample was oven-dried for 48 h at 70°C, cut, crushed into a fine powder with a mortar and pestle, and packed in an Eppendorf capsule. The samples were analyzed in the Stable Isotope Laboratory (Utah State University, USA) for carbon isotope composition according to Buchmann et al. (1997). A 2 mg subsample was combusted and analyzed for $^{13}C/^{12}C$ using an isotope ratio mass spectrometer (delta S, Finnigan MAT, Bremen, Germany). Data are reported as $\delta^{13}C_{root}$ values relative to the Pee Dee Belemnite Standard (PDB). The sample precision of the analysis was ±0.05‰.

Stomatal conductance in the field

In addition, seedling water status in the field was also monitored by means of stomatal conductance measurements (*gs*; mmol $H_2O.m^{-2}$ leaf area.s^{-1}) using a Porometer AP4 (Delta-T Devices Ltd, Cambridge, UK) during March, May, July and September of 2006. Five seedlings per treatment were randomly selected. Measures of *gs* were registered in three leaves per seedling in the morning (from 8:00 to 9:00 A.M. solar time) and at midday (from 13:00 to 14:00 P.M. solar time).

Statistical analysis

The statistical analysis to compare the seedlings produced in the different growing medium types was carried out with SPSS© statistical software v. 15.0 (SPSS Inc. Chicago, Illinois, USA). Data on seedling morphological characteristics at the end of the nursery culture and on seedlings excavated in the experimental plots, volumetric water content of the growing media, predawn xylem water potential of the seedlings and carbon isotope composition in the tap root were compared by means of analysis of variance (one-way ANOVA). Data on stomatal conductance were compared by means of General Lineal Model (GLM) Repeated Measures. Data were transformed when necessary to assure the assumptions of the analysis of variance. An exponential regression was used to assess the relationship between the volumetric water content and the predawn xylem water potential. Data on seedling survival were analyzed by means of the Kaplan-Meier Test (Log Rank—Mantel-Cox).

Results

Morphological characteristics of the seedlings

Mixing the growing media with hydrogel at 0.7% (HS-0.7) and at 1.5% (HS-1.5) favoured a higher shoot height ($p<0.001$) and slenderness index ($p<0.001$) in the seedlings (Table 1). SC-10 did not show significant differences compared with CS. Neither root collar diameter nor aboveground and belowground biomass was affected by the different growing media; as a result, all treatments showed a similar root/shoot ratio and DW_R/SDW ratio (Table 1).

Drought period

Volumetric water content (*VWC*) of the growing media under optimum moisture conditions (first day

Table 1 Morphological characteristics of seedlings at the end of the nursery period

		Growing media type				*F* value
		CS	SC-10	HS-0.7	HS-1.5	
Shoot height	cm	22.6±0.5 b	23.2±0.2 b	27.9±1.2 a	29.5±1.2 a	10.191***
Root collar diameter	mm	4.4±0.2	4.4±0.2	4.3±0.1	4.2±0.1	0.481 ns
Slenderness Index	$cm.mm^{-1}$	5.2±0.2 b	5.5±0.2 b	6.6±0.2 a	7.2±0.3 a	16.107 ***
Leaves dry weight	g	1.2±0.2	0.6±0.0	0.8±0.1	0.8±0.1	2.172 ns
Stem dry weight	g	0.5±0.2	0.4±0.3	0.4±0.3	0.4±0.3	1.237 ns
DW_S	g	1.7±0.3	1.3±0.1	1.1±0.1	1.3±0.1	2.569 ns
Tap root dry weight	g	3.7±0.2	3.8±0.4	3.7±0.4	3.6±0.3	0.061 ns
Fine root dry weight	g	0.7±0.1	0.3±0.0	0.5±0.1	0.5±0.1	1.962 ns
DW_R	g	4.3±0.3	4.1±0.4	4.1±0.4	4.1±0.3	0.098 ns
SDW	g	6.0±0.4	5.1±0.5	5.3±0.4	5.4±0.3	0.837 ns
root / shoot ratio	$g.g^{-1}$	3.3±0.6	4.1±0.3	3.6±0.3	3.5±0.5	0.581 ns
DW_R/SDW	$g.g^{-1}$	0.7±0.0	0.8±0.0	0.8±0.0	0.8±0.0	1.358 ns

Shoot biomass dry weight (DW_S), Root biomass dry weight (DW_R), Seedling dry weight (SDW), Root biomass dry weight/Seedling dry weight (DW_R/SDW). Mean ± standard error ($N=10$). Results of one-way ANOVA and Tukey's HSD *post hoc* test (ns: not significant; *** $P<0.001$)

of the drought period) ranged from 56% to 66%. *VWC* in HS-1.5 and SC-10 was significantly higher than in the CS growing media (p=0.030, Fig. 2), i.e., 17% and 18% higher than CS, respectively. In these optimum moisture conditions, the predawn xylem water potential (Ψ_{pd}) of the seedlings was higher than −0.25 MPa in all cases, showing a tendency towards the highest Ψ_{pd} in HS-1.5 (p= 0.069; Fig. 2). Seven days after the start of the controlled drought period (under drought conditions) the average *VWC* of the root plug decreased notably, ranging between 5% and 19%. These values represent a decrease of between 53 and 46 percentage units. The HS-1.5 and SC-10 growing media presented the highest values (13% and 19% respectively), while CS had the lowest values with an average of 5% (p<0.001, Fig. 2). The average *VWC* of HS-1.5 and CS-10 were 3 and 4 times higher than CS values, respectively. Under drought conditions Ψ_{pd} varied from −0.31 to −1.60 MPa. Seedlings cultivated in SC-10 showed higher Ψ_{pd} than CS (p=0.014; Fig. 2), followed by HS-1.5. In general, *VWC* showed a significant negative exponential relationship with Ψ_{pd} (Fig. 3; R^2=0.926).

Seedling survival and growth in the field

Twenty months after outplanting, the seedlings cultivated with hydrogel at 1.5% (HS-1.5) showed higher survival values than the seedlings cultivated with the control growing media (Kaplan-Meier, Log Rank p=0.040; Fig. 4). HS-0.7 and SC-10 maintained a tendency to higher survival than CS, but significant differences were not found. The CS growing media showed the lowest survival (Fig. 4). The generalized decrease in seedling survival during the second year in the field is undoubtedly linked to the extreme drought of the summer of 2006 (see Fig. 1). However, in spite of this prolonged drought period (90 days, rainfall accumulated: 2.3 mm), survival in the HS-1.5 growing media was higher than in CS, being 57% and 40% respectively at the end of the monitoring period (Fig. 4). In relation to field growth, 20 months after planting no statistical differences in shoot height or root collar diameter were observed, which is probably due to the high variability (high standard deviation) of these values (Fig. 5). The differences observed in shoot height (p<0.01) during the first months were reduced. The average shoot height ranged between 34 and 38 cm and

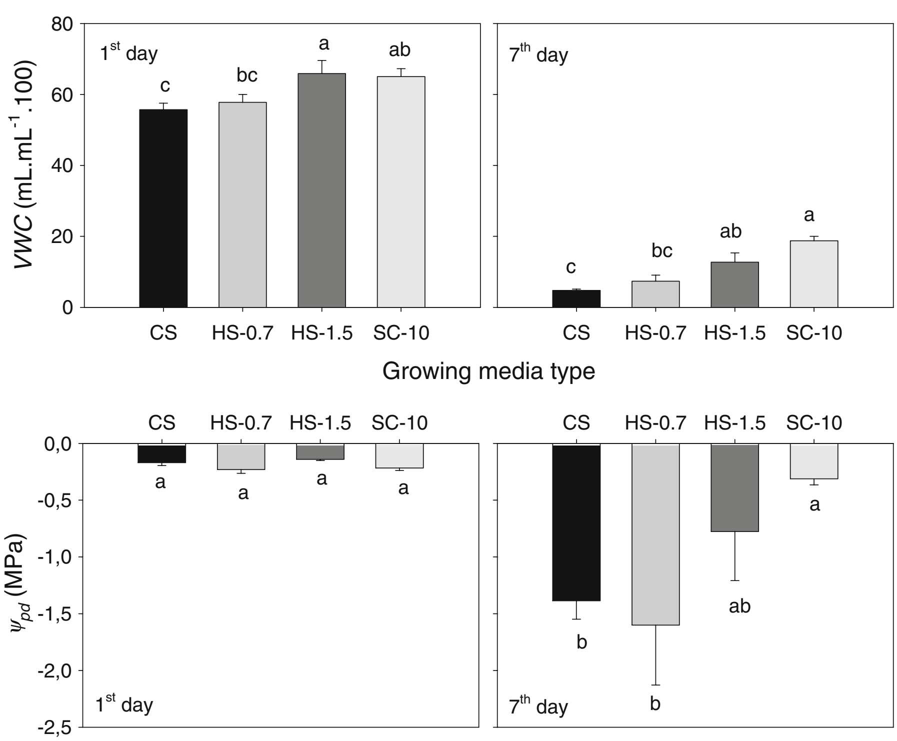

Fig. 2 Volumetric water content (*VWC*) of the root plug (above) and predawn xylem water potential (Ψ_{pd}) of the seedlings (below) during a controlled drought period in the nursery. Results at first day (left) and at seventh day (right). Mean ± standard error. N=5. Results of one-way ANOVA and Tukey's HSD *post hoc* test. *VWC* or Ψ_{pd} values followed by the same letter are not significantly different at p<0.05

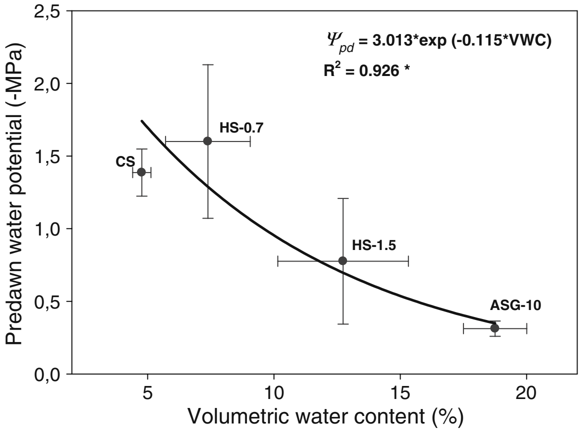

Fig. 3 Exponential functions and regression coefficients for predawn xylem water potential (Ψ_{pd}) of the seedlings as a function of volumetric water content (*VWC*) of substrate in the root plug. Mean ± standard error. N=5

RCD was between 4 and 8 mm, observing a tendency to show the lowest shoot height in CS.

Seventeen months after planting, no significant differences between aboveground and belowground biomass fractions were observed in the seedlings excavated in the field. Although the seedlings cultivated in SC-10 showed the lowest fine-root dry weight values (p=0.04), no differences were found in biomass allocation, expressed as root/shoot ratio and DW_R/SDW ratio. In relation to the new root growth for colonizing the plantation hole, no significant differences between treatments were observed with respect to new root dry weight, root/shoot ratio,

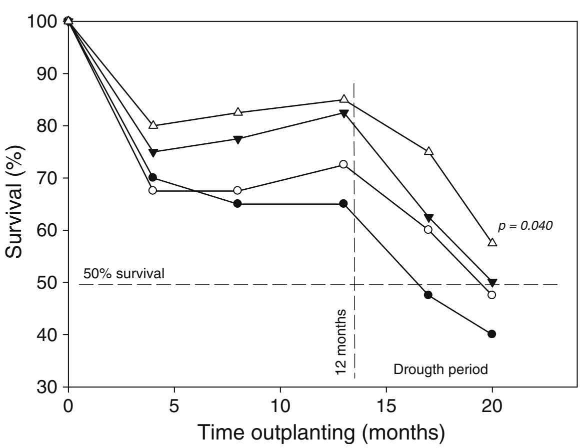

Fig. 4 Seedling survival in the field. Results Kaplan-Meier test, Long Rank *post hoc* (CS: *black circle*; SC-10: *white circle*; HS-0.7: *black triangle down* and HS-1.5: *white triangle up*)

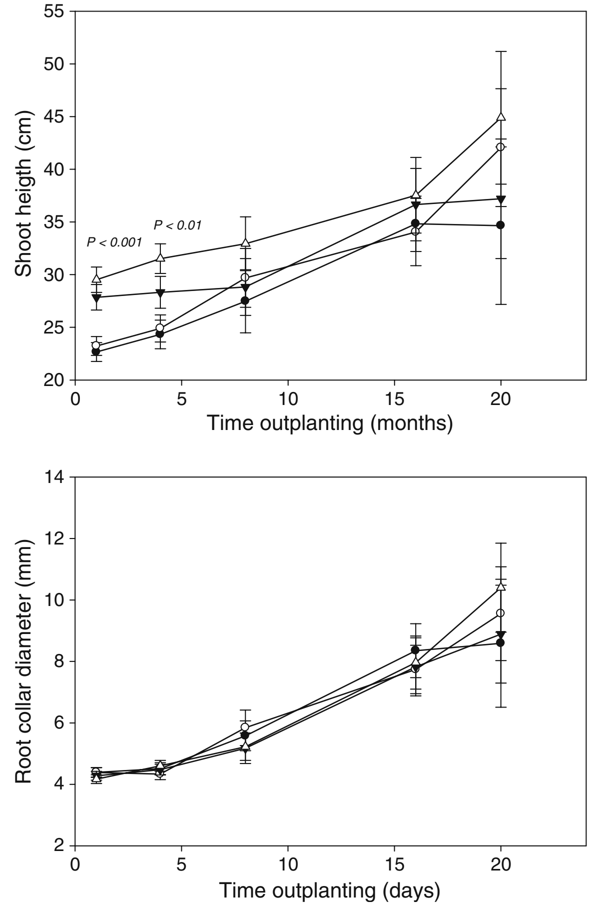

Fig. 5 Growth of seedlings in the field. Shoot height (above) and root collar diameter (below). Mean ± standard error. Results of one-way ANOVA and Tukey's HSD *post hoc* test. (CS: *black circle*; SC-10: *white circle*; HS-0.7: *black triangle down* and HS-1.5: *white triangle up*)

number of new roots growing outside the root plug or maximum rooting depth.

Carbon isotope composition and stomatal conductance

To assess seedling water stress in the field, we determined the carbon isotope composition of the tap root ($\delta^{13}C_{root}$), and we monitored stomatal conductance for 4 months. Our determinations of $\delta^{13}C_{root}$ indicated that CS seedlings showed higher values ($\delta^{13}C_{root}$: −23.96±0.18, p=0.004) than HS-1.5 ($\delta^{13}C_{root}$: −25.48±0.28; Fig. 6), reflecting a higher degree of stress. SC-10 ($\delta^{13}C_{root}$: −24.83±0.29) and HS-0.7 ($\delta^{13}C_{root}$: −24.61±0.18) showed intermediate values which were not significantly different with respect to CS and HS-1.5.

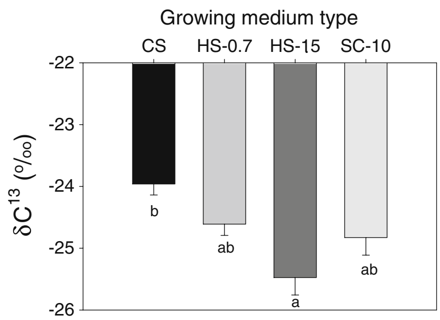

Fig. 6 Carbon isotope composition of the tap root ($\delta^{13}C_{root}$) of seedlings excavated in the field. Mean ± standard error, N=5. Results of one-way ANOVA and Tukey's HSD *post hoc* test. $\delta^{13}C_{root}$ values followed by the same letter are not significantly different at $p<0.05$

Stomatal conductance (*gs*) showed the lowest values during the dry summer, ranging from 99 mmol $H_2O.m^{-2}$ leaf area.s^{-1} in the morning to 77 mmol $H_2O.m^{-2}$ leaf area.s^{-1} at midday, which reflected the drought-stress conditions of this season. Subsequently, these values showed an important recovery in autumn, after the rainfalls (Fig. 7), presenting *gs* values between 254 mmol $H_2O.m^{-2}$ leaf area.s^{-1} in the morning and 432 mmol $H_2O.m^{-2}$ leaf area.s^{-1} at midday. In general, the HS-1.5 treatment showed statistically higher *gs* than CS (G-G′<0.001, Fig. 7) in both measurements, morning and midday. Except in summer, the SC-10 treatment always showed higher *gs* values than the CS treatment, especially in the midday measurements; however, the differences were not statistically significant.

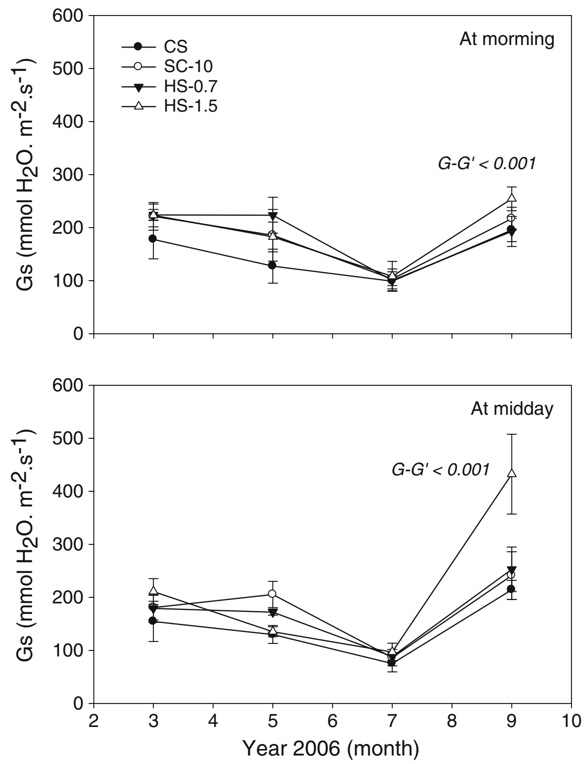

Fig. 7 Stomatal conductance (*Gs*) measured in March, May, July and September of 2006, corresponding to the 3rd, 5th, 7th and 9th month on the x-axis. In the morning (above) and at midday (below). Mean ± standard error, N=5. Results of General Linear Model (GLM) Repeated Measures (CS: *black circle*; SC-10: *white circle*; HS-0.7: *black triangle down* and HS-1.5: *white triangle up*)

Discussion

Changes in the water-holding capacity of the growing media

Mixing sepiolite clay and Stockosorb® hydrogel into a peat-based growing medium increased the water holding capacity of the root plug. Both materials (clay and hydrogel) have a high capacity to absorb, retain and distribute water to the soil or growing media (Chatzoudis and Rigas 1999; Sánchez et al. 2000; Francis et al. 2007). This explains the high water-holding capacity of the root plug of the seedlings cultivated in SC-10 and HS-1.5 during the controlled drought period in the nursery, under both conditions: optimum substrate moisture and drought conditions (Figs. 2 and 3). This result agrees with other experiments using peat-based growing media. A growing medium consisting of a mixture of sphagnum peat and perlite (80:20) mixed with hydrogel at 0.4% showed a higher water content than the control substrate, i.e., 128% in optimum moisture conditions and 150% in drought conditions (Arbona et al. 2005). Using a similar growing medium (sphagnum peat) but mixing it with hydrogel at 0.2% (w/w), Apostol et al. (2009) observed a 0.5–0.9 L increase in water content with respect to the control growing medium. Moreover, using hydrogel as a soil amendment resulted in a linear increase in soil moisture when 0.1, 0.2 and 0.3% of hydrogel was mixed into sandy soil (Akhter et al. 2004) and an increase of 41% in soil water content in saline soils amended with hydrogel at 0.6% (Chen et al. 2004). With respect to the addition of clay in the substrate, Owen et al. (2008) reported that pine bark amended between 0 and 24% (v/v) with a calcined Georgiana palygorksite-bentonite aggregate (0.85–0.25 mm) also produced an increase in the volumetric water content of the growing media.

Effects on seedling growth and morphological traits

Mixing the growing media with hydrogel (HS-07 and HS-1.5) produced seedlings with a higher shoot height and slenderness index at the end of the nursery culture (Table 1), which may be related to a higher water availability in the substrate for seedling growth as a consequence of a higher water holding capacity of the root plug. These results agree with the observations of Hüttermann et al. (1999) in *Pinus halepensis* and Akhter et al. (2004) in *Hordeum vulgare* L., *Triticum aestivum* L. and *Cicer arietinum* L. These authors reported increases in shoot height by increasing the ratio of hydrogel in sandy, loam and loam sandy soils, respectively. However, these authors found no significant differences with respect to root collar diameter growth on using hydrogels, which agrees with our results. Other authors have observed a positive correlation between trunk growth in seedlings of *Fraxinus pennsylvanica* Marsh., *Platyphyllos Betula* and *Acer saccharinum* L. and the water retention porosity of growing media with different proportions of paper mill sludge, wood chips, bark, and peat (Chong and Lumis 2000). On the other hand, the composition of the growing media can also affect the biomass distribution in the seedlings. Previous works have observed a lower root and shoot biomass in *Cupressus glabra* cultivated in a growing media made of a mixture of black peat and sepiolite (60/40 v/v), which was explained by the low air capacity of the mixture (Sánchez et al. 2000). In contrast, hydrogel amendments to substrates with and without soil produced a significant increase in different biomass fractions (Chen et al. 2004; Jobin et al. 2004; Frantz et al. 2005). This increase is particularly important (with respect to our study) when peat moss and hydrogel-amended plugs were used (Ochoa et al. 2009). However in our study, the use of hydrogel and clay did not affect the biomass of roots, leaves and stems, neither their allocation pattern (Table 1).

Seedling survival in the field

Hydrogel use in dryland ecosystems may help to overcome soil moisture limitations at outplanting and improve seedling field performance. In this sense, our field results showed a beneficial effect of hydrogel and clay during the first year in the field. Nevertheless, only the HS-1.5 treatment yielded statistical differences, with an increment of more than 20% in the survival rates compared with the CS growing media (Fig. 4). Similar results have been reported in soils amended with hydrogel. *P. halepensis* seedlings grown in sandy soils amended with hydrogel at 0.4% (w/w) doubled their survival compared to control plants. This result was closely dependent on the concentration of hydrogel (Hüttermann et al. 1999). Similarly *Conocarpus erectus* grown in sandy soil amended with hydrogel at 0.6% showed 3 times higher survival than control plants (Al-Humaid and Moftah 2007). However, fewer survival results have been reported on hydrogel mixed with a peat-based growing medium. *Poncirus trifoliata* L. seedlings (4 months old) and *Citrus reshni* Hort. Ex Tan (1 year old) transplanted to a growing medium consisting of a mixture of sphagnum peat and perlite (80:20) amended with 0.4% hydrogel showed a significantly higher survival rate than seedlings grown in a control substrate, i.e., 67% and 33% respectively (Arbona et al. 2005). Other studies have also reported positive results, e.g., the use of hydrogel to protect the root system of *Picea abies* seedlings during transplanting (Sarvaš 2003), the coating of seeds with hydrogel to increase seedling emergence and field performance in *Agropyron cristatum* (Mangold and Sheley 2007), and the immersing of the root plug in a saturated hydrogel solution to increase survival in *Eucalyptus pilularis* and *Corymbia citriodora* subsp. *variegata* (Thomas 2008).

Water status of seedlings

Previous studies have shown that using hydrogel as a soil amendment or mixing it with a peat-based substrate contribute to improving seedling water status, which is reflected in a higher Ψ_{pd} than in the control substrate (Hüttermann et al. 1999; Al-Humaid and Moftah 2007) or in the highest *gs* values (Arbona et al. 2005). These observations agree with our results (see Figs. 2 and 7), and they can explain how, despite the extreme drought conditions sustained by the outplanted seedlings during the summer of 2006 (2.3 mm in 90 days, rainfall accumulated; see Fig. 1), the seedlings cultivated in HS-1.5 continue to show the highest survival values (Fig. 4). Other studies in similar environmental conditions have indicated that a continuous dry period exceeding 120 days without significant rainfall (>5 mm) can result in a mortality approaching 100% (Vallejo and Alloza 1998).

The leaf carbon isotope composition ($\delta^{13}C_{leaf}$) of the C3 plants is closely related to water availability (Farquhar et al. 1989; Leavitt 1993) and plant transpiration (Dubey and Chandra 2008). $\delta^{13}C_{leaf}$—often reported as leaf carbon isotope discrimination (Δ_{leaf})—has been positively correlated with annual precipitation (Wittmer et al. 2008) and can thus be used as a suitable indicator of the existence of drought periods and stress conditions for plants. Under drought stress, species tend to close their stomata to reduce water loss. Consequently, there is less CO_2 absorption and the plant discriminates less ^{13}C, increasing its concentration in the reserve organs (i.e., leaf and root). In the present study, seedlings cultivated in HS-1.5 showed significantly lower $\delta^{13}C_{root}$ values than those in CS (Fig. 6), which indicates that HS-1.5 seedlings suffered lower drought stress. This can be explained by the higher water availability for seedlings due to the higher water-holding capacity of the substrate mixed with hydrogel at 1.5%. This result agrees with Damesin et al. (1997), who observed lower $\delta^{13}C_{leaf}$ in *Q. ilex* and *Q. pubescens* on the wettest sites (1,134 mm) than on the driest sites (663 mm). In terms of carbon isotope discrimination (Δ_{leaf}), similar results have been reported by Goveiras and Freitas (2008). Both authors found a significant positive correlation between Δ_{leaf} and the rainfall gradient (491–1,299 mm) of the *Q. suber* distribution in Portugal, and linked this result to an adjustment of gas exchange at leaf level according to water availability. The fact that during the second year we observed differences in $\delta^{13}C_{root}$ and *gs* leads us to believe that the hydrogels are still functional and continue to improve the water status of seedlings in the field. These results are reinforced by the fact that we did not observe any significant differences in root colonization in the field.

In conclusion, the use of hydrogel mixed with a peat-based growing medium in the nursery culture is a suitable option for improving the stock quality of seedlings destined for forest restoration in dryland ecosystems. In this paper, the hydrogel Stockosorb® at 1.5% showed the best results, i.e., it increased the water holding capacity of the root plug (high volumetric water content), improved seedling water status (higher predawn xylem water potential and stomatal conductance and lower carbon isotope composition), enhanced seedling drought resistance and increased seedling survival in the field. Considering that the hydrogel did not affect the above and below-ground traits of the seedlings, even in the field, we believe that during the 20 months studied after outplanting, the hydrogel maintained its effect of increasing the water-holding capacity of the root plug. Sepiolite clay, despite increasing the water holding capacity of the root plug, showed moderate results in the field.

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References

Akhter J, Mahmood K, Malik KA, Mardan A, Ahmad M, Iqbal MM (2004) Effects of hydrogel amendment on water storage of sandy loam and loam soils and seedlings growth of barley, wheat and chickpea. Plant Soil Environ 50:463–469

Al-Humaid AI, Moftah AE (2007) Effects of hydrophilic polymer on the survival of buttonwood seedlings grown under drought stress. J Plant Nutr 30:53–66. doi:10.1080/01904160601054973

Alvarez A (1984) Sepiolite: properties and uses. In: Singer A, Galan E (eds) Palygorskite-Sepiolite: occurrences, genesis and uses, vol 37. Elsevier Science Publisher, Amsterdam, pp 253–287

American Soil Technologies Inc. (2010) Agricultural technical use guide for stockosorb® and stockosorb® F. In Products & Services Agriculture. Stockosorb® http://www.americansoiltech.com/products_and_services/agriculture.asp. Accessed 03 august 2010

Apostol KG, Jacobs DF, Dumroese RK (2009) Root desiccation and drought stress responses of bareroot *Quercus rubra* seedlings treated with a hydrophilic polymer root dip. Plant Soil 315:229–240. doi:10.1007/s11104-08-9746-6

Arbona V, Iglesias DJ, Jacas J, Primo-Millo E, Talon M, Gómez-Cárdenas A (2005) Hydrogel substrate amendment alleviates drought effects on young citrus plants. Plant Soil 270:73–82. doi:10.1007/s11104-004-1160-0

Aronson J, Pereira JS, Pausas J (2009) Preface. In: Aronson J, Pereira JS, Pausas J (eds) Cork Oak Woodlands in transition: ecology, management, and restoration of an ancient mediterranean ecosystem. Island Press, Washington DC, pp xv–xvii

Bouranis DL, Theodoropoulus AG, Drossopoulus JB (1995) Designing synthetic polymers as soil conditioners. Commun Soil Sci Plant Anal 26:1455–1480

Buchmann N, Kao WT, Ehleringer J (1997) Influence of stand structure on carbon-13 of vegetation, soils, and canopy air within deciduous and evergreen forest in Utah, United States. Oecologia 110:109–119

Carrasco MS, Rad JC, González-Carcedo S (1995) Immobilization of alkaline phosphatase by sorption on Na-Sepiolite. Bioresour Technol 51:175–181

Chatzoudis GK, Rigas F (1999) Soil salts reduce hydration of polymeric gels and affect moisture characteristics of soil. Commun Soil Sci Plant Anal 30:2465–2474

Chen S, Zommorodi M, Fritz E, Wang S, Hüttermann A (2004) Hydrogel modified uptake of salt ions and calcium in *Populus euphratica* under saline conditions. Trees 18:175–183. doi:10.1007/s00468-003-0267-x

Chirino E, Vilagrosa A, Hernández EI, Matos A, Vallejo VR (2008) Effects of a deep container on morpho-functional characteristics and root colonization in *Quercus suber* L. seedlings for reforestation in Mediterranean climate. For Ecol Manag 256:779–785. doi:10.1016/j.foreco.200805035

Chirino E, Vilagrosa A, Cortina J, Valdecantos A, Fuentes D, Trubat R, Luis VC, Puértolas J, Bautista S, Baeza J, Peñuelas JL, Vallejo VR (2009) Ecological restoration in degraded drylands: the need to improve the seedling quality and site conditions in the field. In: Grossberg SP (ed) Forest management. Nova Publisher, New York, pp 85–158

Chong C, Lumis GR (2000) Mixtures of paper mill sludge, wood chips, bark, and peat in substrates for pot-in-pot shade tree production. Can J Plant Sci 80:669–675

Cortina J, Peñuelas JL, Puertolas J, Savé R, Vilagrosa A (2006) Calidad de planta forestal para la restauración en ambientes mediterráneos: Estado actual del conocimiento. Ministerio de Medio Ambiente, Madrid

Damesin C, Rambal S, Joffre R (1997) Between-tree variations in leaf $\delta^{13}C$ of *Quercus pubescens* and *Quercus ilex* among Mediterranean habitats with different water availability. Oecologia 111:26–35

Di Castri F (1973) Climatographical comparisons between Chile and the western coast of North America. In: Di Castri F, Mooney HA (eds) Mediterranean type-ecosystems. Springer, Berlin, pp 21–36

Donat R (2009) The removal of uranium (VI) from aqueous solutions onto natural sepiolite. J Chem Thermodyn 41:829–835. doi:10.1016/j.jct.2009.01.009

Dubey A, Chandra A (2008) Effect of water stress on carbon isotope discrimination and its relationship with transpiration efficiency and specific leaf area in Cenchrus species. J Environ Biol 29:371–376

Farquhar GD, Ehleringer JR, Hubick KT (1989) Carbon isotopic discrimination and photosynthesis. Annu Rev Plant Physiol Plant Mol Biol 40:503–537

Francis ML, Fey MV, Prinsloo HP, Ellis F, Mills AJ, Medinski TV (2007) Soils of Namaqualand: compensations for aridity. J Arid Environ 70:588–603. doi:10.1016/j.jaridenv.2006.12.028

Frantz JM, Locke JC, Pitchay DS, Krause CR (2005) Actual performance versus theoretical advantages of polyacrylamide hydrogel throughout bedding plant production. HortScience 40(7):2040–2046

Goveiras A, Freitas H (2008) Modulation of leaf attributes and water use efficiency in *Quercus suber* along a rainfall gradient. Trees Struct Funct. doi:1010007/s00468-008-0274-z

Günes T (2007) Effect of polymer on seedlings survival and growth of transplanted tomato under water stress. Asian J Chem 19(4):3208–3214

Hüttermann A, Zommorodi M, Reise K (1999) Addition of hydrogels to soil for prolonging the survival of *Pinus halepensis* seedlings subjected to drought. Soil Tillage Res 50:295–304

IPCC (2007) Cambio climático 2007: Informe de síntesis. Contribución de los Grupos de trabajo I, II y III al Cuarto Informe de evaluación del Grupo Intergubernamental de Expertos sobre el Cambio Climático [Equipo de redacción principal: Pachauri RK y Reisinger A (directores de la publicación)]. IPCC, Ginebra, Suiza, 104 págs. http://www.ipcc.ch/pdf/assessment-report/ar4/syr/ar4_syr_sp.pdf. Accessed 03 August 2010

Jobin P, Caron J, Bernier PY, Dansereau B (2004) Impact of two hydrophilic acrylic-based polymers on the physical properties of three substrates and the growth of *Petunia xhybrida* 'Brilliant Pink'. J Am Soc Hortic Sci 129(3):449–457

Kramer PJ, Boyer JS (1995) Water relations of plants and soils. Academic, San Diego

Leavitt S (1993) Seasonal carbon-13 carbon-12 changes in tree rings species and site coherence and a possible drought influence. Can J For Res 23:210–218

Mañas P, Castro E, De las Heras J (2008) Quality of maritime pine (*Pinus pinaster* Ait) seedlings using waste materials as nursery growing media. New For 37:295–311. doi:10.1007/s11056-008-9125-4

Mangold JM, Sheley RL (2007) Effects of soil texture, watering frequency, and a hydrogel on the emergence and survival of coated versus uncoated crested wheatgrass seeds. Ecol Restor 25(1):6–11. doi:10.3368/er.25.1.6

Marfà O, Lemaire F, Cáceres R, Giuffrida F, Guérin V (2002) Relationships between growing media fertility, percolate composition and fertigation strategy in peat-substitute substrates used for growing ornamental shrubs. Sci Hortic 94:309–321

Marianthi T (2006) Kenaf (*Hibiscus cannabinus* L.) core and rice hulls as components of container media for growing *Pinus halepensis* M. seedlings. Bioresour Technol 97:1631–1639. doi:10.1013/j.biortech.200507.027

Ochoa J, Valdés R, González A, López J, Conesa E, Franco JA, Fernández JA, Bañón S (2009) Effects of type of plug and the growing media on evapotranspiration and growth of potted carnations. Acta Hort (ISHS) 843:367–372. http://www.actahort.org/books/843/843_49.htm

Owen JJS, Warren SL, Bilderback TT, Albano J, Cassel KD (2008) Physical properties of pine bark substrate amended with industrial mineral aggregate. Acta Hortic 779:131–138

Pérez Cueva AJ (1994) Atlas Climático de la Comunidad Valenciana. Consellería d' Obres Publiques, Urbanisme i Transports, Generalitat Valenciana, Valencia, pp 168–183

Pérez-Devesa M, Cortina J, Vilagrosa A, Vallejo R (2008) Shrubland management to promote *Quercus suber* L. establishment. For Ecol Manag 255:374–382

Sánchez A, Cadahia C, Eymar E, Masaguer A (2000) Study of conifer nitrogen nutrition using hydroponic cultures: applications to fertigation systems. J Plant Nutr 23 (7):1007–1024. doi:10.1080/01904160009382077

Sarvaš M (2003) Effect of desiccation on the root system of Norway apruce (*Picea abies* [L.] Karst.) seedlings and a possibility of using hydrogel Stockosorb® for its protection. J For Sci 49(11):531–536

Sarvaš M, Pavlenda P, Takáčová E (2007) Effect of hydrogel application on survival and growth of pine seedlings in reclamations. J For Sci 53(5):204–209

Thomas DS (2008) Hydrogel applied to the root plug of subtropical eucalypt seedlings halves transplant death following planting. For Ecol Manag 255:1305–1314. doi:10.1016/j.foreco.2007.10.035

Trubat R, Cortina J, Vilagrosa A (2010) Nursery fertilization affects seedling traits but not field performance in *Quercus suber* L. J Arid Environ 74:491–497

Vallejo VR, Alloza JA (1998) The restoration of burned lands: the case of eastern Spain. In: Moreno JM (ed) Large forest fires. Backhuys Publishers, Lieden, pp 91–108

Vallejo VR, Aronson J, Pausas J, Cortina J (2006) Restoration of Mediterranean woodlands. In: Van Andel J, Aronson J (eds) Restoration ecology. The new frontier. Blackwell Publication, Oxford, pp 193–207

Viero PWM, Little KM, Oscroft DG (2000) The effect of a soil-amended hydrogel on the establishment of a *Eucalyptus grandis* x *E. camaldulensis* clone grown on the sandy soils of Zululand. Southern Afr For J 188:21–28

Viero PWM, Chiswell KEA, Theron JM (2002) The effect of a soil-amended hydrogel on the establishment of a *Eucalyptus grandis* clone on a sandy clay loam soil in Zululand during winter. Southern Afr For J 193:65–75

Vilagrosa A, Seva JP, Valdecantos A, Cortina J, Alloza JA, Serrasolsas I, Diego V, Abril M, Ferran A, Bellot J, Vallejo VR (1997) Plantaciones para la restauración forestal en la Comunidad Valenciana. In: Vallejo VR (ed) La restauración de la cubierta vegetal en la Comunidad Valenciana. Fundación Centro de Estudios Ambientales del Mediterráneo, Valencia, pp 435–548

Wittmer M, Auerswald K, Tungalag R, Bai YF, Schäufele R, Bai CH, Schnyder H (2008) Carbon isotope discrimination of C3 vegetation in Central Asian Grassland as related to long-term and short-term precipitation patterns. Biogeosciences Discuss 5:903–935

Yang XH, Hu ZO, Gao AL, Wei XF (2006) Remediation of attapulgite on copper contaminated soil. Liaoning Gongcheng Jishu Daxue Xuebao (Ziran Kexue Ban) Journal of Liaoning Technical University (Natural Science Edition) 25:629–631

Yu S, Zinati GM (2006) Physical and chemical changes in container media in response to bark substitution for peat. Compost Sci Util 14:222–230