Physiological mechanisms involved in the recovery of Euonymus and Laurustinus subjected to saline waters

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Abstract

The scarcity of water has frequently led to saline water being reused for the irrigation of ornamental shrubs. However, before the use of such waters can be expanded, the salt tolerance and other characteristics of the ornamentals involved, need to be considered along with their capacity to recover after salinity exposure. For this reason**,** *Euonymus japonica* (euonymus) and *Viburnum tinus* (laurustinus) plants were submitted for twenty weeks to three irrigation treatments applied at 100% water holding capacity: Control (EC < 0.9 dS m^{-1}); NaCl solution, NaCl (EC: 4 dS m^{-1}); and wastewater, WW (EC: 4 dS m^{-1}). This was followed by a recovery period of eight weeks, when all the plants were watered in the control irrigation conditions. The results showed that biomass, leaf number and total leaf area of plants subjected to the saline treatments were lower than in the control at the end of both periods in both species. However, after recovery, only euonymus showed lower growth parameters than those observed in the saline period. The highest Na^+ and Cl^- concentrations were observed in saline plants at the end of saline period for both species, and were higher in shoots than in roots. The opposite was observed for the K^+/Na^+ and the $Ca^{2+/}Na^+$ ratios. In Laurustinus, the ?$_{stem}$ did not diminish in the wastewater-irrigated plants with respect to the control, maintaining osmotic adjustment and a high ?$_{t}$, even after recovery, whereas in euonymus this did not occur at the end of recovery period. In both species the P_n and g_s were similarly reduced during the saline exposure period. However, the recovery of gas exchange in laurustinus irrigated with wastewater might be closely related to the better water status of these plants. Although the aesthetic value and growth decreased in the plants of both species, the chemical properties of the waters applied had different effects in each case, especially as regards the capacity to recover from salinity. These results underline the

importance to studying the physiological mechanisms involved in the recovery of plants.

Keywords: Ornamental plants; Wastewater; Salinity; Gas exchange; Physiology; Nutrient content.

Abbreviations: DW, dry weight; EC, electrical conductivity; g_s, stomatal conductance; P, significance level; P_n, net photosynthesis; RH, relative humidity; Ψ_o, osmotic potential; Ψ_{stem}, stem water potential; Ψ_t, turgor potential; Ψ_{100s}, osmotic potential at full turgor.

1. Introduction

The rising demands for good quality water for domestic and industrial uses in countries with developed economies have already led to the need to re-use marginal water sources. In fact, the application of reclaimed water is a common practise in many areas of the world, especially in arid and semiarid environments where water is a limiting factor (Yermiyahu et al., 2008). However, reclaimed wastewater is usually of poor quality for plant growth, with higher concentrations of salts than fresh water. Several studies have shown the environmental and agronomical interest of using low quality water for irrigation in different crops (Parsons et al., 2001, Pedrero and Alarcón, 2009; Pedrero et al., 2010) but little is known about the requirements to maintain healthy growth and acceptable quality in ornamental plants (Grant et al., 2009; Bañón et al., 2011) irrigated with saline water.

A high concentration of salts in the irrigation water causes water stress due to the decrease in the water potential of the root medium (osmotic effect). In addition, certain ions such Na^+ and Cl^- can be accumulated in the vegetable fabric, where they reach toxic levels (ionic toxicity) and induce nutritional imbalances with the elements that are necessary for the correct functioning of the plant. In some cases, marginal waters also contain high boron concentrations (Feigin et al., 1991), and significant quantities of toxic metals (Barar et al., 2000; Yadav et al., 2002). The weathering of minerals can even increase salt and boron concentrations in the soil solution (Bressler et al., 1982; Keren and Bingham, 1985).

Therefore, salinity affects the establishment, growth and development of plants, leading to a great loss in productivity (Katerji et al., 2003; Mathur et al., 2007), and may also affect the ornamental quality of cultivated and wild species (Morales et al., 2001). To minimize crop loss, it is necessary to identify new irrigation management strategies, such as increased leaching, to maintain high and constant substrate humidity (Bañón et al., 2011), or to use salt-tolerant plants and develop salt-tolerant crops through breeding programmes. One solution to improving the agronomic quality of the reused water could be to blend it with well water (Chaiprapat and Sdoodee, 2007; Gori et al., 2008; Bañón et al., 2011).

It is known that some vegetable species growing under salinity conditions develop different strategies to avoid or mitigate the damage produced by the salts present in the soil and irrigation water. However, salt tolerance varies considerably among the different genotypes of ornamentals used in landscaping (Wu and Dodge, 2005). Therefore, it is necessary to study the resistance or sensitivity of vegetable species since each might develop a particular physiological mechanism to survive. Whatever the case, there are many ornamental species which can tolerate a certain degree of saline stress (Feitosa et al., 2005; Cassaniti et al., 2012). In the case of landscape plants, maximum growth is not always essential and indeed excessive shoot vigor is undesirable, so using an alternative water source (saline water) may even be advantageous to obtain more compact plants without visual damage (Álvarez, et al., 2012; Cassaniti et al., 2012). Indeed, visual quality may or may not be related to biomass production and photosynthetic responses (Zollinger et al., 2007).

Another way to determine the degree of tolerance to salinity could be to study the plant response to a recovery period after saline stress. Cycles of stress and recovery from stress are prevalent processes occurring under natural conditions during different seasons and in different agricultural practices, including irrigation (Vinocur and Altman, 2005). Recovery from water stress is generally characterized by an increase in the leaf water potential followed by a recovery of stomatal conductance, which may be associated with the re- establishment of hormonal balances. However, the physiological mechanisms involved in the recovery of plants subjected to high salinity are poorly understood. It is known that the time period required for photosynthetic recovery after salinity stress is generally much longer (up to 15-20 days) than that following drought (Chaves et al., 2011). Moreover, the intensity and or duration of stresses have particular effects on both the velocity and the extent of recovery after stress relief (Chaves et al., 2009). Also, the differences in salinity tolerance between the species could affect recovery after saline irrigation – a theme that has been hardly studied.

Viburnum tinus L. (laurustinus) is a perennial shrub, autochthonous to the Iberian Peninsula, while *Euonymus japonica* Thunb. (euonymus) is a popular shrub from Japan, well adapted to coastal zones with high concentrations of salt accumulated in the soil. Both species are widely used as ornamental plants.

The aim of this work was to study the response of laurustinus and euonymus to different quality irrigation waters, including reclaimed water and a NaCl solution, both with an EC of 4 dS m^{-1}. Plant growth and quality, water relations, photosynthetic activity and nutrient content were studied to ascertain any differences in the ability of both species to adapt to salinity and to recover from the same. In addition, we wanted to know which of the two species could be cultivated using reclaimed water or saline water, taking into account the origin and characteristics of each species. The results will provide more information on the advantages and disadvantages of using this type of water in shrub species of ornamental and landscape interest.

2. Material and methods

2.1. Plant material and growth conditions

Euonymus plants with an initial height of 15 cm and laurustinus with an initial height of 20 cm, were transplanted on 23 March 2010 into 2.5 L polyethylene pots (diameter 17 cm, height 14 cm) containing a substrate of coconut fibre, black and blond peat, and perlite, (8:7:1) amended with 2 g L^{-1} of Osmocote Plus (14:13:13 N, P, K plus microelements). The pots were placed in a plastic greenhouse equipped with a cooling system and a drip irrigation system in the CEBAS experimental farm located in Santomera (Murcia, Spain). The micro-climatic conditions inside the greenhouse, for the experimental period, recorded with a Hoboware Lite Data Logger (Escort Data Loggers, Inc., Buchanan, Virginia, USA), showed maximum/minimum average temperatures of 20/17 ºC and a maximum/minimum average of 77/50 % RH. A total of 120 plants per specie were randomly attributed to three treatments (40 plants per treatment)

The saline period began on 29 April 2010, five weeks after transplanting. For twenty weeks (saline phase) plants were irrigated with water from different sources..The irrigation treatments were applied at 100% water holding capacity: Control (EC < 0.9 dS m^{-1} indicating no restrictions or slightly restrictions according to FAO classifications); NaCl solution, NaCl (EC: 4 dS m^{-1}); and wastewater, WW (EC: 4 dS m^{-1}) from a sewage treatment plant located in Campotejar (Murcia, Spain). FAO classifications indicate severe restrictions in these two latter types of water. The wastewater treatment plant applies a conventional activated sludge process followed by ultraviolet application as the tertiary treatment. At the beginning of saline period the chemical properties of the irrigation waters were analysed (Table 1). The saline period ended on 15 September 2010. After the saline period, the plants of the NaCl and WW treatments were re-watered, maintaining the same conditions as the control plants, for a further eight weeks (recovery period). The experiment finished on 11 November 2010, thirty three weeks after transplanting.

A multi-outlet emission device, delivering 2 L h^{-1} per pot, was connected to two spaghetti tubes, one on each side of every pot. Plants were irrigated daily and the duration of each irrigation episode depended on the season, climatic conditions and plant development, applying a leaching rate of 10-15% of irrigation water in the control treatment and 30-40% in the saline treatments (NaCl and WW). Water consumption was measured gravimetrically throughout the experimental period and was determined from the difference in weights (weight after irrigation, when drainage stopped, and before irrigating again). The amount of water added to each pot during the saline period was 52833 mL for control and 61700 mL for each saline treatment (NaCl and WW). During the recovery period, the added water was similar in all treatments (19000 mL). The amount of water added was the same for both species.

2.2. Measurements of growth and mineral content

At the end of the saline and recovery periods, the substrate was gently washed from the roots of five plants per treatment for both species. The plants were divided into shoots (leaves and stems) and roots. These were then oven-dried at 80 ºC until they reached a constant weight to measure the respective dry weights (DW). Leaf number was estimated and total leaf area (cm^2) was determined using a leaf area meter (Delta-T; Devices Ltd., Cambridge, UK). Leaf colour was measured in eight plants per treatment throughout the experiment (every 6-10 days) with a Minolta CR-10 colorimeter, giving the colour coordinates lightness (L*), chroma (C*) and hue angle (hº) (McGuire, 1992). Also, the height was measured throughout the experiment, at the same time than colour parameters, in nineteen plants per treatment for both species.

The inorganic solute content of shoots and roots was determined from the dry mass in five plants per treatment at the end of the saline period. The concentration of Cl^- was analysed by a chloride analyzer (Chloride Analyser Model 926, Sherwood Scientific Ltd.) in the aqueous extracts obtained by mixing 100 mg of dry vegetable powder with 40 ml of water before shaking for 30 min and filtering. The concentrations of Na^+, Ca^{2+}, K^+ and B^+ were determined in a digestion extract with HNO_3:$HClO_4$ (2:1, v/v) by Inductively Coupled Plasma optical emission spectrometer (ICP-OES IRIS INTREPID II XDL).

2.3. Water relations and gas exchange

Seasonal changes in stem water potential (? $_{stem}$), osmotic potential (? $_{o}$), osmotic potential at full turgor (? $_{100s}$), turgor potential (? $_{t}$), stomatal conductance (g_s) and net photosynthesis (P_n) at midday were determined in five plants per treatment in laurustinusand six plants per treatment in euonymus throughout the assay (every 6-10 days)..

The ? $_{stem}$ 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). Leaves for ? $_{stem}$ measurements were taken were covered with aluminium foil for at least 2 h before measurements. Leaves from the ? $_{stem}$ measurements were then frozen in liquid nitrogen (-196 °C) and stored at -30 °C. After thawing, the osmotic potential (? $_{o}$) was measured in the extracted sap using a WESCOR 5520 vapour pressure osmometer (Wescor Inc., Logan, UT, USA), according to Gucci et al., (1991). Turgor potential was estimated as the difference between leaf water potential and osmotic potential (? $_{o}$). The osmotic potential at full turgor (? $_{100s}$) was estimated as indicated above for ? $_{o}$, using excised leaves with their petioles placed in distilled water overnight to reach full saturation

Leaf stomatal conductance and net photosynthesis were determined in sunny leaves using a gas exchange system (LI-6400; LI-COR Inc., Lincoln, NE, USA) in greenhouse conditions of temperature, light irradiation, CO_2 concentration and relative humidity.

2.4. Statistics

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 = 0.05). Pearson's correlation analysis was used to test for any relationship between leaf ion concentrations and leaf dry weight.

3. Results

3.1. Growth and mineral content

NaCl and WW affected the biomass parameters and size of the euonymus and laurustinus plants in both periods. However, the response of these species was different when the recovery period was applied (Table 2).

At the end of the saline period, euonymus plants submitted to the saline treatments (NaCl and WW) showed a significant decrease in the total biomass (50% and 63%, respectively), leaf number (66% and 70%, respectively) and total leaf area (59% and 57%, respectively) with respect to the control plants, with no significant differences between the saline treatments. After recovery, the values of the growth parameters of these treatments remained lower than in the control plants and lower than those observed in the saline period (Table 2).

As regards laurustinus, NaCl and WW plants showed a decrease in total biomass (65% and 64% respectively), leaf number (63% and 66%, respectively) and total leaf area (59% and 54%, respectively) with respect to the control. Nevertheless, the values of these parameters in NaCl and WW plants at the end of recovery period were not statistically different from those observed in the saline period, unlike in euonymus plants (Table 2). Both in euonymus and laurustinus, the plants subjected to NaCl and WW treatments were shorter than the control throughout the experimental period (Table 3)

At the end of the saline period, lightness and chroma values were higher and hue angle lower than the control in the plants of euonymus irrigated with both NaCl and WW were observed (Table 3). A contrary pattern was observed at the end of the experiment in the same plants. In laurustinus plants, the lightness and chroma values were the lowest in the NaCl treatment during the saline period. After recovery, only the chroma values were lower in the plants of the WW treatment. There were no significant differences in hue angle values between treatments for either period (Table 3).

As regards solute concentrations (Table 4), at the end of the saline period, the Cl^- and Na^+ accumulated in the shoots and roots of the euonymus plants subjected to the saline treatments were higher than in the control, especially in the NaCl treatment. Only WW plants showed no differences in the Cl^- concentration in roots with respect to control. The highest B^+ values in shoots and roots were found in WW plants. Na^+, Cl^- and B^+ concentrations were higher in shoots than in roots in all the treatments (except Na^+ in control plants).

The K^+/Na^+ and the $Ca^{2+/}Na^+$ ratios were lower in shoots and roots of the euonymus plants irrigated with saline water compared with control plants, especially in the roots of NaCl treatment. In addition, both parameters showed lower values in the roots than in the shoots of all treatments (Table 5).

At the end of saline period, Cl^- and Na^+ were seen to have accumulated in both shoots and roots of the laurustinus plants subjected to the saline treatments, with no differences between both treatments (Table 4). Moreover, Cl^- concentrations were higher in shoots than in roots for all the plants. The WW plants showed the highest B^+ concentration in both shoot and root.

The lowest $Ca^{2+/}Na^+$ ratio corresponded to the NaCl and WW treatments in shoots and roots of laurustinus plants, while no significant effect for K^+/Na^+ ratio in roots was found for WW treatment. Both ratios being lower in roots than in shoots (Table 5).

3.2. Plant water relations and gas exchange

As regards water relations in euonymus plants (Fig. 1), the stem water potential (?$_{stem}$) was significantly lower in the NaCl and WW plants than in the control plants, with no significant differences between the saline treatments in the middle and at the end of the saline period (Fig. 1A). The pressure potential values (?$_{t}$) were higher in the plants of NaCl treatment than in the control in the middle of saline period, whereas only plant of the WW treatment showed higher values at the end of saline period (Fig. 1B). As regards osmotic potential at full turgor (?$_{100s}$) (Fig. 1C) a decrease was observed in WW and NaCl treatments with respect to the control during the saline period, reaching values up to -2.54 MPa for the saline treatments.

At the end of the recovery period, ?$_{stem}$ values were lower in the NaCl and WW plants than in the control plants with no significant differences between the saline treatments (Fig. 1A). The ?$_{t}$ values were higher than in the control only in the WW treatment at the end of the recovery period, while in all ?$_{t}$ values tended to fall at the end of recovery period compared with the end of saline period (Fig. 1B). There were no significant differences in the ?$_{100s}$ values between treatments at the end of recovery period (Fig. 1C).

In laurustinus plants, at the end of saline period, the ?$_{stem}$ values were lower than in the control values only in NaCl plants, with values of around around -1.08 MPa (Fig. 1D). In the middle of the saline period, the ?$_{t}$ was higher in NaCl and especially in WW plants compared with the control, whereas these differences disappeared at the end of saline period (Fig. 1E). The ?$_{100s}$ values decreased in NaCl and WW plants compared with the control in the middle and at the end of saline period (Fig. 1F).

At the end of the recovery period the ?$_{stem}$ values in NaCl plants remained lower than in control plants with no differences between the WW and control treatments (Fig.

1D). There was a greater increase in the ? $_t$ values in the WW treatment (up to 2.71 MPa) (Fig. 1E). The ? $_{100s}$ continued to be lower in the saline treatments, especially in WW with values of -3.5 MPa (Fig. 1F).

As regards gas exchange (Fig. 2), the euonymus plants subjected to saline treatments showed lower stomatal conductance (g_s) and photosynthesis (P_n) values than the control throughout the saline period, reaching values that were half of the control values. Little recovery of these parameters was observed (Fig. 2A,C).

Laurustinus plants subjected to saline treatments also had lower g_s and P_n values than the control throughout the saline period (Fig. 2B,D). At the end of the recovery period, an increase in these values was observed mostly in WW plants, with no significant differences between the WW and control treatments (Fig. 2B,D).

4. Discussion

Previous studies on the effect of reclaimed irrigation water on the growth of ornamental species used for landscaping (Parnell, 1998; Gori et al., 2000), pointed to different behaviours in response to these conditions. Euonymus was considered by Miyamoto et al., (2004) as being moderately tolerant to salinity (able to support 6-8 dS m^{-1}). However, in our case, using water of 4 dS m^{-1}, the decrease in total biomass, leaf number and total leaf area was quite pronounced in spite of the leaching applied, and the effect of salt continued even after two months of watering with the control water. Laurustinus plants also showed a reduction in growth parameters when the wastewater and NaCl solution were applied. A similar behaviour was observed by others authors (Bañón et al., 2012) for this species under saline irrigation, when salinity decreased dry weight of the plants by 60%. However, after the saline stress relief period, the decrease in biomass parameters was not as strong as it was in euonymus. In general, when a severe stress is imposed the recovery of growth is partial and may be related with the recovery of photosynthetic processes, although the maximum photosynthesis rate is not always recovered (Grzesiak, 2004). Saline stress seems to affect photosynthesis more in euonymus plants than in laurustinus plants, accelerating leaf senescence and reducing leaf area as has been observed in other species by Chaves et al., 2011and Navarro et al., 2007.

As regards the colour parameters, leaves of the euonymus plants submitted to salinity were lighter, becoming yellowish green, perhaps as a consequence of the fall in chlorophyll levels in conjunction with other plant related factors (Heiskanen, 2005; Grunenfelder et al., 2006; Navarro et al., 2008). Nevertheless, in laurustinus only the leaves of NaCl plants showed decreased saturation and vividness compared with the control plants, getting a worst visual aspect.

In general, saline conditions, (e.g. a high external NaCl concentration in water and in the substrate) induce an increase in Cl^- and Na^+ levels in roots and leaves, as has been observed in several ornamental species (Cassatini et al., 2009). Rush and Epstein (1981) reported that the maintenance of lower Na^+ levels in shoots than in roots under a high NaCl load is generally considered an adaptive character of halophytes in the face of salt stress. Euonymus accumulated more Na^+ and Cl^- in the shoots than in the roots as a result of the saline treatments, especially the NaCl treatment, and laurustinus accumulated more Cl^- in the shoots than in the roots. These high concentrations in the shoots would have a direct toxic effect on the plant physiology and greatly influence plant growth through osmotic effects and the loss of nutrient availability (Valdez-Aguilar et al., 2009). Moreover, euonymus accumulated more sodium and chlorine in its shoots than laurustinus, three times more sodium in the case of NaCl.

The excessive accumulation of salts may cause an imbalance in the uptake of mineral nutrients as well as phytotoxicity (Hu and Schmidhalter, 2005). The ability to maintain a high K^+/Na^+ ratio is commonly associated with salt resistance (Colmer et al., 2006). It is well established that the control mechanism used by plants under salt stress is a high K^+/Na^+ ratio, which furthermore, is necessary for the maintenance of an appropriate K^+ concentration in plant cells (Wei et al., 2003; Siddiqui et al., 2008; Gorham et al., 2009).

Normally, high levels of NaCl result in a decrease in K^+ and Ca^+ uptake (Chaparzadeh et al., 2003; Niu et al., 1995). In our case, the K^+/Na^+ and $Ca^{2+/}Na^+$ ratios fell in both species as a consequence of saline treatments, and, curiously, this decrease was more marked in roots in the NaCl treatment for both euonymus and laurustinus, presumably due to the non- availability of these ions even when they are present in the plant. Also the Ca^{2+} status plays an important role in saline conditions (Rengel, 1992; Bohnert and Jensen, 1996; Zhu, 2001). The calcium and potassium concentrations in WW water were much higher than in control and NaCl waters. Nevertheless, the Cl^- and Na^+ concentrations dissolved in the WW water could have had a suppressive effect on the Ca^{2+} and K^+ ions, and, as a consequence, reduced their availability for the WW plants, even though the Ca^{2+} and K^+ concentration in leaves was high.

The high B^+ concentration is another problem associated with wastewater use. In both euonymus and laurustinus plants the highest values of B^+ were found in WW plants due to the high content of the irrigation water. Nevertheless, in species showing no B^+ toxicity symptoms, the B^+ concentrations ranged from 100 to 400 mg kg^{-1}, similar to the values to those observed in our assay. No typical boron toxicity symptoms were observed in this experiment; perhaps because salinity mitigated their effect (Bañón et al., 2012).

The leaf water potential and osmotic potential of plants become more negative with salinity, whereas turgor potential increases (Morales et al., 1998; Khan, 2001). In euonymus plants, both kinds of saline water induced a similar decrease in stem water potential and osmotic potential at full turgor, while the turgor potential levels were higher in WW plants than in the control plants, probably because of increased amounts of osmolytes. In laurustinus plants, these parameters were not affected by the WW treatment at the end of the saline period while NaCl treatment showed the lowest values. The data suggest the predisposition of both species to maintain higher pressure potential in the case of saline stress (West et al., 1990). Although, recovery after salinity is generally characterized by an increase in leaf water potential (Chaves et al., 2009) this was not observed in either species, although osmotic adjustment continued during the recovery period in laurustinus, improving the water status of WW plants throughout the experiment

The close association between P_n and g_s in saline conditions suggests that the decline in P_n could be a result of stomatal adjustment. In salinity conditions stomatal closure is generally the main cause of reduced photosynthesis (Flexas et al., 2004; Chaves et al., 2009). In our study, the P_n and g_s in both species were reduced in a similar way.

The recovery of photosynthesis following saline relief determines plant resilience to salinity. Such recovery depends on the intensity of photosynthesis decline and is closely related to a plant’s capacity to avoid or repair membrane damage (Flexas et al., 2006). In our study, the decrease in photosynthesis was similar in both saline treatments for each species. Therefore, the recovery of gas exchange in plants of laurustinus irrigated with WW could be closely related with the behaviour of the water status of these plants, allowing the plants to limit water loss through transpiration and regain higher turgor after relief, as has been demonstrated in other species (Ruiz-Sánchez et al., 1997).

Whatever the case, regardless of the different sources of irrigation water, the above results suggest that the decrease in the aesthetic value was probably more related with ion toxicity and nutritional imbalance. The growth of both species was strongly reduced and injury symptoms such as chlorosis were evident in their leaves, especially in the NaCl treatment.

5. Conclusions

The chemical properties of the saline waters applied had different effects in each species, especially as regards the capacity to recover from salinity. At the end of the recovery period, laurustinus showed no reduction in the total biomass, leaf number or leaf area with respect to the saline period, unlike in euonymus. In laurustinus, the plants irrigated with wastewater developed a more prolonged osmotic adjustment, permitting a better water status and a higher degree of photosynthetic recovery.

These results suggest the importance of studying the physiological mechanisms involved in the recovery of plants subjected to salinity, which may be depend on the sensitivity of a given plant species to salt or different kinds of salts in the irrigation water used.

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Table 1. Chemical analyses of the waters of the different irrigation treatments. Data are values from samples collected at the beginning of saline period.

Physicochemical Analyses	Treatments		
	Control	**NaCl**	**WW**
Na (mg L^{-1})	52.07	801.3	662.30
Chlorides (mg L^{-1})	69.50	1295.90	816.80
B (mg L^{-1})	0.09	0.06	1.08
Ca (mg L^{-1})	94.21	82.54	186.35
K (mg L^{-1})	3.39	4.17	48.27
Mg (mg L^{-1})	41.87	37.79	148.80
P (mg L^{-1})	0.22	<0.1	1.62

Table 2. Percentage of total biomass, leaf number and total leaf area of euonymus (E) and laurustinus (L) submitted to saline treatments with respect to the control plants at the end of saline (S) and recovery period (R).

Measured parameters			**Treatments**			**P**
			Control	**NaCl**	**WW**	
E	**Total biomass DW (%)**	**S**	100 ± 0.00 a	49.49 ± 4.69 bA	62.87 ± 8.69 bA	***
		R	100 ± 0.00 a	29.91 ± 7.65 bB	35.33 ± 7.27 bB	***
	Leaf number (%)	**S**	100 ± 0.00 a	66.29 ± 6.95 bA	69.97 ± 3.83 bA	***
		R	100 ± 0.00 a	32.31 ± 5.15 bB	36.92 ± 7.16 bB	***
	Total leaf area (%)	**S**	100 ± 0.00 a	58.51 ± 5.82 bA	56.85 ± 6.07 b	***
		R	100 ± 0.00 a	26.74 ± 6.95 bB	39.96 ± 7.78 b	***
L	**Total biomass DW (%)**	**S**	100 ± 0.00 a	65.24 ± 5.32 b	63.99 ± 7.51 b	***
		R	100 ± 0.00 a	53.93 ± 8.90 b	65.70 ± 7.59 b	***
	Leaf number (%)	**S**	100 ± 0.00 a	62.66 ± 5.74 b	66.29 ± 5.58 b	***
		R	100 ± 0.00 a	61.83 ± 8.30 b	68.43 ± 7.73 b	***
	Total leaf area (%)	**S**	100 ± 0.00 a	59.47 ± 6.02 b	53.70 ± 8.52 b	***
		R	100 ± 0.00 a	57.62 ± 6.47 b	68.85 ± 6.69 b	***

Values are mean ± S.E. of five plants. Means within a row without a common lowercase letter are significantly different by Duncan$_{0.05}$ test. Means within a column without a common capital letter are significantly different by Duncan$_{0.05}$ test. P, probability level; ns, not significant; * P= 0.05; ** P= 0.01;***P= 0.001

Table 3. Height and colour parameters in euonymus (E) and laurustinus (L) irrigated with water from different sources and of different quality at the end of saline (S) and recovery period (R).

	Measured parameters		Treatments			P
			Control	NaCl	WW	
E	Height (cm)	S	31.68 ± 2.10 a	24.58 ± 1.29 b	24.50 ± 1.35 b	**
		R	36.29 ± 2.02 a	28.13 ± 1.42 b	27.46 ± 1.27 b	**
	L*	S	49.04 ± 0.85 b	52.20 ± 0.56 a	52.41 ± 1.25 a	*
		R	57.91 ± 1.00 a	47.06 ± 0.84 b	47.43 ± 0.85 b	***
	C*	S	23.37 ± 0.79 b	28.35 ± 1.00 a	29.59 ± 1.53 a	**
		R	36.35 ± 0.98 a	23.03 ± 1.25 b	23.53 ± 1.11 b	***
	h°	S	117.79 ± 0.6 a	114.29 ± 0.55 b	113.60 ± 0.97 b	**
		R	108.16 ± 1.00 b	117.33 ± 0.79 a	116.61 ± 0.78 a	***
L	Height (cm)	S	60.74 ± 3.80 a	48.83 ± 2.83 b	50.68 ± 2.25 b	**
		R	64.47 ± 3.66 a	51.44 ± 2.66 b	51.42 ± 2.21 b	**
	L*	S	43.74 ± 0.68 a	40.41 ± 0.86 b	41.86 ± 0.77 ab	*
		R	43.91 ± 0.56	44.34 ± 0.89	41.46 ± 1.05	ns
	C*	S	18.03 ± 0.71 a	12.68 ± 1.57 b	14.92 ± 1.09 ab	*
		R	18.13 ± 1.04 a	17.51 ± 1.43 a	11.79 ± 1.36 b	**
	h°	S	119.40 ± 1.02	120.91 ± 1.76	120.64 ± 1.48	ns
		R	115.42 ± 1.12	115.76 ± 1.50	118.30 ± 3.41	ns

Values are mean ± S.E. of nineteen plants for height and eight plants for colour. Different lowercase letters indicate significant differences between treatments according to $Duncan_{0.05}$ test. L*, lightness; C*, chroma; h*, hue angle; P, probability level; ns, not significant; * P= 0.05; ** P= 0.01;***P= 0.001

Table 4. Shoot and root Na^+, Cl^- and B^+ concentration in euonymus (E) and laurustinus (L) irrigated with water from different sources and of different quality at the end of the saline period.

Solutes ($mg\ kg^{-1}$ DW)			Treatments			P
			Control	**NaCl**	**WW**	
E	**Na^+**	**Shoot**	4,944 ± 669 c	32,057 ± 3457 aA	20,681 ± 2,762 bA	***
		Root	4,002 ± 204 b	10,907 ± 1102 aB	8,606 ± 948 aB	***
	Cl^-	**Shoot**	21,120 ± 2185 cA	47,520 ± 1786 aA	38,480 ± 2,218 bA	***
		Root	12,960 ± 3113 bB	31,680 ± 6641 aB	15,680 ± 1,562 bB	*
	B^+	**Shoot**	176.57 ± 3.52 bA	202.16 ± 14.53 bA	249.24 ± 6.79 aA	***
		Root	84.85 ± 1.52 bB	100.62 ± 7.54 bB	158.5 ± 14.63 aB	***
L	**Na^+**	**Shoot**	1,403 ± 383 bB	11,644 ± 1901 a	13,787 ± 1,172 a	***
		Root	5,122 ± 322 bA	15,100 ± 920 a	10,623 ± 1,290 a	***
	Cl^-	**Shoot**	15,200 ± 1124 bA	33,440 ± 5370 aA	32,880 ± 1,907 aA	**
		Root	9,760 ± 744 bB	16,000 ± 876 aB	16,480 ± 1,076 aB	*
	B	**Shoot**	297.70 ± 5.55 bA	257.63 ± 3.16 cA	325.25 ± 12.76 aA	***
		Root	130.32 ± 1.59 bB	116.14 ± 2.13 bB	142.68 ± 3.07 aB	***

Values are mean ± S.E. of five plants. Means within a row without a common lowercase letter are significantly different by $Duncan_{0.05}$ test. Means within a column without a common capital letter are significantly different by $Duncan_{0.05}$ test. P, probability level; ns, not significant; * P= 0.05; ** P= 0.01;***P= 0.001

Table 5. Shoot and root K^+/Na^+ and Ca^{2+}/Na^+ ratio in euonymus (E) and laurustinus (L) irrigated with water from different sources and of different quality at the end of the saline period.

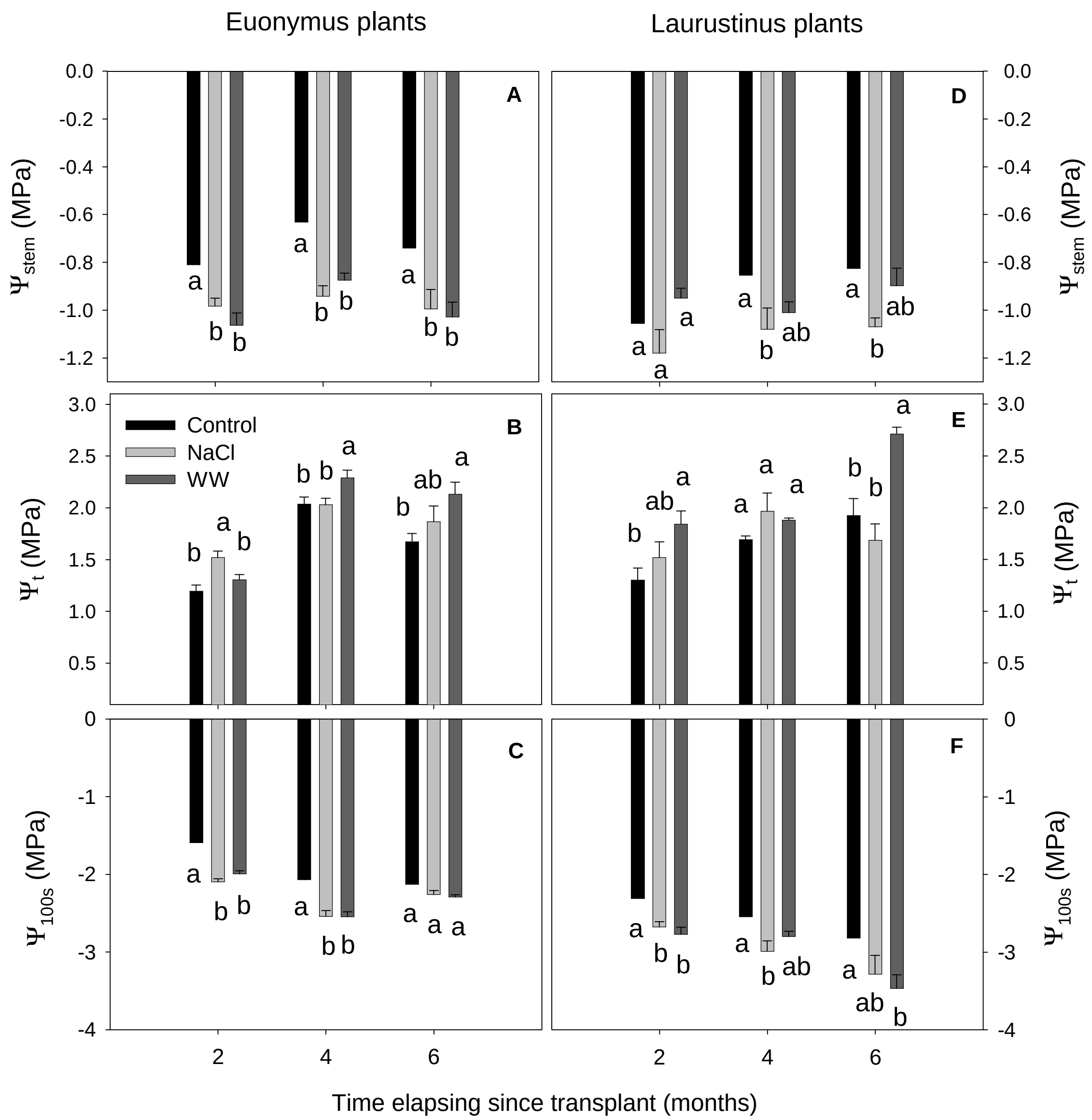
Measured parameters			Treatments			P
			Control	**NaCl**	**WW**	
E	**K^+/Na^+**	**Shoot**	6.24 ± 1.03 aA	0.78 ± 0.06 bA	1.27 ± 0.28 bA	***
		Root	0.74 ± 0.04 aB	0.24 ± 0.04 cB	0.38 ± 0.05 bB	***
	Ca^{2+}/Na^+	**Shoot**	6.11 ± 1.01 aA	1.14 ± 0.07 bA	1.69 ± 0.30 bA	***
		Root	0.84 ± 0.06 aB	0.39 ± 0.04 cB	0.63 ± 0.08 bB	**
L	**K^+/Na^+**	**Shoot**	29.37 ± 7.04 aA	2.57 ± 0.36 bA	2.16 ± 0.23 bA	***
		Root	0.79 ± 0.21 aB	0.25 ± 0.05 bB	0.55 ± 0.07 abB	*
	Ca^{2+}/Na^+	**Shoot**	14.07 ± 4.20 aA	1.18 ± 0.18 bA	0.99 ± 0.09 bA	**
		Root	0.60 ± 0.05 aB	0.20 ± 0.02 bB	0.33 ± 0.05 bB	***

Values are mean ± S.E. of five plants. Means within a row without a common lowercase letter are significantly different by $Duncan_{0.05}$ test. Means within a column without a common capital letter are significantly different by $Duncan_{0.05}$ test. P, probability level; ns, not significant; * P= 0.05; ** P= 0.01;***P= 0.001

Figure captions

Fig.1 Evolution of stem water potential (Ψ_{stem}) in euonymus (A) and laurustinus (D) plants, turgor potential (Ψ_t) in euonymus (B) and laurustinus (E) plants, and osmotic potential at full turgor (Ψ_{100s}) in euonymus (C) and laurustinus (F) plants, irrigated with water from different sources and of different quality, at the middle (two months), at the end of saline period (four months) and at the end of recovery period (six months). Values are means of six and five plants per treatment in euonymus and laurustinus, respectively. Vertical bars indicate standard errors and different lower case letters indicate significant differences between treatments according to $Duncan_{0.05}$ test.

Fig.2. Evolution of stomatal conductance (g_s) in euonymus (A) and laurustinus (B) plants, and net photosynthetic rate (P_n) in euonymus (C) and laurustinus (D) plants at midday irrigated with water from different sources and of different quality at the middle (two months), at the end of saline period (four months) and at the end of recovery period (six months). Values are means of six and five plants per treatment in euonymus and laurustinus, respectively. Vertical bars indicate standard errors and Asterisks indicate statistically significant differences between treatments by $Duncan_{0.05}$ test.



Euonymus plants
Laurustinus plants
Ψ_{stem} (MPa)
Ψ_t (MPa)
Ψ_{100s} (MPa)
A
B
C
D
E
F
Control
NaCl
WW
Time elapsing since transplant (months)

Fig. 1

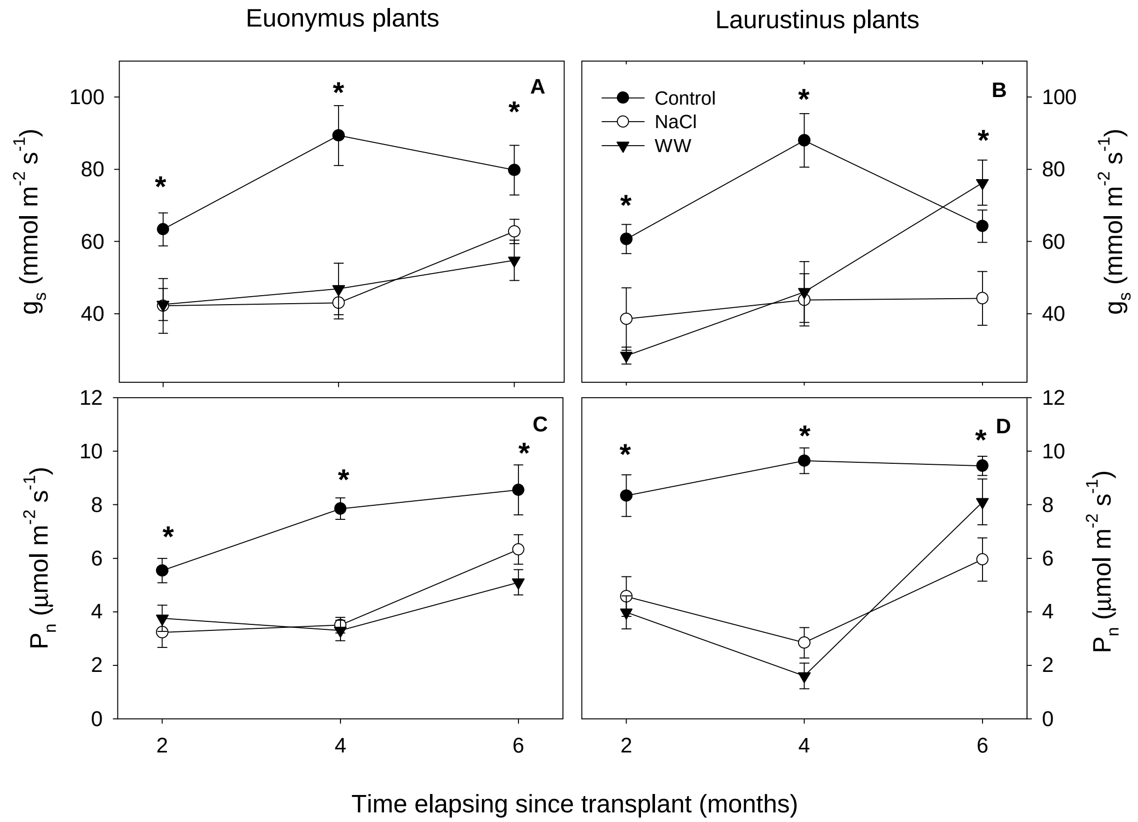

Euonymus plants
Laurustinus plants
A
B
C
D
Control
NaCl
WW
gs (mmol m-2 s-1)
Pn (µmol m-2 s-1)
100
80
60
40
12
10
8
6
4
2
0
*
Time elapsing since transplant (months)

Fig. 2