Photosynthetic Response, Biomass Distribution and Water Status Changes in *Rhamnus alaternus* Plants during Drought

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Abstract

***Rhamnus alaternus* is used as an ornamental plant in Mediterranean regions. The objective of this research was to analyse the ability of *R. alaternus* to overcome water stress in terms of adjusting its physiology and morphology. For this, potted plants were grown in a greenhouse and subjected to water stress by reducing irrigation water by 50% compared with the control (irrigated to container capacity). The water stress produced the smallest plants at the end of the experiment. After ten months of drought, the leaf area, leaf number, total dry biomass and height had decreased, although the root/shoot ratio and succulence were not affected. Total root length decreased with water stress, a reduction observed for all sizes of root. Biomass distribution in the different parts of the plant (root, shoot and leaf) was similar in both treatments. An improvement in water use efficiency was seen in water deficit plants. Throughout the experiment, leaf color, chlorophyll, fluorescence (F_v/F_m) and ion leakage (membrane damage) were not affected by deficit treatment. Water stress lowered the predawn and midday leaf water potentials, although the decrease in the latter parameter was more affected by the climatic conditions. It is concluded that water deficit leads to morphological changes in this species as an adaptation to Mediterranean conditions.**

INTRODUCTION

Low water availability is considered the main environmental factor limiting plant´ growth and productivity in semiarid areas (Gulías et al., 2002). In these areas, as a result of climate changes, Mediterranean species will have to face more severe drought conditions due to increase temperature and water deficit (Houghton et al., 2001). Although growth patterns of Mediterranean species are well adapted to these environmental conditions (De Herralde et al., 1998), they may suffer considerable stress during the dry season (Lo Gullo and Salleo, 1988), which might affect the survival and distribution of the species. Consequently, a prerequisite for any plant species selected for use in these areas is that it should show ecophysiological traits that confer drought resistance through the optimization of water use.

Rhamnus alaternus is an evergreen sclerophyll shrub distributed throughout the Mediterranean area, where it is well adapted to high temperature, high solar radiation and vapor pressure deficit (VPD) (Martínez-Sánchez et al., 2008), it has a high ornamental value because of its bright green leathery foliage, which, with its small red fruits, creates a very pleasing effect. It is also well adapted to growing in pots, which is of interest for landscaping and gardening. The purpose of this work was to study physiological patterns in *R. alaternus* plants exposed to water deficit. This was achieved by measuring the changes in growth, ornamental characteristics, water relations, gas exchange, and photosynthetic efficiency developed by this species in order to help it adapt to conditions of drought stress.

MATERIALS AND METHODS

Plant Material, Treatments and Experimental Conditions

Seedlings (50) of 2-year-old *Rhamnus alaternus* were grown in 3.6 L plastic pots filled with a 2:1:1 (v/v/v) mixture of coconut fibre:black peat:perlite, amended with 2 g Osmocote® Plus (14:13:13 N,P,K plus microelements) per litre of substrate. The experiment was conducted in 2007 at Santomera (Murcia, Spain) in a plastic greenhouse equipped with a cooling system. After eight weeks in the greenhouse, plants were subjected to two irrigation treatments (25 plants per treatment) using a computer-controlled drip irrigation system from January-September 2007. The irrigation treatments consisted of watering to 100% water holding capacity [leaching 15% (v/v) of the applied water] (Control, C), or applying 50% of the control-level of irrigation water (Water stress, WS). One drip nozzle, delivering 2 L h^{-1} per pot, was connected to two spaghetti tubes (one on each side of every pot) and the duration of each irrigation episode was used to vary the amount of water applied, which depended on the season and on climatic conditions.

Biomass and Ornamental Measurements

At the end of the experimental period, the substrate was gently washed from the roots of eight plants per treatment and each plant was divided separately into shoots (i.e., leaves and stems) and roots. These were then oven-dried at 80ºC, until they reached a constant weight, to measure their respective dry weight (DW). Succulence, leaf numbers, and leaf areas (cm^2), using a leaf area meter (Delta-T Devices Ltd., Cambridge, UK), were determined in the same eight plants per treatment. Also, plant height was determined in 20 plants per treatment. The roots were cleaned by low pressure water applied through a flat nozzle. The cleaned root systems were then placed in a metacrylate tray coupled to a double scanner connected to a computer with a Root System Analyser (Winrhizo LA 1600 Regent Inc., USA). The root systems were put in an oven to dry immediately after the root length measurements.

Leaf color was measured using a Minolta CR-10 colorimeter, which provided the color coordinates of hue angle (HUE), chroma (C) and lightness (L). Three leaves were measured on each plant, and eight plants were studied per treatment. The relative chlorophyll content (RCC) was measured using a Minolta SPAD-502 chlorophyll meter at the midpoint of each mature leaf using the same leaves as were used for the colorimetric measurements. The rates of passive ion leakage from stressed sensitive plant tissue were used as a measure of alterations in membrane permeability, (Lafuente et al., 1991).

Water Status and Chlorophyll Fluorescence Measurements

Seasonal changes in leaf water potential at predawn (Ψ_{pd}) and at midday (Ψ_{md}), leaf osmotic potential at full turgor (Ψ_{100s}), photosynthesis (P_n) and stomatal conductance (g_s) were measured in five plants per treatment. Leaf water potential (Ψ_l) was estimated according to Scholander et al. (1965), using a pressure chamber (Model 3000; Soil Moisture Equipment Co., Santa Barbara, CA, USA) in which leaves were placed in the chamber within 20 s of collection and pressurised at a rate of 0.02 MPa s^{-1} (Turner, 1988). Leaf osmotic potential at full turgor (Ψ_{100s}) was estimated according to Gucci et al. (1991), using excised leaves with their petioles placed in distilled water overnight to reach full saturation. Leaves from the Ψ_{100s} measurements were frozen in liquid nitrogen (-196°C) and stored at -30°C. After thawing, the osmotic potential was measured in the extracted sap using a WESCOR 5520 vapour pressure osmometer (Wescor Inc., Logan, UT, USA). Leaf stomatal conductance (g_s) and net photosynthetic rate (P_n) were determined at midday in attached leaves using a gas exchange system (LI-6400; LI-COR Inc., Lincoln, NE, USA). Water-use efficiency (WUE) was calculated at the end of the experiment by dividing the increase in the total dry weight by the water used (total dry weight (g)/water (L)). The values of chlorophyll fluorescence were taken according to

Camejo et al. (2005). The values of F_v/F_m were read directly in the fluorometer (OS-30 OptiScience Inc., Tyngsboro).

Statistical Analysis

The experiment consisted of a randomized block design, where a single block referred to a single treatment. The data were analysed by one-way ANOVA using Statgraphics Plus for Windows 5.1 software. Ratio and percentage data were subjected to an arcsine square-root transformation before statistical analysis to ensure homogeneity of variance. Treatment means were separated with Duncan's Multiple Range Test ($P \leq 0.05$).

RESULTS AND DISCUSSION

Growth and Plant Quality

At the end of the experiment, water deficit was seen to have significantly altered *R. alaternus* growth although the changes differed depending on the parameters studied (Table 1). Drought induced a significant decrease in total plant biomass (dry weight) of 44%, in height of 23% and in total leaf area of 51%. Also, leaf number was modified in plants subjected to water stress. As regards biomass distribution in the root, stems and leaves, no differences were found between both treatments; however, the greatest accumulation of dry matter in relation to total plant dry matter was seen in the roots both in the control and in stressed plants. Total root length decreased with water stress (59%), a reduction observed in all sizes of root, particularly in those with a diameter greater than 2 mm (71% of reduction) (Table 2). This behaviour has also been described by Bañón et al. (2003) in the same species. Independently of root length, the root distribution in each root system was not modified by the irrigation. Growth reductions as a result of water deficit have been reported widely in many ornamental species (Sánchez-Blanco et al., 2002; Franco et al., 2006), but the intensity of the plant response can vary, depending on the species and the level and duration of the stress (Cameron et al., 1999). Stanhill and Albers (1974) suggested that the volume of water used by plants is generally related to the incoming radiation and air movement and, in this sense, the reduction of aerial part growth observed in our stressed plants may be considered an adaptive response to limit the loss of water via transpiration and to contribute to the water economy of the plant (De Herralde et al., 1998).

During the experimental period, leaf color parameters (L, C, HUE), RCC and membrane damage, assessed by ion leakage, were not affected by the water deficit treatment (Fig. 1), suggesting that plants can cope with water shortage without losing their ornamental value (Sánchez-Blanco et al., 2009).

Water Relationships

Water use efficiency (WUE) increased in the stressed plants (0.1054 g L^{-1} and 0.1556 g L^{-1} for control and stressed plants, respectively), an observation that has been associated with the application of deficit irrigation regimes (Cameron et al., 2006; Álvarez et al., 2009) to maintain leaf water status of these species (Hessini et al., 2008).

During the experiment, significant differences in Ψ_l levels at predawn and at midday were noted between treatments, although the influence of environmental factors was higher in stressed plants (Fig. 2A). At the end of experimental period, the values of Ψ_{md} decreased (-1.49 and -1.91 MPa in control and WS) as the evaporative demand of the atmosphere increased (in July and August). No differences in Ψ_{100s} were found between treatments (Fig. 2B), suggesting that osmotic adjustment occurred in the deficit irrigation treatment. According to Tognetti et al. (2000), many Mediterranean shrubs show a low degree of active osmotic adjustment and/or, slight adjustments during drought resistance.

Photosynthetic Parameters

The chlorophyll fluorescence (F_v/F_m) values of R. *alaternus* remained constant, irrespective of drought, at approx. 0.8 in both treatments (Fig. 2C), indicating the lack of drought-induced damage of the photochemistry PSII, as has been reported for many species (Cornic, 1994; Munné-Bosch et al., 2009). According to Corlett and Choudhary (1993), the photochemical efficiency measured as F_v/F_m is only affected when the water stress in horticultural species is very severe. Drought caused decreases in stomatal conductance (g_s) and in net photosynthesis (P_n), (Fig. 3).

We conclude that *R. alaternus* shows several traits that limit plant biomass production under water deficit conditions. Also, the stomatal response may enable to delay plant dehydratation. However, the species' ornamental value remains inaltered.

ACKNOWLEDGEMENTS

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Literature Cited

Álvarez, S., Navarro, A., Bañón, S. and Sánchez-Blanco, M.J. 2009. Regulated deficit irrigation in potted *dianthus* plants: effects of severe and moderate water stress on growth and physiological responses. Sci. Hort. 122:579-585.

Bañón, S., Ochoa, J., Franco, J.A., Sánchez-Blanco, M.J. and Alarcón, J.J. 2003. Influence of water deficit and low humidity in the nursery on survival of *Rhamnus alaternus* seedling following planting. J. Hort. Sci. Biotechnol. 78:518-522.

Camejo, D., Rodríguez, P., Morales, M.A., Dell'Amico, J., Torrecillas, A. and Alarcón, J.J. 2005. High temperature effects on photosynthetic activity of two tomato cultivars with different heat susceptibility. J. Plant Physiol. 162:281-289.

Cameron, R.W.F., Harrison-Murray, R.S. and Scott, M.A. 1999. The use of controlled water stress to manipulate growth of container-grown *Rhododendron* cv. Hoppy. J. Hort. Sci. Biotechnol. 74:161-169.

Cameron, R.W.F., Harrison-Murray, R.S., Atkinson, C.J. and Judd, H.L. 2006. Regulated deficit irrigation: a means to control growth in woody ornamentals. J. Hort. Sci. Biotechnol. 81:435-443.

Corlett, J.E. and Choudhary, R. 1993. Chlorophyll fluorescence for water deficit detection in horticultural crop? Acta Hort. 335:241-244.

Cornic, G. 1994. Drought stress and high light effects on leaf photosynthesis. p.297-313. In: N.R. Baker and J.R. Bowyer (eds.), Photoinhibition of photosynthesis: from molecular mechanisms to the field. Bios Scientific Publishers Limited, Oxford, U.K.

De Herralde, F., Biel, C., Savé, R., Morales, M.A., Torrecillas, A., Alarcón, J.J. and Sánchez-Blanco, M.J. 1998. Effect of water and salt stresses on the growth, gas exchange and water relations in *Argyranthemum coronopifolium* plants. Plant Sci. 139:9-17.

Franco, J.A., Martínez-Sánchez, J.J., Fernández, J.A. and Bañón, S. 2006. Selection and nursery production of ornamental plants for landscaping and xerogardening in semi-arid environments. J. Hort. Sci. Biotechnol. 81:3-17

Gucci, R., Xiloyannis, C. and Flore, J.A. 1991. Gas exchange parameters water relations and carbohydrate partitioning in leaves of field-grown *Prunus domestica* following fruit removal. Physiol. Plant. 83:497-505.

Gulías, J., Flexas, J., Abadía, A. and Medrano, H. 2002. Photosynthetic responses to water deficit in six Mediterranean sclerophyll species: possible factors explaining the declining distribution of *Rhamnus ludovici-salvatoris*, an endemic Balearic species. Tree Physiol. 22:687-697.

Hessini, K., Ghandour, M., Albouchi, A.A., Soltani Koyro, H.W. and Abdelly, C. 2008. Biomass production, photosynthesis, and leaf water relations of Spartina alterniflora under moderate water stress, J. Plant Res. 121:311-318.

Houghton, J.T., Ding, Y., Griggs, D.J., Noguer, M., Van der Linden, P.J., Dai, X., Maskell, K. and Johnson, C.A. 2001. Climate change 2001: the scientific basis. Third assess-

ment report of Intergovernmental Panel on Climate Change. Cambridge, Cambridge University Press.
Lafuente, M.T., Belver, A., Guye, M.G. and Saltveit Jr., M.E. 1991. Effect of the temperature conditioning on chilling injury of cucumber cotyledons. Plant Physiol. 95:443-449.
Lo Gullo, M.A. and Salleo, S. 1988. Different strategies of drought resistance in three Mediterranean sclerophyllous trees growing in the same environmental conditions. New Phytologist 108:267-276.
Martínez-Sánchez, J.J., Franco, J.A., Vicente, M.J., Muñoz, M., Bañón, S., Conesa, E., Fernández, J.A., Valdés, R., Miralles, J., Ochoa, J., Aguado, M., Esteva, J., López, J. and Aznar, L. 2008. Especies silvestres mediterráneas con valor ornamental. Selección, producción viverística y utilización en jardinería. Consejería de Agricultura y Agua, Región de Murcia. p.224.
Munné-Bosch, S., Falara, V., Pateraki, I., López-Carbonell, M., Cela, J. and Kanellis, A.K. 2009. Physiological and molecular responses of the isoprenoid biosynthetic pathway in a drought-resistant Mediterranean shrub, *Cistus creticus* exposed to water deficit. J. Plant Physiol. 166:136-145.
Sánchez-Blanco, M.J., Rodríguez, P., Morales, M.A., Ortuño, M.F. and Torrecillas, A. 2002. Comparative growth and water relation of *Cistus albidus* and *Cistus monspeliensis* plants during water deficit conditions and recovery. Plant Sci. 162:107-113.
Sánchez-Blanco, M.J., Álvarez, S., Navarro, A. and Bañón, S. 2009. Changes in leaf water relations, gas exchange, growth and flowering quality in potted geranium plants irrigated with different water regimes. J. Plant Physiol. 166:467-476.
Scholander, P.F., Hammel, H.T., Bradstreet, E.D. and Hemingsen, E.A. 1965. Sap pressure in vascular plants. Scientia 148:339-346.
Stanhill, G. and Albers, J.S. 1974. Solar radiation and water loss from greenhouse roses. J. Amer. Soc. Hort. Sci. 99:107-110.
Tognetti, R., Raschi, A. and Jones, M.B. 2000. Seasonal patterns of tissue water relations in three Mediterranean shrubs co-occurring at a natural CO_2 spring. Plant Cell Environ. 23:1341-1351.
Turner, N.C. 1988. Measurement of plant water status by the pressure chamber technique. Irrig. Sci. 9:289-308.

Tables

Table 1. Influence of irrigation treatment on growth, water use efficiency (WUE) and ion leakage in *R. alaternus* plants at the end of experiment.

Parameters	Treatments		P
	C	WS	
Plant height (cm)	16.05±0.71 b[1]	12.37±1.05 a	**
Total dry weight (g plant^{-1})	9.00±0.77 b	5.07±0.42 a	***
Root/shoot ratio	1.11±0.14 a	1.01±0.14 a	ns[2]
Leaf succulence	2.39±0.08 a	2.37±0.03 a	ns
Leaf number	268±42 b	149±15 a	*
Leaf area (cm^2)	165.4±14.7 b	81.3±11.6 a	***
Leaf size (mm^2)	65.96±5.21 a	54.97±4.48 a	ns
Leaf part (%)	20.2±2.9 a	24.5±3.4 a	ns
Stem part (%)	20.8±1.1 a	26.9±3.5 a	ns
Root part (%)	51.0±3.4 a	48.6±3.4 a	ns
WUE (g_{DW} L^{-1})	0.105±0.009 a	0.156±0.013 b	**
Ion leakage (%)	30.117±1.557	31.563±0.993	ns

[1] Means within a row without a common letter are significantly different by Duncan $_{0.05}$ test.

[2] ns, not significant according to Duncan test (P>0.05).

Table 2. Influence of irrigation treatment on root morphology in *R. alaternus* plants at the end of experiment.

Parameters	Treatments		P
	C	WS	
Root total length (cm)	5359±730 b[1]	2184±262 a	**
RL<0.5 mm ϕ (cm)	3098±435 b	1372±186 a	**
RL 0.5-2.0 mm ϕ (cm)	2026±314 b	735±75 a	**
RL >2.0 mm ϕ (cm)	234.4±34.3 b	69.2±15.3 a	**

[1] Means within a row without a common letter are significantly different by Duncan $_{0.05}$ test.

Figures

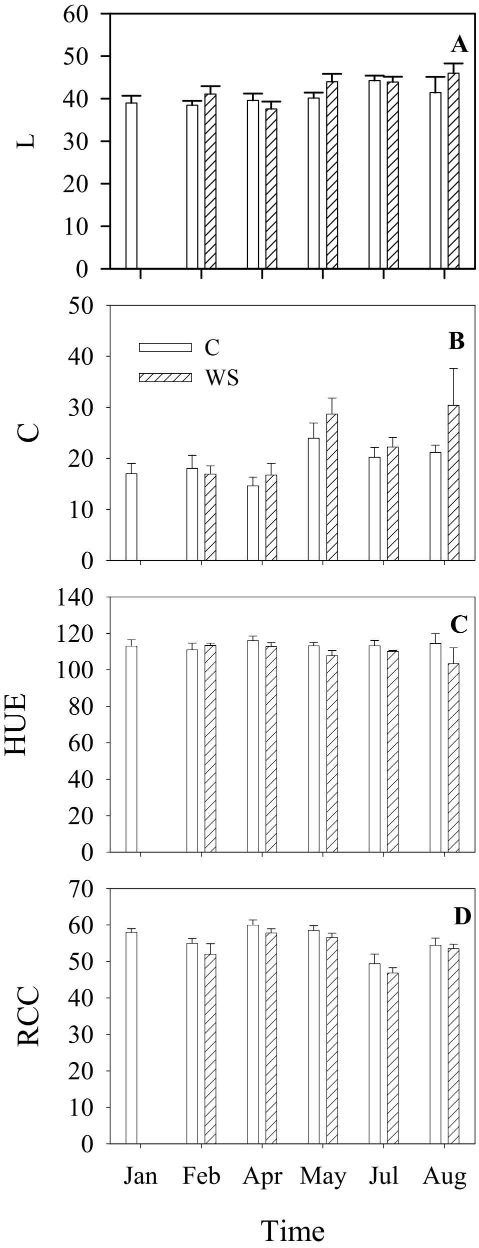

Fig. 1. Influence of irrigation treatment on leaf color parameters [A (lightness), B (chroma), C (angle)] and relative chlorophyll content (D) in *R. alaternus* plants under different irrigation treatments.

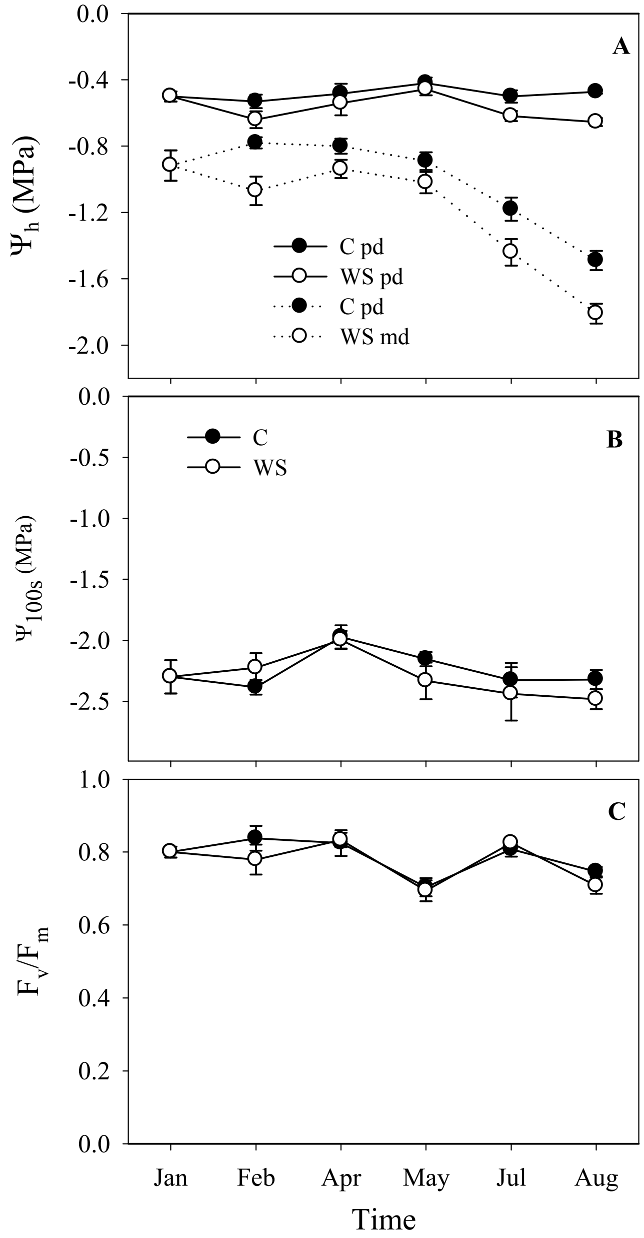

Fig. 2. Seasonal patterns of leaf water potential at predawn (Ψ_{pd}) and midday (Ψ_{md}) (A), leaf osmotic potential al full turgor at midday (Ψ_{100s}; B) and chlorophyll fluorescence (F_v/F_m; C) in *R. alaternus* plants under different irrigation treatments.

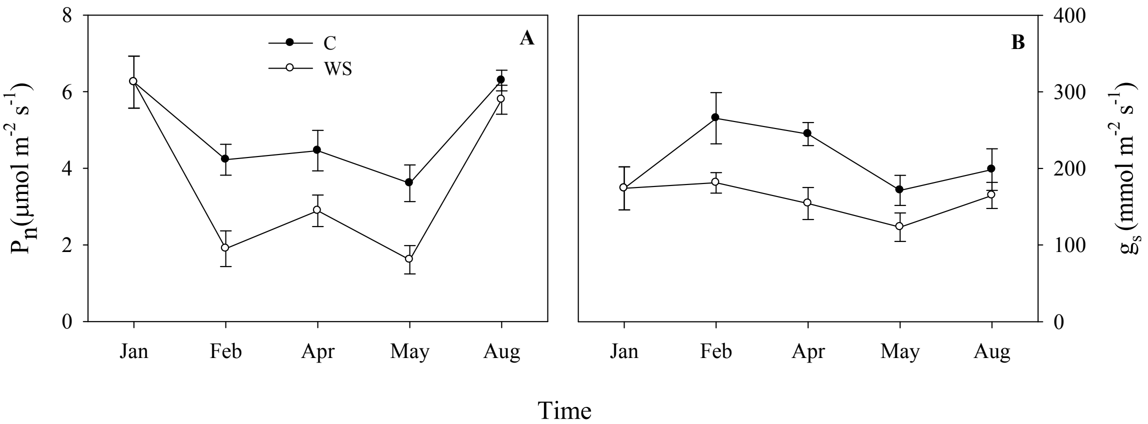

Fig. 3. Seasonal pattern of photosynthesis (P_n; A) and stomatal conductance (g_s; B) in *R. alaternus* plants under different irrigation treatments.