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ORIGINAL PAPER

Effects of nursery preconditioning through mycorrhizal inoculation and drought in *Arbutus unedo* L. plants

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Abstract The influence of a water deficit treatment and mycorrhizal inoculation with *Pisolithus tinctorius* (Pers.) Coker and Couch on the water relations, gas exchange, and plant growth in *Arbutus unedo* L. plants was studied in order to evaluate the hardening process during the nursery period. The ability to withstand the adverse conditions after transplantation was also studied. Mycorrhizal and non-mycorrhizal seedlings of *A. unedo* were pot-grown for 4 months in a greenhouse (nursery period), during which time two irrigation treatments, well watered (100% water holding capacity, leaching 20% of the applied water) and deficit irrigation (50% of the well watered), were applied. Subsequently, the plants were transplanted to the field and well irrigated (transplanting period), after which and until the end of the experiment they received no water (establishment period). At the end of the nursery period, both water deficit and mycorrhizae were seen to have altered the plant morphology. Mycorrhizal plants had lower leaf area and improved leaf color parameters, while the water deficit increased root dry weight and the root/shoot ratio. Mycorrhizal plants had higher leaf water potential values than non-inoculated plants. Mycorrhizae increased stomatal conductance and photosynthesis values, especially in stressed plants. Drought led to an osmotic adjustment and a decrease in the leaf water potential values at turgor loss point in the mycorrhizal plants. Cell wall rigidity, measured as increased bulk modulus of elasticity, was decreased by the mycorrhizae effect. After transplanting, no differences were found in the water relations or gas exchange values between treatments. During the establishment period, the plants that had been exposed to both drought and mycorrhizae showed a better water status (higher leaf water and turgor potential values) and higher gas exchange values. In conclusion, water deficit and mycorrhizal inoculation of *A. unedo* plants in nursery produced changes in tissue water relations, gas exchange, and growth, related with the acclimation process in the seedlings, which could provide better resistance to drought and stress conditions following planting.

Keywords Strawberry tree · *Pisolithus tinctorius* · Hardening · Water stress · Gas exchange · Arbutoid mycorrhiza

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Introduction

The morphological, nutritional, and physiological shoot and root characteristics of plants grown under nursery conditions

and their performance following transplantation may be critical for optimizing high-quality plants and essential for their successful establishment and survival (Franco et al. 2006). Numerous reports have determined that deficit irrigation during nursery affects several aspects related to the hardening of ornamental plants, such as reductions in leaf area, changes in root morphology, osmotic adjustment, and stomata closure (De Herralde et al. 1998).

Mycorrhizal symbiosis also affects the root morphology and its functioning, which often causes physiological changes in host plant in both well-watered (Danneberg et al. 1992; Drüge and Schönbeck 1992) and water-stressed (Goicoechea et al. 1995, 1996) conditions. This symbiosis can increase drought resistance of the host, improving the plant water status as a result of changes in water relations due to a mechanism related with water uptake (Ruíz-Lozano and Azcón 1995; Duan et al. 1996; Morte et al. 2000; Dell'Amico et al. 2002) and/or phosphorus nutrition (Fitter 1988). Several authors concluded that increases in stomatal conductance and transpiration of plants infected by mycorrhizae are related to higher leaf P concentrations (Nelsen and Safir 1982; Duan and Augé 1992), while others maintain that these responses are not mediated by the P status (Augé et al. 1986; Augé and Stodola 1990).

Modifications of host plant–water relations induced by mycorrhizal fungi have been reported for arbuscular mycorrhizal symbiosis, but less information exists about the effects caused by arbutoid mycorrhizae. *Pisolithus tinctorius* (Pers.) Coker and Couch forms arbutoid mycorrhiza with *Arbutus unedo* L., characterized by a cruciform structure usually branched in pinnate pattern, a few hyphae along the cruciform roots without forming a true mantle (Navarro et al. 2009). These hyphae penetrate between the epidermical cells, forming the Hartig net, and invade the epidermical cells. *A. unedo* roots have two layers of cortical cells, and the hyphae penetrate some cells of the first cortical layer but never the endodermis or central cylinder (Navarro et al. 2009).

A. unedo is a sclerophyllus evergreen shrub, characteristic of Mediterranean coastal scrub vegetation, which is of great interest in gardening and landscaping. Its commercial value as a potted plant has also been evaluated (Navarro et al. 2007). It is a species well adapted to Mediterranean climatic conditions, and the effect of drought on water relations, leaf gas exchange, and growth in this species has been widely reviewed (Araújo-Alves et al. 2000; Munné-Bosch and Peñuelas 2004; Serrano et al. 2005). However, the effects of the arbutoid mycorrhiza on *A. unedo* plants and the combined effect of water deficit and mycorrhiza have not been studied.

Therefore, the purpose of this work was to test the effects of water deficit on the response of *A. unedo* seedlings associated with *P. tinctorius*. Changes in growth, ornamental characteristics, nutritional status, water relations, and gas exchange of *A. unedo* seedlings during the nursery period were evaluated and related with its ability to withstand adverse conditions during establishment in field conditions.

Materials and methods

Plant material

A. unedo (native of SE Spain) seedlings of 2 years from multi-alveolar trays of black polyethylene with dimensions for alveolus of 50×50×115 mm filled with black peat were acquired. Only seedlings of 20–22 cm height were chosen for transplanting in order to homogenize the plant material used in the experiment.

Fungal inoculum

A spore suspension of *P. tinctorius* (from ripe sporocarps collected from a *Quercus rotundifolia* orchard in Campus de Espinardo, University of Murcia, Spain) was used as mycorrhizal inoculum. The spore suspension was prepared according to Castellano and Molina (1989) with a concentration of approximately 10^7 spores per plant.

Plant growth conditions

Seedlings were transplanted into 2.5-l plastic pots filled with a mixture of clay-loam soil, vermiculite, and black peat (5:3:2, v/v/v) previously sterilized by autoclaving (115°C, 60 min) on three alternate days and amended with 2 gl^{-1} substrate of osmocote plus (10:11:18 N, P, K+ microelements). The experiment was conducted during 2004 in Santomera (Murcia, Spain) in a plastic greenhouse equipped with a cooling system. The plants were irrigated three to five times per week, depending on the evapotranspirational demand, using a drip irrigation system. The micro-climatic conditions were registered with an Escort Junior Data Logger (Escort Data Loggers, Inc., Buchanan, VA, USA). The maximum/minimum average temperatures in the greenhouse were 33/18°C and the relative humidity ranged between 55% and 80%. The average maximum photosynthetically active radiation was 960 μmol $m^{-2}s^{-1}$.

At 2 months after transplanting (26 February 2004), 120 plants (30 per treatment) were subjected to four treatments lasting 4 months (from 26 February to 30 June 2004): two inoculation treatments (mycorrhizal, M and non-mycorrhizal, NM) by two irrigation treatments (deficit, D and well-watered, WW). Mycorrhizal plants were inoculated with a spore suspension of *P. tinctorius* by placing inoculum close to the roots. The remaining plants were not

Table 1 Effects of water regime (W), mycorrhizal inoculation (M), and their interaction (W×M) on mycorrhizal colonization (%) and growth of *A. unedo* seedlings at the end of the nursery period

Parameters	Water regime (W)		Mycorrhizal inoculation (M)		Significance		
	Well-watered	Deficit	M plants	NM plants	W	M	W×M
Mycorrhization (%)	21.07	17.59	34.40	4.26	ns	**	ns
Total plant dry weight (g plant^{-1})	11.58	15.85	12.71	13.73	*	ns	ns
Shoot dry weight (g plant^{-1})	8.88	10.44	8.53	9.79	ns	ns	ns
Root dry weight (g plant^{-1})	2.70	5.41	4.17	3.94	***	ns	ns
Root/shoot ratio	0.34	0.54	0.49	0.38	**	*	ns
Leaf area (cm^2)	336.65	345.20	309.64	372.21	ns	*	ns
Leaf number	102	107	94	114	ns	ns	ns

NM non-mycorrhizal plants

*P<0.05, **P<0.01, ***P<0.001, *ns* non-significant, according to Duncan's multiple range test. Values are means (n=10)

inoculated. Well-watered irrigation treatment plants were watered to 100% water holding capacity (leaching 20% of the applied water), while deficit irrigation plants received 50% of the well-watered irrigation.

On 30 June 2004 (end of nursery period), 15 plants per treatment were transplanted to the field. Prior to transplanting, the plants were watered abundantly and after transplanting were well irrigated by drip using one emitter per plant, each delivering 1 lh^{-1} until 27 July 2004 (transplanting period), after which and until the end of the experiment (2 September 2004) they received no water (establishment period).

Fungal colonization assessment

At harvest (end of the nursery period), root samples were collected from five root systems per treatment and examined for mycorrhizal colonization. Roots were observed by stereomicroscope (Olympus SZH) to determine the pattern of the mycorrhizal system and the presence of a mantle along the mycorrhizal roots.

To quantify the intracellular *P. tinctorius* colonization, the root samples were cleared and trypan blue-stained according to Phillips and Hayman (1970) but using lactic acid instead of lactophenol, and 100 root segments per plant were mounted on slides, squashed by pressing on the cover glasses and quantified for colonization according to McGonigle et al. (1990). This quantification was carried out 1 month after mycorrhizal application and at the end of the nursery period to check the presence or absence of mycorrhiza.

Growth and ornamental characters

At the end of the nursery period, the relative chlorophyll content (RCC) and leaf color were determined in three leaves per plant and ten plants per treatment. RCC was measured at the midpoint of mature leaves with a Minolta SPAD-502 chlorophyll meter (Konica Minolta Sensing Inc., Osaka, Japan). Leaf color was measured with a Minolta CR-10 colorimeter (Konica Minolta Sensing Inc.), giving the color coordinates lightness (L*), chroma (C*), and hue angle (h°) (McGuire 1992).

Table 2 Effects of water regime (W), mycorrhizal inoculation (M), and their interaction (W×M) on the relative chlorophyll content (RCC) and color CIELAB parameters (L, chroma, and hue angle) in *A. unedo* seedlings at the end of the nursery period

Parameters	Water regime (W)		Mycorrhizal inoculation (M)		Significance		
	Well-watered	Deficit	M plants	NM plants	W	M	W×M
RCC	37.65	36.82	35.96	38.52	ns	ns	ns
Lightness (L*)	43.01	42.72	43.82	41.91	ns	*	ns
Chroma (C*)	32.03	31.80	33.60	30.22	ns	**	ns
Hue angle (h°)	114.52	113.56	113.57	114.51	ns	ns	ns

NM non-mycorrhizal plants

*P<0.05, **P<0.01, *ns* non-significant, according to Duncan's multiple range test. Values are means (n=10)

Table 3 Effects of water regime (W), mycorrhizal inoculation (M), and their interaction (W×M) on mineral concentrations in leaves and roots of *A. unedo* seedlings at the end of the nursery period

Parameters	Part plant	Water regime (W)		Mycorrhizal inoculation (M)		Significance		
		Well-watered	Deficit	M plants	NM plants	W	M	W×M
N (mg g^{-1})	Leaves	6.80	6.40	6.70	6.40	ns	ns	ns
	Roots	6.70	6.80	6.90	6.60	ns	ns	ns
B (mg g^{-1})	Leaves	0.482	0.552	0.558	0.478	ns	*	ns
	Roots	0.226	0.238	0.245	0.220	ns	ns	ns
Ca (mg g^{-1})	Leaves	13.60	14.49	14.27	13.82	ns	ns	ns
	Roots	19.41	16.98	19.76	16.63	*	ns	ns
Cu (mg g^{-1})	Leaves	0.015	0.012	0.014	0.013	**	ns	ns
	Roots	0.030	0.028	0.032	0.027	ns	*	ns
Fe (mg g^{-1})	Leaves	0.250	0.213	0.242	0.221	ns	ns	ns
	Roots	0.777	0.684	0.814	0.647	ns	ns	ns
K (mg g^{-1})	Leaves	14.13	7.698	11.59	10.24	***	*	ns
	Roots	1.771	4.011	3.269	2.153	***	*	ns
Mg (mg g^{-1})	Leaves	4.206	4.539	4.499	4.247	ns	ns	ns
	Roots	3.977	3.493	4.184	3.285	ns	*	ns
Mn (mg g^{-1})	Leaves	0.027	0.026	0.032	0.020	ns	***	ns
	Roots	0.042	0.032	0.050	0.023	ns	***	ns
Na (mg g^{-1})	Leaves	2.259	1.091	1.873	1.477	***	ns	ns
	Roots	2.652	2.382	2.825	2.209	ns	ns	ns
P (mg g^{-1})	Leaves	1.678	1.824	1.738	1.864	ns	ns	ns
	Roots	0.850	1.178	1.103	0.925	*	ns	ns
S (mg g^{-1})	Leaves	2.177	3.229	2.841	2.565	***	ns	ns
	Roots	1.427	1.524	1.554	1.397	ns	ns	ns
Zn (mg g^{-1})	Leaves	0.085	0.098	0.086	0.097	*	*	ns
	Roots	0.051	0.047	0.053	0.045	ns	*	ns

NM non-mycorrhizal plants

*$P<0.05$, **$P<0.01$, ***$P<0.001$, *ns* non-significant, according to Duncan's multiple range test. Values are means ($n=10$)

The substrate was gently washed from the roots, ten plants per treatment were divided into leaves, stems, and roots, and the fresh weights were measured. Leaf area and leaf number were measured using a Delta-T Leaf Area Meter (Device Ltd., Cambridge, UK). Later, the different parts of the plants were oven-dried at 70°C until they reached a constant mass to measure the respective dry weights. With these data, the root/shoot ratio was determined.

Mineral content

At the end of the nursery period, B, Ca, Cu, Fe, K, Mg, Mn, N, Na, P, S, and Zn were determined in leaves and roots of ten plants per treatment. The determination of the mineral content was carried out on dry material, which was milled to a particle size able to pass through a 0.5-mm-diameter mesh and stored in plastic containers until later chemical analysis. B, Ca, Cu, Fe, K, Mg, Mn, Na, P, S, and Zn (mg g^{-1}) were determined in the extract, obtained after nitric perchloric digestion (HNO_3:$HCLO_4$, 2:1, v/v) of dry vegetable powder, with an atomic absorption spectrophotometer (Perkin-Elmer mod. 5500, USA). Plant tissue N was determined using an elemental analyzer (Thermo Finnigan Flash EA 1112 Series, USA).

Water relations

Daily evolutions of leaf water (Ψ_l), leaf osmotic (Ψ_s), and leaf turgor (Ψ_t) potential were registered on 30 June (end of the nursery period), 27 July (end of transplanting period), and 2 September 2004 (1 month after establishment) in five plants per treatment from dawn to sunset at 2-hour intervals. To measure Ψ_l, the sampled leaves were enclosed in a plastic bag (Turner 1988), which was immediately placed in a pressure chamber (Soil Moisture Equipment Co., Santa Barbara, CA, USA) according to Scholander et al. (1965), raising the pressure at a rate of 0.02 MPa s^{-1}

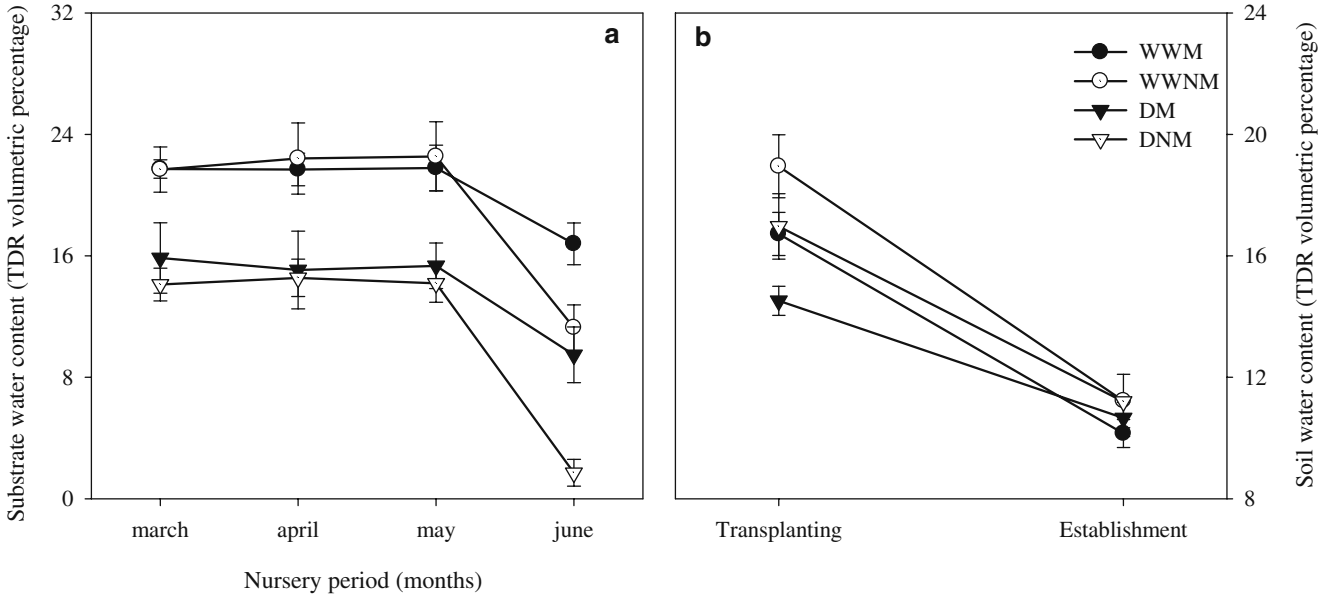

Fig. 1 Seasonal patterns of substrate and soil water content (TDR volumetric percentage) during the nursery period (**a**) and during the transplanting and establishment periods (**b**) in well-watered irrigation mycorrhizal (*WWM*), well-watered irrigation non-mycorrhizal (*WWNM*), deficit irrigation mycorrhizal (*DM*), and deficit irrigation non-mycorrhizal (*DNM*) treatments. Values are means (n=5) and *vertical bars* indicate standard errors

using nitrogen gas. Ψ_s was estimated using a vapor pressure osmometer (Wescor 5520, Wescor Inc., Logan, UT, USA) in excised leaves harvested and immediately frozen in liquid nitrogen (−170°C) and stored at −30°C. Before the measurements, samples were thawed and leaf sap was extracted for immediate Ψ_s determination according to Gucci et al. (1991). Ψ_t was estimated as the difference between Ψ_l and Ψ_s.

Estimates of the bulk modulus of elasticity (ε) at 100% RWC, relative water content at zero turgor (RWC_0), osmotic potential at full turgor (Ψ_{100s}), and zero turgor (Ψ_{0s}) were obtained at the end of the nursery period in four leaves per plant and five plants per treatment via pressure–volume analysis of leaves, as outlined by Hinckley et al. (1980). Bulk modulus of elasticity (ε) at 100% RWC was calculated using the formula:

$$\varepsilon = (RWC_0 \times \Psi_{100s})/(100 - RWC_0)$$

where ε is expressed in megapascals, Ψ_{100s} is the osmotic potential at full turgor (MPa), and RWC_0 is the relative water content at zero turgor.

Leaves were excised in the dark, placed in plastic bags, and allowed to reach full turgor by dipping the petioles in distilled water overnight (Davis and Mooney 1986; Yoon and Richter 1990). Pressure–volume curves were obtained from periodic measurements of leaf weight and balance pressure as leaves dried on the bench at constant temperature of 20°C. The leaf drying period in each curve was about 3–5 h.

Soil and substrate water content

Substrate and soil water content were measured each month in the pots and the field by measuring the volumetric content of water in the substrate and soil (θ_v) in the top 15 cm using time domain reflectometry probes (TDR) (Topp et al. 1980, 1982) attached to a TEKTRONIX 1502C in five replicates per treatment.

Gas exchange

Net photosynthesis (P_n) and stomatal conductance (g_s) were determined on the same days and in the same plants as Ψ_l using a portable gas-exchange system (LI-6400, LI-COR Inc., Lincoln, NE, USA). Measurements were made from sunrise to sunset, at 2-hourly intervals on the abaxial surface of well-developed, sun-exposed leaves.

Experimental design and statistical analyses

In the nursery period, each treatment comprised 30 plants divided into three randomly distributed blocks of ten plants. In the transplanting and establishment period, each treatment comprised 15 plants divided into three randomly distributed blocks of five plants.

The data were analyzed by two-way analyses of variance using Statgraphics Plus 5.1 for Windows. Treatment means were separated with Duncan's multiple range test (P<0.05). Ratio and percentage data were arcsine-transformed before statistical analysis to ensure homogeneity of variance.

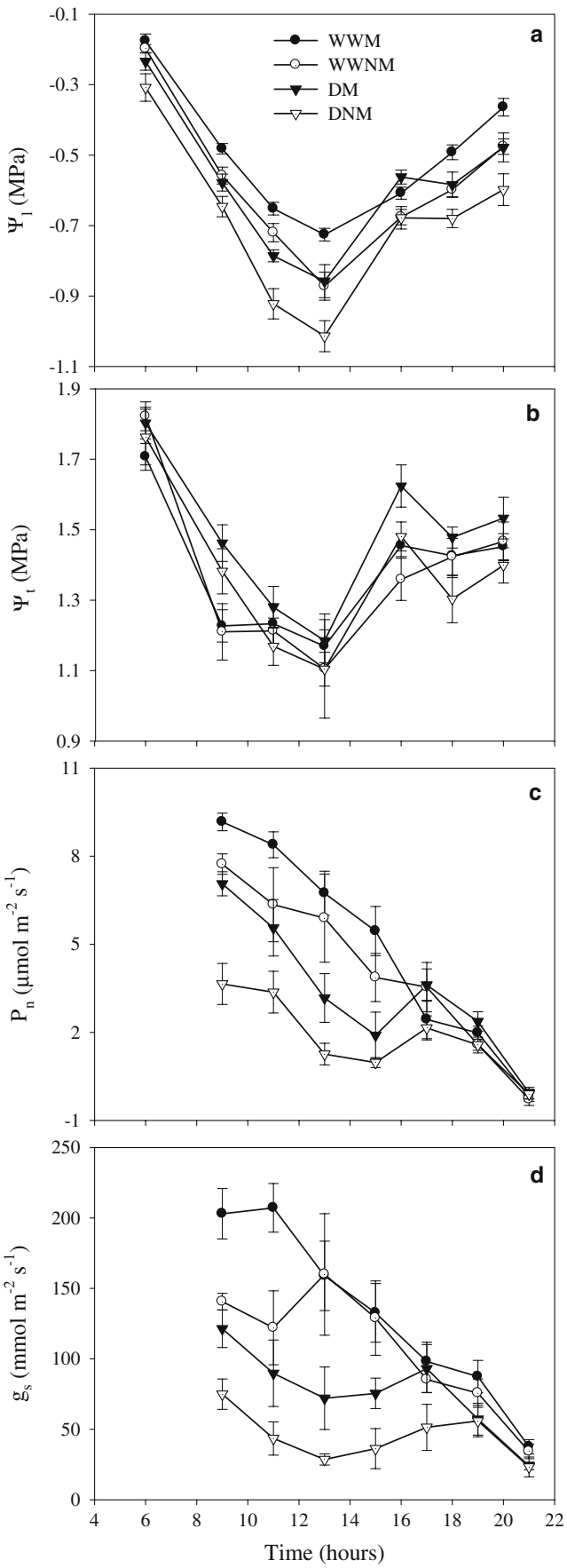

Fig. 2 Diurnal patterns of leaf water potential (Ψ_l; **a**), leaf turgor potential (Ψ_t; **b**), net photosynthetic (P_n; **c**), and stomatal conductance (g_s; **d**) at the end of the nursery period in *A. unedo* seedlings with well-watered irrigation mycorrhizal (*WWM*), well-watered irrigation non-mycorrhizal (*WWNM*), deficit irrigation mycorrhizal (*DM*), and deficit irrigation non-mycorrhizal (*DNM*) treatments. Values are means (n=5) and *vertical bars* indicate standard errors

Results

Nursery period

Mycorrhizal colonization reached values of 34% (Table 1), which neither the drought effect nor the interaction between drought and mycorrhizae influenced. The water deficit treatment significantly increased root dry weight (100%) and root/shoot ratio (59%), but did not affect leaf area or leaf number. While leaf area of mycorrhizal plants decreased, the root/shoot ratio increased. Growth parameters were statistically unaffected by the interaction of both factors (Table 1).

Changes in RCC and in color characteristics were unaffected by the water deficit. However, M plants, independently of soil water conditions, had higher chroma and lightness values (Table 2).

With respect to the mineral nutrition, the leaves of M plants showed higher concentrations of B, K, and Mn than non-mycorrhizal (NM) plants (Table 3), with increases of 17%, 13%, and 60%, respectively, but a lower Zn concentration (11%). The deficit irrigation induced lower Cu, K, and Na concentrations and higher S and Zn concentrations (Table 3). This effect was particularly pronounced in the K and Na concentrations, which decreased by 46% and 51%. The roots of M plants had higher Cu, K, Mg, Mn, and Zn concentrations than the NM plants (Table 3). Deficit irrigation decreased the Ca concentration by 13% and increased K and P by 126% and 39%, respectively, compared with the well-watered irrigation treatment (Table 3).

The soil water content decreased in deficit irrigation treatments, although the differences between M and NM plants were not evident until the end of the nursery period (June), coinciding with high temperatures (Fig. 1a).

The water deficit and mycorrhizal colonization caused significant differences in the water relations of the *A. unedo* plants (Figs. 2 and 3). The diurnal patterns of leaf water potential (Ψ_l) and turgor potential (Ψ_t) showed the highest values at early morning and the lowest at midday (Fig. 2a, b), after which the values recovered. Significant differences in Ψ_l levels were noted between treatments. The values of Ψ_l were, in general, always higher in the inoculated well-watered plants and lower in the non-inoculated water deficit plants. The well-watered NM plants and the water deficit M plants had similar values. The differences between treat-

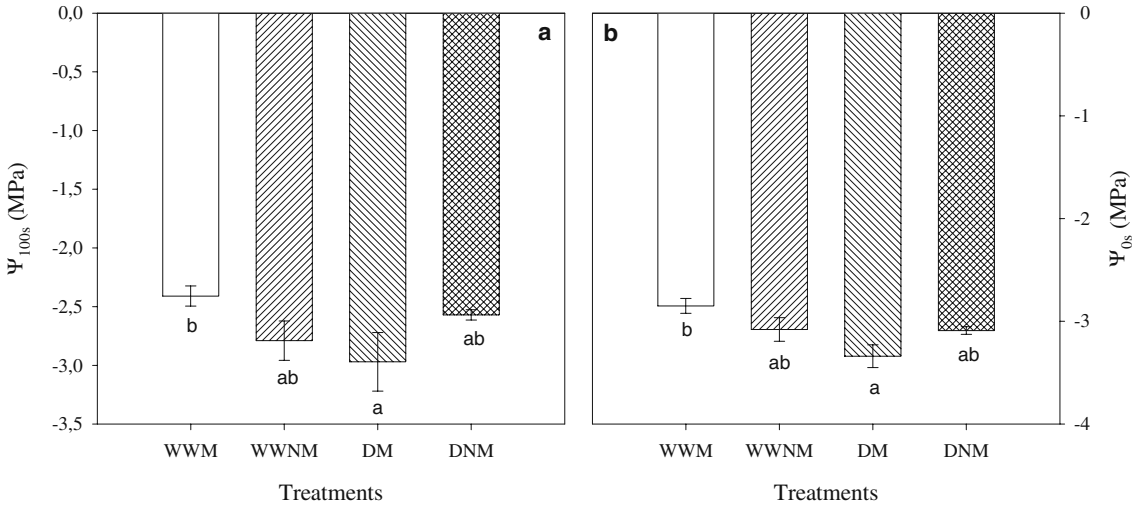

Fig. 3 Osmotic potential at full turgor (Ψ_{100s}; **a**) and osmotic potential at zero turgor (Ψ_{0s}; **b**) in *A. unedo* seedlings at the end of nursery period with well-watered irrigation mycorrhizal (*WWM*), well-watered irrigation non-mycorrhizal (*WWNM*), deficit irrigation mycorrhizal (*DM*), and deficit irrigation non-mycorrhizal (*DNM*) treatments. Each histogram represents the mean of five values and the *vertical bars* indicate standard errors

ments for Ψ_t values were lower than those observed for Ψ_l (Fig. 2b).

The highest values of g_s and P_n corresponded to the well-watered inoculated plants, as did Ψ_l. Both g_s and P_n decreased to a similar extent in both water deficit treatments, but more significantly in NM plants (Fig. 2c, d). In all treatments, a marked decrease in g_s and P_n values was observed in the afternoon. From this moment, there were no differences between treatments in these parameters.

Parameters derived from the pressure–volume curves are shown in Fig. 3. The leaf osmotic potential at full turgor (Ψ_{100s}) decreased in the water deficit inoculated plants (Fig. 3a), indicating leaf osmotic adjustment. In these same plants, leaf potential at zero turgor (Ψ_{0s}) was lower, showing values of −3.34 MPa (Fig. 3b). The bulk modulus of elasticity (ε) decreased from 12.58 to 9.76 MPa (data not shown) as a result of mycorrhizal colonization.

Transplanting and establishment period

After the nursery period, when all the plants had been transplanted and were well irrigated, the differences in the soil water content between irrigation treatments diminished, with similar recorded in all treatments (19%) except for the DM treatment, where it was about 15% (Fig. 1b). These soil conditions induced similar Ψ_l and Ψ_t diurnal patterns in all treatments. High and similar values of Ψ_l and Ψ_t at dawn and minimum values at midday were observed in all the treatments (Fig. 4a, b). The diurnal patterns of g_s and P_n (Fig. 4c, d) showed much greater variability, although inoculated plants tended to have the highest values. At the end of the experiment (establishment period), a significant decrease in the soil water content was observed and no differences between treatments were found (Fig. 1b) since irrigation was withheld from all the plants for 1 month. In general, Ψ_l and Ψ_t reflected the dehydration suffered during the field conditions (Fig. 5a, b), both showing lower values than during the transplanting period. Nevertheless, the highest values of Ψ_l during the first hours of the day were for the plants that had been exposed to deficit irrigation during nursery, concretely when combined with mycorrhizae (Fig. 5a). As the day progressed, lower values of Ψ_l and Ψ_t were seen coinciding with increasing vapor pressure deficit, after which these values were not recovered in the NM plants (Fig. 5a, b). In this period, although the values of both gas exchange parameters were lower than those for the transplanting period in all treatments, the lowest values of P_n and g_s were for the plants that had been well irrigated and non-inoculated during nursery period (Fig. 5c, d).

Discussion

Mycorrhizal colonization percentage in *A. unedo* (34%) was similar to those obtained in eucalyptus (28%; Mason et al. 2000) and in pine (22%; Walker 2001) with the same fungal species *P. tinctorius*. The host–fungus compatibility between *A. unedo* and *P. tinctorius* was previously demonstrated (Navarro et al. 2009). Moreover, deficit irrigation did not affect the mycorrhizal colonization percentage. Previous studies have shown that the percentage of roots colonized by mycorrhizal fungi is not affected by water deficit (Nelsen and Safir 1982; Davies et al. 1992; Bryla and Duniway 1997; Morte et al. 2000).

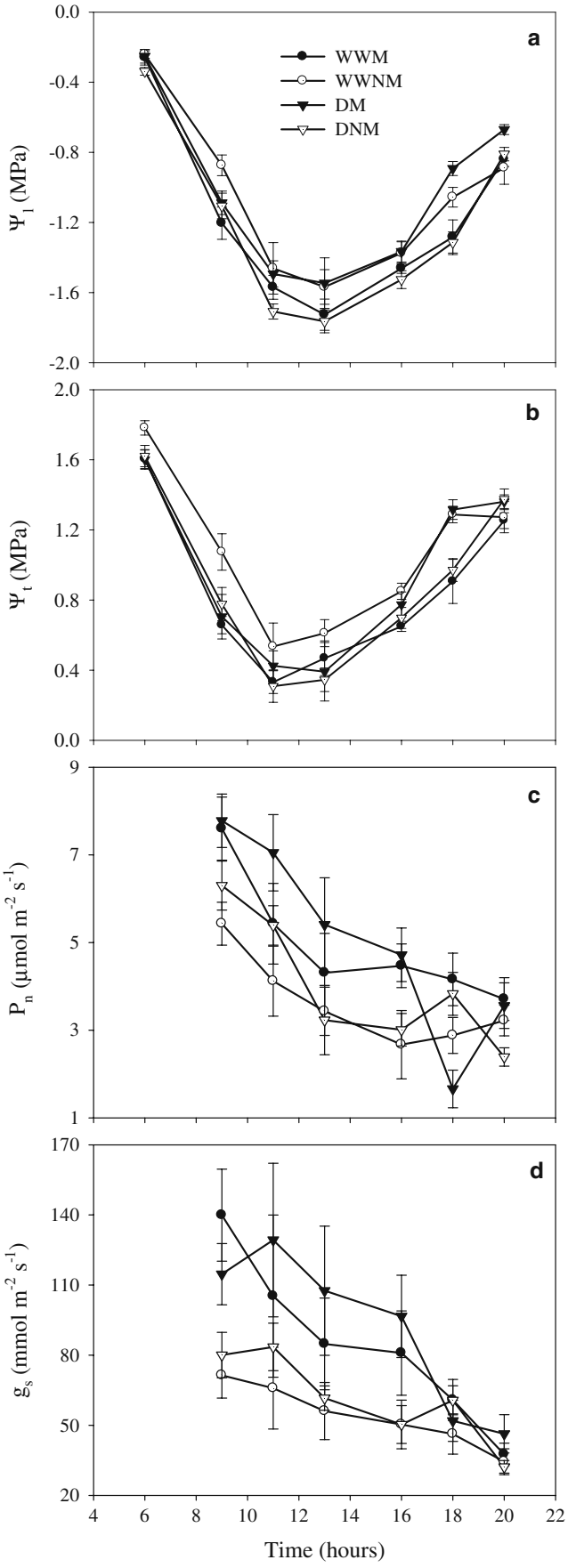

Fig. 4 Diurnal patterns of leaf water potential (Ψ_l; **a**), leaf turgor potential (Ψ_t; **b**), net photosynthetic (P_n; **c**), and stomatal conductance (g_s; **d**) after transplanting in *A. unedo* seedlings with well-watered irrigation mycorrhizal (*WWM*), well-watered irrigation non-mycorrhizal (*WWNM*), deficit irrigation mycorrhizal (*DM*), and deficit irrigation non-mycorrhizal (*DNM*) treatments. Values are means (n=5) and *vertical bars* indicate standard errors

The plants subjected to deficit irrigation showed a different distribution of biomass between the aerial part and the root system. Significant increases in root growth and the root/shoot ratio were seen in response to drought (Table 1), responses that have been observed by several authors in different species (Molyneux and Davies 1983; Khalil and Grace 1992; Palta and Gregory 1997). Under drought conditions, this behavior may improve the ability of a plant to obtain water and to acquire nutrients. The morphological characteristics of plant growth in nursery conditions may determine its ability to survive and grow following transplantation in different environmental conditions (Leskovar 1998). The morphology of *A. unedo* changed when combined with mycorrhizae (Table 1). *P. tinctorius* has been associated with significant growth reductions in the host plant (Tonkin et al. 1989; Eltrop and Marschner 1996), as was observed in the aerial part (leaf area) in our conditions. It also represents an important adaptation to drought since it reduces the transpiration demand (Sánchez-Blanco et al. 2004a). Also, mycorrhizal colonization induced morphological changes to the root system of the plants, which must be considered positive; this has been seen to affect the successful establishment of many ornamental woody species, particularly in adverse environmental conditions (Hooker et al. 1992; Maestre et al. 2002; Goicoechea et al. 2004). However, other authors observed that *P. tinctorius* has no effect in plant development in other host species (Svenson et al. 1991; Mason et al. 2000; Walker 2001).

Deficit irrigation did not cause changes in RCC or the color characteristics of *A. unedo* (Table 2), which is an important observation since it is used as an ornamental species. The leaf color of M plants was lighter (increased lightness) and gained in saturation or vividness (increased chroma) compared with the NM plants (Table 2), thus improving their ornamental quality. This suggests that the benefit granted by *P. tinctorius* inoculation would affect aerial part quality rather than quantity.

Changes in plant growth were associated with root colonization by *P. tinctorius*, as a response that has frequently been correlated with increases in phosphorus nutrition of host plants (Rousseau et al. 1992; Burgess et al. 1993; Thomson et al. 1994), although this was not observed in our experiment. In contrast, increases in the Mn, K, and Zn concentrations of roots and leaves were detected (Table 3). *P. tinctorius* therefore seems to participate in

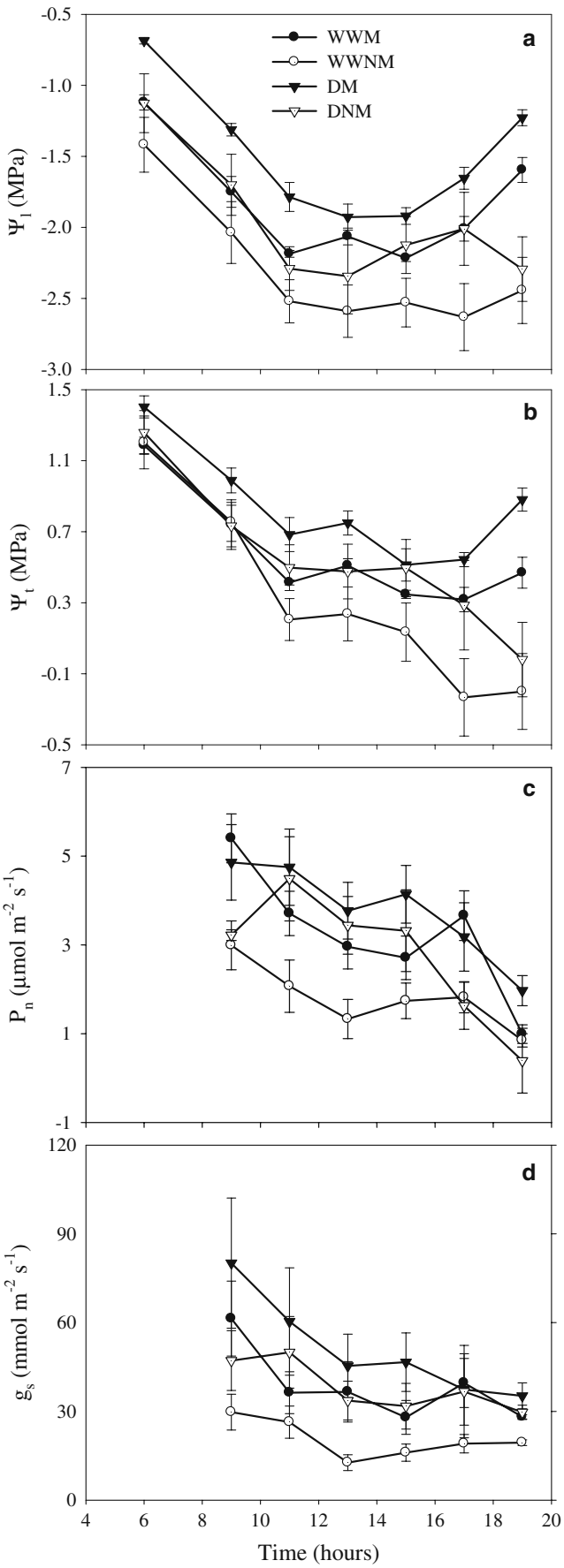

Fig. 5 Diurnal patterns of leaf water potential (Ψ_l; **a**), leaf turgor potential (Ψ_t; **b**), net photosynthetic (P_n; **c**), and stomatal conductance (g_s; **d**) during establishment in *A. unedo* seedlings with well-watered irrigation mycorrhizal (*WWM*), well-watered irrigation non-mycorrhizal (*WWNM*), deficit irrigation mycorrhizal (*DM*), and deficit irrigation non-mycorrhizal (*DNM*) treatments. Values are means ($n=5$) and *vertical bars* indicate standard errors

mineral transformations that increase the availability of some nutrients in the soil (Cairney and Ashford 1989). There is direct evidence that *P. tinctorius* increased Mn accumulation in *Pinus virginiata* plants (Miller and Rudolph 1986) and may displace K from the soil and make it more available to the plants, probably through secretion of oxalate (Paris et al. 1995, 1996). Increases in K and Mg (Hall 1978; Azcón and Ocampo 1981), Ca (Pai et al. 1994), Cu, and Mn (Sylvia et al. 1993) have also been observed in arbuscular mycorrhizal plants. Indirectly, these increments may have an impact on host plant drought resistance.

Under drought conditions, the availability of nutrients for the plant is limited, fundamentally due to the low mobility of mineral ions in the soil (Subramanian 1997). This was seen from the Cu, K, and Na leaf concentrations, which may participate in the enzymatic processes involved in amino acid transformation in plants and improve the protection of plants when they are subjected to unfavorable environmental situations. The contrary occurred for Zn and S concentrations.

The K concentration of plants was increased by the effect of mycorrhizal colonization (Table 3). Potassium plays an important role in the drought tolerance of plants and is related to stomatal movement in response to the changes produced in leaf water status (Premachandra et al. 1993). Ruíz-Lozano et al. (1995) observed that the protection of M plants against water stress is partially related to K uptake. The significant increase in P levels observed in the roots of non-mycorrhizal plants subjected to deficit irrigation should be considered as a positive effect for maintaining root growth and prolonging the period of root development (Li et al. 2001).

Osmotic adjustment plays an important role in the response of plant to hardening (Bañón et al. 2006). This mechanism was developed by *A. unedo* in response to a combination of mycorrhiza and water deficit conditions and these plants therefore presented higher leaf turgor values (Fig. 3b). This was accompanied by lower Ψ_{0s} values, which indicates that the turgor loss point is reached at lower leaf water potential. These same plants exhibited Ψ_l values that were higher throughout the day than in NM plants under water deficit conditions, which were also observed by Subramanian et al. (1995). The higher Ψ_l values in mycorrhizal plants may be due to a higher water uptake capacity by the roots as a result of better hydraulic conductivity in the root system (Sánchez-Blanco et al. 2004b). This was also reported in this same species by Navarro et al. (2009).

Turgor can also be maintained by changes in the elasticity of the cellular walls (Radin 1983). In this sense, mycorrhizal colonization decreased the bulk modulus of elasticity in the *A. unedo* plants, independently of the substrate water conditions (data not shown), and seems to induce an increase in the elasticity of the cell walls. Similar results but in alfalfa plants inoculated with *Glomus fasciculatum* and/or *Rhizobium meliloti* were found by Goicoechea et al. (1997).

As many other Mediterranean shrubs, *A. unedo* shows a conservative strategy in the use of water (Tenhunen et al. 1990), mainly based on the avoidance of drought stress through reducing stomatal conductance, as was observed in water deficit (M and NM) plants (Fig. 2d). M plants, in general, had higher gas exchange levels that NM plants. This effect has also been observed in other species (Augé et al. 1992; Ebel et al. 1997; Morte et al. 2001) and could be related to the better water status since it has been associated with altered root or whole plant hydraulic conductivity (Allen et al. 1981). Besides, mycorrhizal colonization induced plants subjected to the deficit irrigation to show higher stomatal conductance than NM plants. This effect has been reported in cultivated pot plants and in field conditions (Thomas et al. 1976; Ackerson 1980; Augé et al. 1986; Sánchez-Blanco et al. 2004b).

After the plants had left the nursery and after rewatering (transplanting period), all plant showed a similar water status, although the Ψ_l values at midday were lower than in the nursery period due the higher evaporative demand in the transplanting period. During the drought period (establishment), the mycorrhizal plants showed a significantly better water status and the water deficit treatment produced a g_s above that of the well-watered condition (Fig. 5d), that is, they showed a higher degree of stomatal opening than non-hardened plants (Spence et al. 1986). The parallel behavior of P_n and g_s during the experiment explains why the reduction in photosynthetic rates under drought has been mainly attributed to stomatal conductance reductions (De Herralde et al. 1998).

Conclusions

A. unedo plants responded to deficit irrigation through a water conservation strategy, which involved reducing stomatal conductance and the increasing root/shoot ratio. The mycorrhizal symbiosis had a beneficial effect on plant water status and increased photosynthetic activity.

The combined effect of mycorrhizal inoculation and drought in the nursery produced a hardening process in *A. unedo* seedlings through osmotic adjustment and stomatal regulation. This improved the water status of plants (higher leaf water and turgor potential) and induced a lower turgor loss point, which could provide better resistance to drought and stress conditions following planting.

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