UNIVERSIDAD POLITÉCNICA DE CARTAGENA

DEPARTAMENTO DE PRODUCCIÓN VEGETAL

Efecto del uso de aguas de distinta calidad en el desarrollo, aspectos fisiológicos, nutricionales y ornamentales de plantas de *Euonymus japonica* Thunb. y *Viburnum tinus* L.

M^a José Gómez Bellot
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Que la referida Tesis Doctoral, ha sido realizada por D^a M^a José Gómez Bellot, dentro del programa de doctorado "Técnicas Avanzadas en Investigación y Desarrollo Agrario y Alimentario", dando mi conformidad para que sea presentada ante la Comisión de Doctorado para ser autorizado su depósito.

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Gómez-Bellot, M.J., Castillo, M., Álvarez, S., Acosta, J.R., Alarcón, J.J, Ortuño, M.F., Sánchez-Blanco, M.J. Effect of different quality irrigation water on the growth, mineral concentration and physiological parameters of *Viburnum tinus* plants. 2nd Symposium on Horticulture in Europe. Angers, France. July 2012.

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ABSTRACT

This thesis evaluates the effect of using water of different quality in the development, physiological and agronomical behaviour of two ornamental species of different origin, euonymus (*Euonymus japonica* Thunb.) and laurustinus (*Viburnum tinus* L.), growing in both soil and pots. It also evaluates the beneficial effects of arbuscular mycorrhizal fungi on plants under these conditions. The experiments performed were designed to: 1) determine the response of euonymus and laurustinus irrigated with water of different qualities and different saline levels, growing in pots under greenhouse conditions and during both saline and recovery periods. 2) study the application and effectiveness of the mycorrhizal fungus *Glomus iranicum* var. *tenuihypharum* on euonymus plants growing in pots in greenhouse conditions and irrigated with two types of water of different sources. 3) evaluate the daily behaviour pattern of euonymus and the use of two techniques to measure of canopy temperature (manual measurement with thermographic camera and continuous measurement with infrared thermometers) to estimate the water status of plants irrigated with two types of water from different source and quality, under pot and greenhouse conditions, and 4) analyze the effectiveness of *Glomus iranicum* var. *tenuihypharum* in laurustinus plants growing in soil under field conditions irrigated with water from different source and quality.

The results showed the following: 1) The use of water from different sources (saline water and reclaimed wastewater) but with similar electrical conductivity (4 dS m^{-1}) seriously affected the development and growth of euonymus and laurustinus. However, after a recovery period, laurustinus plants were more resistant than euonymus plants, maintaining gas exchange and stem water potential values close to those of the control plants, while realizing osmotic adjustment during the recovery period. 2) The application of reclaimed wastewater with the same electrical conductivity (4 dS m^{-1}) but with a composition differing from that of previous experiment did not have a negative effect on both growth and the ornamental value of euonymus because the toxic effects produced by the high saline content were offset by other beneficial ions. Therefore, the reclaimed wastewater components maintained the good nutritional status of plants. However, the application of *Glomus iranicum* var. *tenuihypharum* in the same plants had no additive effect. The positive effect of

mycorrhizae was more evident in plants irrigated with Control water (1 dS m^{-1}), which improved the uptake of phosphorus into the plant and the water status. 3) The daily physiological behavior of euonymus plants irrigated with reclaimed wastewater reflected osmotic stress felt as a result of salts accumulation in the plant. However, parameters such as stomatal conductance and fluorescence tended to recover after midday, even reaching values above the control plants, confirming the resistance of the photosynthetic apparatus to salinity in these conditions. The canopy temperature of the plant (T_c) throughout the day was influenced by the high concentration of salts from the reclaimed wastewater treatment. In addition, the T_c measured by thermal imager was closely correlated with the measured by infrared thermometer, validating both techniques in the diagnosis of water status. 4) The AMF *Glomus iranicum* var. *tenuihypharum* successfully colonized the roots of laurustinus growing in soil and showed a high percentage of enzymatic activity. As a result, the soil structure improved, beneficial nutrients were accumulated in the leaves, toxic ion levels decreased and the water status increased in plants irrigated with reclaimed wastewater.

In conclusion, obtaining plants irrigated with reclaimed wastewater with an acceptable ornamental value depends to a great extent on the species selected and the source, type of treatment and composition of the treated water. The use of water of a moderate salinity in resistant species, together with a suitable leaching level, could minimize the negative effects of this kind of water. Furthermore, the physiological mechanisms developed by these plants during both saline and recovery period vary between species. From the results obtained, mycorrhizal inoculation of the plant is most effective when the irrigation water applied contains a higher concentration of toxic ions and is poor in nutrients. However, its effectiveness depends not only on the intensity and duration of the salt stress, but also on other factors such as the plant species used, the type of AMF selected and the association between them, the characteristics of the soil or substrate and the water regime applied.

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BACKGROUND

1. Use of reclaimed wastewater in agriculture

1.1. The current situation

Water supply and water quality degradation are global concerns that will intensify with population growth, climate change, water demand and the industrialization of developed countries. As a result there has been and will be an increase in water imbalance in different parts of the world, especially important in arid and semi-arid areas (Delgado Díaz 2003).

The natural water resources in Spain are estimated to be around 100,000 hm³/year, 74% corresponding to surface water and 26% underground water, while the present demand is estimated as 35,310 hm³ year⁻¹ (Ministry of Agriculture, Fish and Food, 2008). Despite the existing regulations, the irregular distribution of water resources among regions causes serious deficit situations in much part of the country (Pedrero et al. 2010a). The present demand for water in Spain is distributed across different sectors (urban, agriculture, industrial, cooling, etc.), the agriculture demand being the largest one (around 24,094 hm³/year). In these conditions, and especially for some regions of Spain with a structural water deficit situation, the use of marginal-quality water in agriculture is of great importance, because it contributes to more effective water resources management.

Indeed, all over the world, marginal-quality water is becoming an increasingly important component of agricultural water supplies, particularly in water-scarce countries (Qadir et al. 2007). One of the major types of marginal-quality water is the wastewater from urban and peri-urban areas.

Wastewater has been recycled for agricultural use for centuries as a means of disposal in cities such as Berlin, London, Milan and Paris (AATSE, 2004). In developed countries, there are programs developed by public institutions that include policies to improve the management of reclaimed wastewater for agriculture. If such programs are put into effect, the use of reclaimed wastewater in agriculture will become a growing practice that may help ensure safe and sustainable crops.

Spain is one of the countries which reuses large amounts of water, although in insignificant quantities (no more than 5 % of the volume of wastewater captured), but, according to recent studies, the potential of re-use is about 10 times higher than the current level (Hochstrat et al. 2005). Among the causes of the low level of re-use, are the absence of treatment protocols for reclaimed water in wastewater treatment plants (EDAR), the absence of quality indicators for reusable water depending on the uses has been remedied by the publication of a specific legislation, Royal Decree 1620/2007, which establish the legal regimen for the reuse of treated wastewater. In this decree, the following uses are distinguished: urban, agricultural, industrial, recreational and environmental and Annex I differentiated quality criteria are set according to usage, providing mandatory limits. The defined criteria are considered enforceable mandatory minimum, including physicochemical and health parameters. Also, for the other parameters need to treated water necessary to meet the wastewater discharge conditions as set out in Royal Decree 1315/1992 (BOE, 1992).

The greenhouse and nursery industries are also facing increasing political and financial pressures due to restrictions on the release of effluents and the cost of improving the quality of local water resources. In an attempt to address political, economic, and environmental issues, many growers have begun recycling these waters for use in agricultural systems. Local crop production can save energy and maintain local economic and environmental resources if irrigation and drainage issues are addressed (Carter et al. 2005).

1.2. Benefits and detriments of using reclaimed wastewater for ornamental plants

According to Mujeriego (1990), the use of reclaimed wastewater may have multiple benefits: 1) a reduction in the contribution of pollutants to natural water courses, particularly when the treated water is used for landscaping or forestry; 2) greater reliability and regularity of the water flow available, since the flow of reclaimed wastewater is generally much more controllable than natural watercourses; 3) an extra supply of nutritional substances and organic matter in the water, especially for gardening irrigation, constituting a contribution of fertilizer to plants (Kiziloglu et al.

2007). In fact, ornamental plant production requires a high amount of nutrients such as nitrogen and phosphorus, which would permit an increase in nutrient recycling (Lubello et al. 2004).

On the other hand, reclaimed wastewater reuse for the production of ornamental plants has some peculiarities compared to the same practice in other agricultural fields. First, the ornamental plant production is a high value added activity and it involves that higher economic margins are possible in order to refine reclaimed wastewater. Moreover the potential physical, chemical or biological problems that, may, albeit rarely, can be associated with effluent applied to vegetable or fruit crops are not as applicable to landscape plant production. However, the high value of plants requires that the effect of reclaimed wastewater irrigation has to be carefully evaluated. The use of low quality water affects plants in different ways, and depends on source, kind of treatment applied, as well as the degree of salt tolerance of the species (Acosta-Motos et al. 2014a, 2014b). Reclaimed water may contain high salt concentrations and municipal wastewater treatment plants located near the Mediterranean coast tend to produce wastewater with a high electrical conductivity. In addition, heavy metals and microorganisms are normally present in higher quantities in reclaimed wastewater than in fresh water (Feigin et al. 1991).

Several ornamental plants and cut flower crops, such as *Limonium*, *Dianthus*, *Celosia*, *Gypsophila*, *Matthiola*, *Chrysanthemum*, *Antirrhinum*, *Polygala myrtifolia* and *Ficus benjamina* have been identified as suitable for water reuse systems (Shillo et al. 2002; Carter et al. 2005; Grieve et al. 2006; Friedman et al. 2007; Carter and Grieve 2008; Bañón et al. 2011; Valdés et al. 2012). However, other research projects have provided controversial results probably due to the different cultivation techniques and environments or to the different characteristics of the species under observation (Fox et al. 2005; Gori et al. 2000; Gori et al. 2008; Bañón et al. 2011; Miralles et al. 2011). Whatever the case, advances in methods of cultivation and management along with successes in breeding and selection techniques (Franco et al. 2006), have increasingly enabled growers to use recycled irrigation waters for ornamental and cut flower crop production with no loss of yield or quality.

1.3. Salinity effects

According to the Unesco Water Portal (2007), it is estimated that 6% of the world's land and 30% of the world's irrigated areas already suffer from salinity problems. The expansion of agriculture into semi-arid and arid regions through of intensive irrigation will increase secondary salinization as a result of changes in the hydrologic balance of the soil between the water applied (irrigation or rainfall) and the water used by crops (transpiration) (Chaves et al. 2009).

Particularly in the Mediterranean region, protected horticultural and ornamental crops have to cope with increasing salinization of the irrigation water, because the intensive use of scarce water resources has increased the problems of salinity in both the soil and ground water.

Salinity affects the establishment, growth and development of plants, thus leading to a great loss in productivity (Giri et al. 2003; Katerji et al. 2003; Mathur et al. 2007) and may also affect the ornamental quality of cultivated and wild species (Morales et al. 2001). The earliest response to salinity involves hormonal changes, reduction in the activity of certain enzymes and impaired photosynthesis (Munns 2002, Acosta-Motos et al. 2014a, 2014b), leading to a reduction in the rate of leaf surface expansion, followed by a cessation of expansion as the stress intensifies. The accumulation of salts in the root zone causes a decrease in the osmotic potential and, as a consequence, a decrease in the water potential, diminishing the water available to the root zone (Franco et al. 2011; Sánchez-Blanco et al. 2014). Moreover, the high accumulation of ions such Na^+ and Cl^- in the plant tissue may be metabolically toxic, resulting in "scorched" or "burned" leaves (Shannon and griev 1998). When salts are present in the soil solution, they cause nutrient imbalances, inducing inhibition in the uptake of essential nutrients like K^+ , Ca^{2+} and NO_3 from the roots (Ashraf 2004; Meloni et al. 2008; Zhu 2001) because the toxic ions alter the uptake and translocation of the nutrients or due to a direct interaction with them. As a result of the above, the growth, productivity and quality of plants diminish (Akram et al. 2008; Álvarez et al. 2012; Gómez-Bellot et al. 2013b).

Nevertheless, the response to salinity varies with each plant growth stage, growing conditions including climatic and soil factors, agronomic and irrigation management and the degree of tolerance of the species (Alarcón et al. 1994; Katerji et al. 2003; Gómez Bellot et al., 2013a, Álvarez and Sánchez-Blanco et al. 2014), even within the different cultivars of the same species (Sánchez-Blanco et al. 2003).

1.4. Growth conditions: pots and soil

In general, greenhouse trials for studying salt tolerance are less labour-intensive, less costly, and the environmental conditions can be more easily controlled, while in field studies environmental conditions, such as temperature, light intensity, humidity, and wind speed, can considerably affect plant responses to salinity (Fox et al. 2005; Niu et al. 2007; Zollinger et al. 2007). In field experiments, where plants are transplanted and grow in soil, salinity is a dynamic property in the root zone, more dependent on the evapoconcentration of the soil solution, water extraction, selective plant uptake from plant roots, and replenishment by irrigation or rainfall (Tanji 2002). In pot experiments, in contrast, variation between adjacent plots is eliminated. The control of potted plants and the salinized substrates (Miralles et al. 2012; Valdés et al. 2014a, 2014b) is easier than when plants are grown in field conditions. Whatever the case, the salt accumulation in both soil and substrate is influenced by common factors such as the soil's physical-chemical properties, plant water use, irrigation characteristics and the drainage and/or leaching fraction (Bernstein et al. 2006; Niu and Rodriguez 2006a, 2006b; Miralles et al. 2012; Valdés et al. 2014a, 2014b).

To deal with salinity and guarantee production, scientists have searched for new salt-tolerant plants (Brown et al. 1999; Colmer et al. 2006). Plants that can survive high concentrations of salt in the rhizosphere, grow and complete their life cycle are called halophytes (Parida and Das 2005). Such plants have developed biochemical and molecular mechanisms to tolerate salt stress (Parida and Das 2005) such as ion exclusion or compartmentalization and the synthesis of compatible solutes (Tester and Davenport 2003), changes in the photosynthetic pathway, alterations in membrane structure and the induction of antioxidative enzymes and

hormones. All halophytes must meet the challenge of osmotic adjustment to a low external water potential. Since monovalent ions are judged toxic at the concentrations required for osmotic adjustment, it is generally assumed that Na^+ and Cl^- are compartmentalized, predominantly in the vacuoles. In this way, the concentrations in the cytoplasm are maintained within tolerable limits (Wyn Jones and Gorham 2002). Therefore, it is possible to equilibrate the osmotic plant-soil gradient, allowing the entry of water and solutes into cells in situations in which the osmotic potential of the soil decrease (Flowers and Colmer 2008).

Nevertheless, the genetically complex responses to abiotic stresses are multigenic, and thus more difficult to control. This is dependent on longer term plant performance with respect to biomass, yield data (Álvarez and Sánchez-Blanco 2014) and the degree of recovery from stress. Cycles of stress and recovery from stress are the prevalent processes occurring under natural conditions during different seasons, and in agricultural practices such as irrigation and salt leaching. Thus, the degree of recovery from stress, which also has its molecular basis, is as relevant as the response to stress (Chaves et al. 2009). The carbon balance of a plant during a period of salt/water stress and recovery may depend as much on the velocity and degree of photosynthetic recovery, as it depends on the degree and velocity of photosynthesis decline during water depletion. In summary, the factors limiting photosynthesis recovery after water and salt stress are multiple (Galmés et al. 2007) and vary strongly according to the species, the intensity and duration of the stress as well as leaf age (older leaves are more affected since they accumulate higher amounts of salt) and the plant species (Lawlor and Cornic 2002; Munns 2002; Chaves et al. 2003; Flexas et al. 2004; Galmés et al. 2007).

2. Use of mycorrhizal inoculation in saline conditions

In order to deal with saline water and mitigate the negative effects of salt stress derived from using reclaimed wastewater, farmers usually irrigate their crops after mixing it with well water and use water management techniques such as controlled leaching. Farmers also use genetic breeding, improving the resource use efficiency,

and desalinizing seawater, (Muralev et al. 1997) although some of these methods employed to fight against salt stress are expensive. In this respect, biological processes to alleviate salt stress would be a sustainable option.

Plants are able to establish mutually beneficial associations with some microorganisms present in the rhizosphere (Aroca et al. 2013). One of the most well studied beneficial plant-microorganism associations is that established with arbuscular mycorrhizal fungi (AMF) (Smith and Read 2008). These fungi are so named because they produce characteristic finely branched hyphal structures, termed arbuscules, inside cortical cells of plant roots. The AMF genera *Gigaspora* and *Scutelospora* only produce arbuscules and inter- and intracellular hyphae, whereas *Glomus*, *Entrophospora*, *Acaulospora* and *Sclerocystis* also produce vesicles which are terminal, globose lipid-rich structures in intracellular areas of the root cortex (Strullu et al. 1983).

Interestingly, the vast majority of land plants, including most agricultural crop species, are able to develop symbiotic associations with AMF (Smith and Read, 2008). In symbiosis with the host plant, AMF produce a very extensive network of hyphae in the soil. The intraradical colonization of plant roots results in the formation of some specialized structures. The arbuscules act as organelles for the exchange of nutrients with the host plant, while vesicles, the storage organelles, may enhance the water and nutrient absorbing capacity of the root (Rillig and Mummey 2006).

Many authors have reported that AMF improve plant growth by increasing water and nutrient uptake from the soil, increasing the photosynthetic capacity, improving the water status of plants (Sánchez-Blanco et al. 2004; Franco et al. 2011) and enhancing the stability of soil aggregates while alleviating plant stress caused by biotic and abiotic factor (Deressa et al. 2008; Miransani et al. 2009). In fact, AMF can actually increase the use of different forms of N by plants (Hodge et al. 2001) and improve the P nutrition of host plants growing with sparingly soluble P forms (Evelin et al. 2009). This is largely due to the external hyphae absorbing P beyond the depletion zones around the roots and root hairs, and transporting it to root tissues (Smith and Gianinazzi- Pearson 1988). In addition, AMF secrete a glycoprotein called glomalin, which is a direct and substance contribution source of organic C and N in soil (Rillig et

al. 2001). This protein plays a relevant role in the stability of soil aggregates, since it is able to gather the particles of clay, silt and sand with the soil organic matter added to create high stability (Rillig et al. 2001).

Nevertheless, a negative growth response of host plants to AM colonization has also been described (Tawarayama 2003; Smith and Read 2008; Smith et al. 2009).

In saline conditions AMF can alleviate the adverse effect of salinity on plant productivity by employing various mechanisms, such as defending roots against soil-borne pathogens, improving rhizospheric and soil conditions, modifying microbial communities, enhancing antioxidant enzyme activity, stimulating plant growth regulators, enhancing plant nutrient acquisition, maintaining the K/Na ratio and inducing biochemical, physiological and molecular changes (Fernandez-Martín 2003; Sheng et al., 2008; Evelin et al. 2009; Hajiboland et al. 2010; Franco et al. 2011; Talaat and Shawky 2011; Abdel- Fattah and Asrar 2012; Cekic et al. 2012).

However, salinity not only negatively affects the host plant but also the AMF as it can reduce the colonization capacity, spore germination and the growth of fungal hyphae (Jahromi et al. 2008). In addition, neither do all AMF behave identically in a given environment, nor is a given fungus is the most effective for all environments. Therefore, it is essential to understand the biology and ecological requirements of fungal species, in order to use the most appropriate, depending on the species, cultivar, type of stress and the ecosystem where the inoculated plants are placed (Navarro et al. 2011; Mardukhi et al. 2011).

3. Situation of the ornamental plant sector. Species studied.

In Spain, despite the low number of hectares dedicated to ornamental plants and flowers compared to other uses such as vegetables, citrus and fruit trees, the economic importance of ornamental species is very high. The export figures for plants and flower are around 218 million euros per year (MAGRAMA 2009). Moreover, the production of ornamental plants in Spain has experienced an upward trend, increasing by 37% in the last decade (Fig 1).

Fig 1. Production and surface of ornamental plants in Spain

One of the latest trends in gardening and landscaping under semiarid conditions is the use of designs with low irrigation requirements, so that it is necessary to use ornamental plants adapted to water deficit or salinity.

Although native plants have tended to be forgotten in the design of gardens and landscapes, the use of wild native species from the Mediterranean area in xeriscape, revegetation and landscaping projects is of increasing interest. These species can be considered a wise choice in semi-arid climates due to their resistance to pests and diseases, high tolerance to salinity, high efficiency in water use and growth patterns well adapted to the soil and climatic conditions in these areas (Franco et al. 2006). Therefore, this work focuses on the study of *Viburnum tinus*, a native species to Mediterranean area, and *Euonymus japonica*, a foreign species.

3.1. *Euonymus japonica*

Japanese euonymus or japanese spindle (*Euonymus japonica* Thunb) is a perennial shrub of the family Celastraceae which is native to Japan, Korea and China. The shrub can grow to 4 m in height, but is most commonly seen about 2 to 2.5 m high (Gilman 1999). The leaves are 3-7 cm long, oval, dark green and shiny. The

flowers, which appear from May until June, are inconspicuous, greenish-white, 5 mm diameter and the orange fruit hangs below the flaring pink seed coverings.

Within genus *Euonymus*, there are about 130 species and within the species *Euonymus japonica* there are many cultivars with different types of leaves, from totally green (such as the species studied here), to cultivars with variegated yellow leaves (such as *Euonymus japonica aureomarginata*) or variegated white leaves (such as *Euonymus japonica albomarginata*).

Picture 1. Euonymus plant and flowers

E. japonica is appreciated for its attractive foliage and its uses in gardening are multiple, since it can be cultivated as a border plant, for mass planting, screen, container or above-ground planter, near a deck or patio and espalier. The wood of some cultivars were traditionally used to make spindles for spinning wool, a use that is the origin of the English name of these shrubs.

Although this species is native to Asia, it is widely demanded and used in Mediterranean gardening, occupying top place in the production in nurseries.

3.2. *Viburnum tinus*

Viburnum tinus, which is known as laurustinus, is an evergreen shrub within the family Adoxaceae and native to the Mediterranean area of Europe and North Africa. The genus *Viburnum* consists of about 150 species of small trees and shrubs distributed primarily in the northern hemisphere but extending south in the mountains

of south-east Asia and South America (Mabberley 1997; Donoghue et al. 2004). *Viburnum tinus* has three subspecies: *Viburnum tinus* subsp. *Subcordatum* from the Azores, *Viburnum tinus* subsp. *rigidum* (syn. *V. rigidum*) from the Canary Islands, and our studied species, *Viburnum tinus* subsp. *Tinus* from the Mediterranean region.

The shrub can reach 2-4 m high and is rounded in shape. The elliptic leaves are disposed in opposite pairs, 4–10 cm long and 2–4 cm broad, with an entire margin. The face colour is bright green and lighter on the underside, with hair strands in the crotches of nerves leaves. The small flowers are slightly pinkish white and disposed in umbel shaped inflorescences, reaching about 10 cm in diameter. The fragrant flowers are bisexual and pentamerous. The fruits are metallic blue drupes 5-7 mm in length.

Picture 2. Flowers of laurustinus

V. tinus is a very popular shrub in gardens due to its spectacular flowering in winter, easy care and resistance. As it tolerates pruning well it is often sold as topiary specimens, although even moderate clipping significantly reduces flowering ability.

Furthermore, as a Mediterranean shrub, this species is used for biodiversity conservation, providing greater resistance to the environmental stress imposed by the conditions of semi-arid climatic zones.

The plants have medicinal properties and the main active ingredients are viburnina and tannins, which have been used as diuretic to remove fluid buildup in the body and as a purgative.

CHAPTER 1

**Morphological, nutritional and physiological responses of
Euonymus Japonica Thumb. and *Viburnum tinus* L.
plants under different saline waters.**

Chapter 1.1

Water relations, nutrient content and developmental responses of *Euonymus* plants irrigated with water of different degrees of salinity and quality

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Abstract

Euonymus japonica plants to different irrigation sources were studied. Four irrigation treatments were applied at 100 % water holding capacity: control (electrical conductivity (EC): 0.9 dS m^{-1}); irrigation water normally used in the area (irrigator's water) IW (EC: 1.7 dS m^{-1}); NaCl solution, NaCl (EC: 4 dS m^{-1}); and reclaimed wastewater, WW (EC: 4 dS m^{-1}). This was followed by a recovery period of 13 weeks, when all the plants were rewatered with the same amount and quality of irrigation water as the control plants. Despite the differences in the chemical properties of the water used, the plants irrigated with NaCl and WW showed similar alterations in growth and size compared with the control even at the end of the recovery period. Leaf number was affected even when the EC of the irrigation water was of 1.7 dS m^{-1} (IW), indicating the salt sensitivity of this parameter. Stomatal conductance (g_s) and photosynthesis (P_n), as well as stem water potential (Ψ_{stem}), were most affected in plants irrigated with the most saline waters (NaCl and WW). At the end of the experiment the above parameters recovered, while IW plants showed similar values to the control. The higher Na^+ and Cl^- uptake by NaCl and WW plants led them to show osmotic adjustment throughout the experiment. The highest amount of boron found in WW plants did not affect root growth. Reclaimed wastewater can be used as a water management strategy for ornamental plant production, as long as the water quality is not too saline, since the negative effect of salt on the aesthetic value of plants need to be taken into consideration.

Keywords: Gas exchange; NaCl; Reclaimed wastewater; Quality plants; Water relations.

1. Introduction

In the Iberian Peninsula, particularly in Mediterranean areas, the increasing shortage of water resources and their low quality mean that greater effort is needed for their management to satisfy the needs of crops (Jordan et al. 2009; Ruiz-Sánchez et al. 2010). Indeed, the use of low quality water, in many species is becoming an alternative, in order to guarantee irrigation. Several studies have shown the environmental and agronomical interest of using reclaimed water for irrigation in different crops (Parsons et al. 2001; Aiello et al. 2007). Nevertheless, in ornamental plant production the high concentration of toxic ions that this kind of water contains can decrease the quality and landscape value of these plants (Wu and Dodge 2005). Depending on their source and treatment, such waters may have a high salt content, especially sodium and chloride and significant quantities of toxic metals (Barar et al. 2000; Yadav et al. 2002). Their long-term use therefore may result in the toxic accumulation of heavy metals with unfavourable effects on plant growth (Rattan et al. 2005). Phosphorus concentration levels may also be very high, contributing to surface water eutrophication (Kalavrouziotis et al. 2005) and high levels of boron can cause phytotoxic problems in crops. For example, numerous articles have demonstrated that B^+ reduces citrus tree growth and productivity and contributes to defoliation and yellow leaves (Chapman 1968, Aucejo et al. 1997; Barar et al. 2000). This kind of water may also contain high K^+ and S^- levels that can induce magnesium and phosphorus deficiency, respectively (Rattan et al. 2005) reducing the growth and development of plants. Nevertheless, potential irrigation problems associated with low water quality will depend on the duration of its application (Bansal et al. 1992; Palaniswami and Sree Ramulu 1994) and the different characteristics of the plant species. One alternative that has produced goods results and avoided many of the problems associated with wastewater use in agriculture was the blending reclaimed water with well water (Pedrero 2010b; Bañón et al. 2011).

Salinity can cause imbalances in the uptake of mineral nutrients as well as a gradual accumulation of electrolytes (mainly Na^+ and Cl^-) in the aerial parts, causing

damage to the plant metabolism, when no compartmentation of saline ions in the vacuole takes place. One of the main mechanisms that plants use to adapt to osmotic stress is osmotic adjustment which maintains the positive turgor required for stomata opening and cell enlargement (Torrecillas et al. 2003; Navarro et al. 2007; Álvarez et al. 2012). When osmotic adjustment occurs, the osmotic potential decreases and, as a consequence, the water potential falls, diminishing the availability of water to the root, affecting growth and vegetal development (Greenway and Munns 1980; Tanji 1990; Neumann 1997).

If poor quality water is to be used, it is important to select plants that have mechanisms which help tolerate osmotic and saline damage without damaging their development.

Euonymus japonica is a popular compact shrub, which is usually cultivated as a hedge to adorn gardens. It is well adapted to coastal zones where high concentrations of salt accumulate in the soil. The objectives of this work were: 1) to study the effect of different irrigation water sources on plant growth and quality, water relations, gas exchange and nutrient content in *Euonymus japonica*, and 2) to evaluate whether reclaimed water with a high salinity level can be used as an alternative source of water and nutrients for *Euonymus* plant production. The results will increase the little information that exists on the impact of low quality water of different chemical properties on shrub species of ornamental and landscape interest in the Mediterranean region.

2. Material and methods

2.1. Plant material and growth conditions

Euonymus japonica plants (n= 160) with an initial height of 15 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 in the CEBAS

experimental farm located in Santomera (Murcia, Spain), equipped with a cooling system (picture 1). The micro-climatic conditions, recorded with a Hoboware Lite Data Logger (Escort Data Loggers, Inc., Buchanan, Virginia, USA), showed maximum/minimum average temperatures of 20/17 °C and maximum/minimum average RH of 70/50 %. During the experimental period, the average values of the air temperature, RH and vapour pressure deficit (VPD) were around 22 °C, 64% and 1.3 KPa, respectively.

Irrigation with saline water began on 29 April 2010, five weeks after transplanting. For twenty weeks (saline phase) plants of different irrigation sources were studied. Four irrigation treatments were applied at 100% water holding capacity: Control ($EC < 0.9 \text{ dS m}^{-1}$, leaching 10-15% of the applied water); irrigation water normally used in the area (irrigator's water, reclaimed wastewater blended 50% with well water), IW ($EC: 1.2\text{-}1.8 \text{ dS m}^{-1}$, leaching 20-25%); NaCl solution, NaCl ($EC: 4 \text{ dS m}^{-1}$, leaching 30-40%); and reclaimed wastewater, WW ($EC: 4 \text{ dS m}^{-1}$, leaching 30-40%) from a sewage treatment plant located in Campotejar (Murcia, Spain). The wastewater treatment plant applies a conventional activated sludge process followed by ultraviolet application for tertiary treatment. The saline period ended on 15 September 2010.

Picture 1. Euonymus plants in greenhouse (A) and the Hoboware Lite Data Logger (B)

After the saline period, the plants of the IW, NaCl and WW treatments were rewatered maintaining the same conditions as the control plants for a further thirteen weeks (recovery period). The experiment finished on 16 December 2010, 38 weeks after transplanting.

One drip nozzle, 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. 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).

2.2. Water and substrate analyses

The inorganic solute content, pH and EC of the irrigation waters were assessed at the beginning of the experiment. The samples were collected in glass bottles and stored at 5°C before being processed for chemical analyses. pH was measured with a Cryson-507 pH-meter (Crisom Instruments S.A. Barcelona, Spain); EC and total dissolved solids (TDS) were determined using the multirange equipment, Cryson-HI8734 (Crisom Instruments S.A. Barcelona, Spain); turbidity was measured with a Dinko-D-110 turbidity meter (Dinko Instruments S.A., Barcelona, Spain); the concentrations of macronutrients (Na^{+} , K^{+} , P, Ca^{2+} and Mg^{2+}) and micronutrients (B⁺ and S) were determined by inductively coupled plasma optical emission spectrometer (ICP-ICAP 6500 DUO Thermo, England) and anions (chloride, nitrate, phosphate and sulphate) were analysed by ion chromatography with a Metrhom Chromatograph (Switzerland);

Five substrate samples per treatment were collected and sent to an external analysis laboratory (Antonio Abellán Caravaca S.L. (Fitosoil)) at the end of the saline period and at the end of the recovery period. The substrate was dried at room temperature for a week. Na^{+} , Ca^{2+} and Mg^{2+} were determined by Inductively Coupled Plasma ICP-AES in a saturated soil extract and Cl^{-} was determined by ion chromatography. EC was determined on saturated soil paste.

2.3. Measurements of growth, ornamental characters 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. The plants were divided into shoots (leaves and stems) and roots. These were then oven-dried at 80 °C until they reached a constant weight to measure the respective dry weights (DW). Leaf number was counted directly, while succulence was calculated as the shoot fresh weight/ shoot dry weight ratio, and leaf area (cm²) was determined in the same plants, using a leaf area meter (Delta-T; Devices Ltd., Cambridge, UK). Root length was analyzed by a root system analyzer (Winrhizo LA 1600 Regent Inc., USA).

Picture 2. Destructive sampling of euonymus plants (A) and measure of leaf area (B)

At the end of the saline and recovery periods all the plants were visually evaluated as follows: (1) PIC, percentage of plants in ideal condition; (2) PAC, percentage of plants in acceptable condition; (3) PDB, percentage of plants with dry branches; and (4) DP, percentage of dry plants.

The inorganic solute content of leaves 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⁺, K⁺, Ca²⁺, Mg²⁺ and B⁺ were determined in a digestion extract obtained by

mixing 1 g of dry plant material with 10 mL of concentrated HNO_3 and 5 mL of concentrated HClO_4 . After digestion, they were filtered through Whatman filter paper and a volume of 100 mL was obtained by adding distilled water (Rashid 1986). Na^+ , K^+ , Ca^{2+} , Mg^{2+} and B^+ were analyzed by Inductively Coupled Plasma optical emission spectrometer (ICP-OES IRIS INTREPID II XDL).

The absorption rate of Na^+ , Cl^- and B^+ ions by the root system (J) was calculated considering the total salt content of five plants per treatment at harvest, expressed as mmol Na^+ , Cl^- and B^+ and the mean root weight, using the formula described by Pitman (1975):

$$J = (M_2 - M_1) / (WR * t)$$

where M_1 and M_2 correspond to concentration in mmol of Na^+ , Cl^- or B^+ in the total plant at the beginning and at the end of saline period, respectively, t corresponds to time in days and WR is the logarithmic mean root biomass, calculated as $(WR_2 - WR_1) / \ln(WR_2 / WR_1)$, with WR_1 and WR_2 being the dry weight of roots at the beginning and at the end of saline period respectively.

2.4. Water relations

Seasonal changes in leaf stomatal conductance (g_s), net photosynthesis (P_n), stem water potential (Ψ_{stem}) and leaf osmotic potential at full turgor (Ψ_{100s}), were determined at midday in six plants per treatment periodically during the assay.

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.

Stem water potential was estimated immediately in the same leaves as g_s and P_n were measured, 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 20s of collection and pressurised at a rate of 0.02

MPa s⁻¹ (Turner 1988). Leaves for Ψ_{stem} were taken from the north facing side and were covered with aluminium foil for at least 2 h before measurements. Leaves from plants were excised with their petioles and placed in distilled water overnight to reach full saturation before being frozen in liquid nitrogen (-196 °C) and stored at -30 °C. After thawing, the osmotic potential at full turgor (Ψ_{100s}) was measured in the extracted sap using a WESCOR 5520 vapour pressure osmometer (Wescor Inc., Logan, UT, USA), according to Gucci et al. (1991).

Picture 3. Measurement of gas exchange

2.5. Statistics

For the experiment, 40 plants were randomly attributed to each 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 ($P \leq 0.05$). Pearson's correlation analysis was used to test for any relationship between leaf ion concentrations and leaf dry weight.

3. Results

3.1. Chemical characteristics of water and substrate

At the beginning of the experiment, the physicochemical properties of the irrigation waters were analysed (Table 1). The NaCl and WW waters had similar pH, EC, and TDS values. The highest EC value of both waters compared with the control water was due to the higher salt content. The sodium and the chloride content of the NaCl water was about 15 and 18-times higher, respectively, than the corresponding values of the control water, meaning that Nephelometrical Turbidity Units (NTU) were up to 4 times higher than in the control treatment. The highest boron, calcium, potassium, magnesium, phosphorus, sulphur and sulphate values were observed in the WW water. The IW water showed intermediate values between the control and the highest saline waters (NaCl and WW) for most ions.

Table 1. Physicochemical analyses for the irrigation treatments. Data are values from samples collected at the beginning of saline period.

Physicochemical Analyses	Treatments			
	Control	IW	NaCl	WW
pH	7.97	8.17	7.78	7.67
EC (dS m ⁻¹)	0.87	1.71	4.24	4.19
TDS (mg L ⁻¹)	383	720	1626	1785
Turbidity (NTU)	2.10	2.88	8.16	3.2
B (mg L ⁻¹)	0.09	0.20	0.06	1.08
Ca (mg L ⁻¹)	94.21	92.45	82.54	186.35
K (mg L ⁻¹)	3.39	10.51	4.17	48.27
Mg (mg L ⁻¹)	41.87	54.14	37.79	148.80
Na (mg L ⁻¹)	52.07	187.90	801.3	662.30
P (mg L ⁻¹)	0.22	0.7	<0.1	1.62
S (mg L ⁻¹)	85.6	112.90	64.81	310.30
Chlorides (mg L ⁻¹)	69.50	600.70	1295.90	816.80
Sulphates (mg L ⁻¹)	220.12	750.20	157.87	1044.03

At the end of the saline period irrigation with NaCl and WW treatment caused an accumulation of Na^+ and Cl^- in the substrate, especially of Cl^- in the NaCl treatment (Table 2). There were no significant differences in Ca^{2+} content between the three saline treatments, while the Mg^{2+} content was only higher than the control in the WW treatment.

Table 2. Physicochemical analyses of substrate collected from plants irrigated with water from different sources and of different quality at the end of the saline (S) and recovery (R) periods. Values are means $\pm$ SEM (n=5 plants).

Measured parameters	Treatments				P
	Control	IW	NaCl	WW	
Na^+ (mg kg^{-1} DW)	S 213.89 $\pm$ 37.62 b	375.70 $\pm$ 55.64 b	2073.06 $\pm$ 260.19 a	1523.56 $\pm$ 333.82 a	***
	R 330.66 $\pm$ 71.28	412.37 $\pm$ 113.14	254.68 $\pm$ 21.02	577.09 $\pm$ 103.74	ns
Cl^- (mg kg^{-1} DW)	S 334.82 $\pm$ 69.12 c	625.78 $\pm$ 73.85 c	3936.68 $\pm$ 558.18 a	2390.78 $\pm$ 495.44 b	***
	R 460.58 $\pm$ 99.50	563.62 $\pm$ 166.00	355.96 $\pm$ 26.93	807.80 $\pm$ 197.41	ns
Ca^{2+} (mg kg^{-1} DW)	S 306.31 $\pm$ 73.37	396.57 $\pm$ 36.73	327.05 $\pm$ 48.48	381.11 $\pm$ 43.26	ns
	R 450.68 $\pm$ 42.31 a	423.03 $\pm$ 80.00 a	92.05 $\pm$ 22.95 b	457.98 $\pm$ 38.78 a	***
Mg^{2+} (mg kg^{-1} DW)	S 153.27 $\pm$ 40.74 b	199.38 $\pm$ 19.88 b	181.10 $\pm$ 34.98 b	350.02 $\pm$ 73.30 a	*
	R 291.11 $\pm$ 29.05 a	221.04 $\pm$ 49.92 a	88.65 $\pm$ 58.94 b	253.32 $\pm$ 27.28 a	*
EC (ds m^{-1})	S 2.97 $\pm$ 0.58 b	4.35 $\pm$ 0.40 b	10.43 $\pm$ 2.05 a	10.25 $\pm$ 1.75 a	**
	R 2.64 $\pm$ 0.50 ab	4.61 $\pm$ 0.99 ab	2.44 $\pm$ 0.35 b	6.13 $\pm$ 0.83 a	*

Means within a row without a common letter are significantly different by Duncan_{0.05} test.

P, probability level; ns, not significant; * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.

The salt content increased the EC values of the substrate in the NaCl and WW treatments to around 10 dS m^{-1} . At the end of the experiment, after irrigation with control water, Na^+ and Cl^- concentrations were similar in all treatments. Only the NaCl treatment showed lower Ca^{2+} and Mg^{2+} levels than the other three treatments.

3.2. Growth, ornamental characteristics and mineral content

At the end of saline period, the aerial biomass of *euonymus* plants irrigated with NaCl and WW water was statistically lower than in the control plants due to the lower in leaf DW (11.02 ± 0.69 g plant⁻¹ and 14.35 ± 2.03 g plant⁻¹ in NaCl and WW treatments, respectively), as well as a decrease in leaf number (36% and 25% lower than the control treatment in NaCl and WW treatments, respectively) and total leaf area (43% and 36% lower than the control treatment in NaCl and WW treatments, respectively). An increase in succulence (40% and 21% higher than the control treatment in NaCl and WW treatments, respectively) was observed in these treatments (Table 3). As regards the root/shoot ratio, this parameter only increased in plants irrigated with NaCl due to the marked reduction in leaf DW in this treatment. In contrast, root DW did not show significant differences between all the treatments, although a significant decrease in total root length was observed in NaCl and WW plants (Table 3), more specifically in thin ($\varnothing \leq 0.5$ mm) and medium thickness ($0.5 < \varnothing \leq 2.0$ mm) roots (data not shown). No growth parameters in the IW treatment were significantly altered during this time, except for leaf number (Table 3).

Table 3. Growth and development parameters in *Euonymus japonica* plants irrigated with water from different sources and of different quality at the end of saline (S) and recovery (R) periods. Values are means $\pm$ SEM (n=5 plants).

Measured parameters		Treatments								P
		Control		IW		NaCl		WW		
Leaf DW (g plant ⁻¹)	S	24.38 ± 2.90	a	18.12 ± 3.34	ab	11.02 ± 0.69	bA	14.35 ± 2.03	bA	**
	R	25.21 ± 2.46	a	26.69 ± 4.39	a	5.37 ± 1.06	bB	8.80 ± 2.30	bB	***
Leaf number	S	344 ± 33	a	226 ± 23	bB	220 ± 12	bA	258 ± 19	bA	**
	R	407 ± 31	a	474 ± 69	aA	121 ± 15	bB	173 ± 38	bB	***
Total leaf area (dm ²)	S	17.82 ± 2.20	a	13.03 ± 1.59	ab	10.11 ± 1.24	bA	11.41 ± 1.82	bA	*
	R	17.28 ± 2.87	a	22.21 ± 3.22	a	4.29 ± 0.63	bB	7.60 ± 1.77	bB	***
Root DW (g plant ⁻¹)	S	9.07 ± 1.54	B	7.95 ± 1.31	B	5.80 ± 0.34		5.72 ± 0.71		ns
	R	17.18 ± 1.38	aA	15.60 ± 2.59	aA	5.36 ± 1.29	b	7.92 ± 1.77	b	***
Root/Shoot ratio	S	0.26 ± 0.04	bB	0.32 ± 0.02	ab	0.38 ± 0.04	a	0.27 ± 0.02	bB	*
	R	0.45 ± 0.03	A	0.22 ± 0.09		0.52 ± 0.05		0.48 ± 0.04	A	ns
Total root length (cm)	S	5008 ± 514	aB	4214 ± 603	abB	3124 ± 291	bc	2161 ± 508	cB	**
	R	12611 ± 425	aA	7989 ± 661	bA	3117 ± 783	c	5544 ± 1305	bcA	***
Suculence	S	2.40 ± 0.10	bB	2.39 ± 0.13	b	3.37 ± 0.23	a	2.92 ± 0.16	aB	**
	R	2.62 ± 0.04	bA	2.80 ± 0.12	b	3.67 ± 0.21	a	3.76 ± 0.18	aA	***

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 $\leq$ 0.05; ** P $\leq$ 0.01; ***P $\leq$ 0.001.

Picture 4. Scanning of Control (A) and RW roots (B)

At the end of recovery period, leaf DW in the two most saline treatments (NaCl and WW) continued to be significantly lower than in the control treatment, and the values were even lower than at the end of the saline period (Table 3). The same behaviour was observed for leaf number and total leaf area.

As regards root growth, neither the dry weight nor the total length of the roots of NaCl and WW plants recovered (Table 3) by the end of the experiment although the root/shoot ratio did not show significant differences between any of the treatments.

As regards the visual characteristics, both at the end of the saline and the recovery periods, a high percentage of PIC were observed in the control treatment (around 94%), followed by plants irrigated by IW (around 81%) (Fig. 1a, b). In contrast, the NaCl treatment produced 68% of PAC at the end of saline phase (Fig. 1a), a percentage that decreased by the end of the assay, when the number of DP or PDB had increased. A similar response was seen in the plants from the WW treatment, where the quantity of DP increased from 6.45% in the saline phase to 23.33% at the end of the recovery period (Fig. 1a, b).

Fig.1 Percentage of plants according to the visual characteristics of *Euonymus japonica* plants irrigated with water from different sources and quality at the end of the saline (a) and recovery period (b). PAC, percentage of plants in acceptable conditions; PDB, percentage of plants with dry branches; PIC, percentage of plants in ideal conditions and DP, percentage of dry plants.

Cl^- and Na^+ accumulated in the leaves of the plants subjected to the two most saline treatments, especially in the NaCl treatment. No differences between the control and IW were observed at the end of the saline period in this respect (Table 4). Sodium concentrations were higher in leaves than in roots in the NaCl treatment, while the Cl^- concentration was similar in both leaves and roots. The highest K^+ values in roots were found in IW plants. B^+ only accumulated in WW plants (in both leaves and roots). In general, K^+ and Ca^{2+} accumulation was greater in the leaves than in the roots in all plants. While the contrary was observed for Mg^{2+} .

The highest rates of Na^+ and Cl^- absorption by roots were observed in plants of the NaCl and WW treatments (Fig. 2a, b), especially the NaCl treatment. As regards B^+ absorption by roots, WW had the highest values, although no statistically significant differences were found with respect to the control (Fig. 2c). The lowest rates of B^+ absorption were found for the NaCl and IW treatments.

Table 4. Leaf and root Na^+ , Cl^- , B^+ , Ca^{2+} , K^+ and Mg^{2+} concentration in *Euonymus japonica* plants irrigated with water from different sources and of different quality at the end of saline period. Values are means $\pm$ SEM (n=5 plants)

Solutes (mg kg ⁻¹ DW)		Treatments								P
		Control		IW		NaCl		WW		
Na^+	Leaf	1943 $\pm$ 445	cB	1565 $\pm$ 320	cB	21385 $\pm$ 3098	aB	12538 $\pm$ 2403	b	***
	Root	4002 $\pm$ 204	cA	4163 $\pm$ 209	cA	10907 $\pm$ 1102	aA	8606 $\pm$ 948	b	***
Cl^-	Leaf	10560 $\pm$ 1434	c	8560 $\pm$ 627	c	27200 $\pm$ 1374	a	23040 $\pm$ 1366	bA	***
	Root	12960 $\pm$ 3113	b	10960 $\pm$ 3360	b	31680 $\pm$ 6641	a	15680 $\pm$ 1562	bB	*
B^+	Leaf	92.64 $\pm$ 4.37	b	87.87 $\pm$ 2.92	b	103.87 $\pm$ 8.57	b	127.69 $\pm$ 6.70	a	**
	Root	84.85 $\pm$ 1.52	b	82.78 $\pm$ 1.34	b	100.62 $\pm$ 7.54	b	158.5 $\pm$ 14.63	a	***
Ca^{2+}	Leaf	8671 $\pm$ 497	bA	8740 $\pm$ 613	bA	11539 $\pm$ 644	aA	9432 $\pm$ 681	b	*
	Root	5816 $\pm$ 402	bB	5714 $\pm$ 208	bB	7049 $\pm$ 314	bB	8969 $\pm$ 703	a	***
K^+	Leaf	10568 $\pm$ 236	A	10756 $\pm$ 486	A	11305 $\pm$ 772	A	11698 $\pm$ 597	A	ns
	Root	5046 $\pm$ 346	bB	6485 $\pm$ 456	aB	4195 $\pm$ 372	bB	5280 $\pm$ 351	bB	**
Mg^{2+}	Leaf	1296 $\pm$ 105	bB	1296 $\pm$ 235	bB	1695 $\pm$ 80	abB	1867 $\pm$ 124	aB	*
	Root	5470 $\pm$ 365	bA	4964 $\pm$ 265	bA	4578 $\pm$ 207	bA	6495 $\pm$ 305	aA	**

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; * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.

Fig.2 Absorption rate (J) of Na^+ (a), Cl^- (b) and B^+ (c) ions by roots in *Euonymus japonica* plants irrigated with water from different sources and of different quality at the end of the saline period. Values are mean of five plants. Different lower case letters indicate significant differences between treatments according to Duncan_{0.05} test.

3.3. Plant water relations and gas exchange

During the saline period, midday Ψ_{stem} in the control plants ranged between -0.6 and -1.0 MPa, but was significantly lower for the most experimental period in NaCl and WW plants, reaching values of -0.87 to -1.15 MPa for NaCl and of -0.8 to -1.20 MPa for WW (Fig. 3a). The Ψ_{stem} values of IW plants were close to those of control plants. Significant differences in Ψ_{100s} values were found between the two highest saline treatments and the control along almost throughout the assay (Fig. 3b), pointing to the plants osmotic adjustment which induced higher pressure potential values (data not shown). No differences were observed in the Ψ_{100s} values between the control and IW treatment. When control water was applied to all the plants (recovery phase), neither the Ψ_{stem} nor the Ψ_{100s} values of the NaCl and WW treatments recovered (Fig. 3a, b). As regards gas exchange, the control plants had the highest stomatal conductance and photosynthesis values during the saline period (Fig. 3c, d). Both parameters were significantly lower in the other treatments, particularly in NaCl and WW, which showed similar values.

Fig.3 Evolution of stem water potential (Ψ_{stem}) (a) osmotic potential at full turgor (Ψ_{100s}) (b), stomatal conductance (g_s) (c) and net photosynthetic rate (P_n) (d) at midday in *Euonymus japonica* plants irrigated with water from different sources and of different quality at the end of the saline period. Values are means of six plants per treatment. Asterisks indicate statistically significant differences by Duncan_{0.05} test.

Both g_s and P_n tended to recover at the end of the experiment in NaCl and WW plants (Fig. 3c, d). Statistical differences in the g_s values of IW plants compared with control disappeared, while the P_n was higher at the end of the experiment (Fig. 3c, d).

4. Discussion

As seen in many other ornamental species submitted to saline conditions (Gori et al. 2000), the toxic effect of the salt accumulated as a result of using NaCl and WW delayed the growth and development of *Euonymus* plants. There are few references to the effect of using reused water for irrigation on the physiological response of ornamental plants, although Bañon et al. (2011) observed that the growth of lantana and polygala plants was reduced when saline reused water (5.11 dS m^{-1}) was applied, but differently in each species. In our case, growth reductions were very similar in the plants of both treatments (NaCl and WW), despite the different results obtained for the chemical properties of the waters (Table 1) and the differences in salt accumulation in the substrate, especially in the case of Cl^- (Table 2). As regards the development parameters the root/shoot ratio was higher in the plants irrigated with NaCl than with WW. Similar behaviour was observed in herbaceous perennials under saline irrigation (Niu and Rodriguez 2006a; Navarro et al. 2008). Salinity affects leaf number and, in our case, this occurred even when the EC of the irrigation water was around 1.7 dS m^{-1} (IW). One of the first symptoms of plants exposed to high salinity is a restriction in leaf expansion with a subsequent decrease in leaf area (Navarro et al. 2007). It can be explained by a decrease in leaf turgor, changes in cell wall properties or decreased photosynthesis rates (Rodriguez et al. 2005). It has been demonstrated that a sudden increase in soil salinity causes cells to lose water, although the loss of cell volume and turgor is transient, as was observed in our conditions. With time, the cells regain their original volume and turgor owing to osmotic adjustment, but despite this, cell elongation rates are reduced (Passioura and Munns 2000). Reductions in cell elongation and cell division lead to leaves appearing more slowly and to a smaller final size. Cell dimensions change, with greater reductions in area than depth, so leaves are smaller and thicker. Most of these biomass related responses (reductions in leaf DW and leaf number) were especially marked during the recovery period (Table 3), indicating that the observed effects were not reversible, even though

leaching was efficient, as seen from Cl^- and Na^+ accumulation in the substrate during the recovery phase (Table 2).

At the end of the saline period root dry weight was the only biomass parameter to remain unaltered in treated plants, indicating that shoots and roots responded differently to salinity (Álvarez et al. 2012). In polygala, shoot growth was more sensitive to saline reused water than root growth (Bañón et al. 2011). This indicates that the changes that take place in the cell wall properties of roots differ from those in leaves, although the mechanism is unknown. With time, the initiation of new seminal or lateral roots is probably reduced, as seen in other ornamental species submitted different saline levels (Fornes et al. 2007). In our experiment, total root length was reduced at the end of the saline period, especially in thin roots which take up water and nutrients for the plant. This reduction can be considered a mechanism to avoid the entry of toxic ions and heavy metals into the plant, although in our experiment the amounts of heavy metals were small, with no significant differences between treatments, implying no risk to plants. Nonetheless, an increase in toxic ions, mainly sodium and chloride in irrigation water was evident. Ouzounidou et al. (1995) suggested that the inhibitory action of toxic ions and heavy metals on root length, shoot height and leaf area seems principally to be due to chromosomal aberrations and abnormal cell division. This could also be correlated with the metal-induced inhibition of photosynthesis and respiration in the shoot and protein synthesis in the root (Iannelli et al. 2002, Maria and Tadeusz 2005). However, after the two month recovery period, the effect of the salt continued, since these parameters (including root size) in WW and, especially, in NaCl treated plants were lower than in the control plants, meaning that the toxic effects of salts were not reversible during the time studied (Rodríguez et al. 2005).

Injury symptoms, like chlorosis and senescence, were a consequence of Na^+ and Cl^- accumulation in the leaves of NaCl and WW plants, causing the death of 20% by the end of experiment (Fig.1). Chloride has been described as being more toxic than Na^+ when it accumulates in excess in the leaves (Bennet, 1993). At cellular level, high amounts of Na^+ and Cl^- in leaves can be tolerated through anatomical adaptations, such as increased succulence due to increases in vacuole volume,

which was observed in our experiment (Table 3), although it did not mitigate the damage caused by these ions.

Frequently, the presence of high concentrations of Na^+ and Cl^- in the medium leads to a decrease in the uptake of K^+ and Ca^{2+} , given the competition that exists with Na^+ for membrane transporters. It is not clear whether this occurred in our plants since Ca^{2+} was not displaced but accumulated in the leaves of NaCl plants, and K^+ concentrations were similar in the leaves of the plants of all treatments. The same results were obtained by Fornes et al. (2007) in *Petunia*, *Calendula* and *Calceolaria* plants submitted to saline irrigation.

The differences found in other elements in the water of the NaCl and WW treatments, such as boron, magnesium and sulphur (Table 1), did not differently affect the growth and quality of the plants of these treatments (Table 3; Fig. 1). According to Wu and Dodge (2005), these elements and other heavy metals are rarely found in reclaimed water at levels that are damaging to landscape plants. In the specific case of boron, its high rate of absorption by roots (Fig. 2) and its accumulation in the leaves and roots of the plants irrigated with WW did not inhibit a leaf and root growth (Cervilla et al. 2009) compared with NaCl plants. Bañón et al. (2012) showed that plant tolerance to B^+ differs widely among species and cultivars. In species with no B^+ toxicity symptoms, the B^+ concentrations ranged from 100 to 400 mg kg^{-1} , which is similar to the values observed in our assay (Table 3).

Plants responded to salinity by decreasing stomatal aperture (Fig. 3c), and showing a lower Ψ_{stem} . However, in salt-treated plants the osmotic adjustment observed throughout the experiment was able to maintain leaf cell turgor. Salinity affects stomatal conductance immediately and transiently owing to perturbed water relations (Munns and Tester 2008). The reduction in P_n in NaCl and WW plants was related with a lower g_s , which could be related to the high concentration of Cl^- and Na^+ accumulated in the leaves. In extremely saline situations photosynthesis is depressed due to reductions in stomatal and mesophyll conductance to CO_2 (Flexas et al. 2004). The inhibition of photosynthesis observed in the saline treatments was more marked at the end of the experiment, as reflected in the inhibition of photo-assimilation and dry matter production (Table 3) even through leaf turgor was maintained.

5. Conclusions

In conclusion, our results indicate that, regardless of irrigation sources, no differences in the aesthetic and growth response were found between WW and NaCl plants due to the high salt content in the irrigation water of both treatments. Considering our results and the fact that after a two month recovery period 20% of the plants had died, *Euonymus* should not be irrigated with EC values exceeding to 4 dS m⁻¹, especially for long periods. The use of reclaimed water with a moderate EC (IW treatment) could be regarded as a safe water management strategy, since the problems that are associated with this water are of little importance in landscape plants.

Chapter 1.2

Effect of different quality irrigation water on the growth, mineral concentration and physiological parameters of *Viburnum tinus* plants

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Abstract

Treated wastewater may be considered an alternative source of water and fertilizer nutrients for landscape plants. However, NaCl, the principal compound in this water, can be detrimental to plants. *Viburnum tinus* plants were submitted for 4 months to 4 irrigation treatments from different sources: control (Control) ($EC < 0.9 \text{ dS m}^{-1}$); NaCl solution (NaCl) ($EC = 4 \text{ dS m}^{-1}$); irrigation water normally used in the area (IW) ($EC = 1.2\text{-}1.8 \text{ dS m}^{-1}$) and reclaimed water (RW) ($EC = 4 \text{ dS m}^{-1}$). During a recovery period of two months, all plants were irrigated with the control water. The results showed that both leaf area, plant height and biomass was different among plants of NaCl, IW and RW treatments, and decreased at the end of saline period. These changes were more pronounced in the NaCl treatment. The growth alterations evident after the recovery period indicate that salts continued to be present in the substrate. Compared with the control, NaCl and RW plants showed a greater rate of Na^+ and Cl^- absorption by roots. Stem water potential was mainly affected by the NaCl treatment, which was the only treatment that did not recover their stomatal conductance and photosynthesis rate values at the end of experiment. Plants of the IW treatment showed slight changes in gas exchange with respect to the control. Hence, using different sources of water with similar EC, it is important to know the exact composition, since the toxic effects produced by high concentrations of Na^+ and Cl^- might be offset by the effect of other ions like magnesium, potassium and phosphorus. In the physicochemical analysis of water, the highest concentrations of these ions were observed in RW and as consequence, their concentrations in plants were not reduced by the Na^+ and Cl^- effect; phosphorus even increased, improving the plant nutritional balance.

1. Introduction

The use of non-conventional water resources, such as reclaimed water, is common in many areas of the world, especially in arid and semiarid environments (Yermiyahu et al. 2008) and is becoming an alternative to guarantee the irrigation (Elbana et al. 2010). Nevertheless in ornamental plant production, the high concentration of toxic elements present in this kind of water could cause damage to soil quality and nutrient balance inducing a decrease of quality and landscape value of these plants. Most landscape plantings include a variety of different plant species with different abilities to tolerate salt in irrigation water. So, it is important to select plants which have some mechanisms that help to tolerate the osmotic and saline damage, without being affected in their aesthetic value.

A high concentration of salts in the root system causes a water deficit in plants due to the decrease of water potential in root medium (osmotic effect) (Alvarez et al. 2012). In addition the absorption of certain ions induces their accumulation in the plant to toxic levels which also affect the nutritional balance. As a consequence, salinity affects important process such as photosynthesis, respiration, protein synthesis, enzymatic activities and transport of solutes (Chaves et al. 2011).

Viburnum tinus (durillo) is a perennial shrub, native to the Iberian Peninsula which grows in Mediterranean regions. It is cultivated for gardening forming hedges or as an individual plant. This species has a low water requirement, but it has been classified as a salt-sensitive (Bañón et al. 2012). The aim of this work was to study the effect of irrigation water from different sources, including reclaimed water, on plant growth and quality, water relations, gas exchange and photosynthetic activity of *Viburnum tinus*.

2. Material and methods

2.1. Plant material and experimental conditions

Viburnum tinus plants (n=160) with an initial height of 20 cm were transplanted on 23th 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 into a plastic greenhouse in the CEBAS experimental farm located in Santomera (Murcia, Spain), equipped with a cooling system with three extractor fans with a flow of 38000 m³ h⁻¹ and a humidity panel.

Picture 1. Laurustinus plants in the greenhouse

The program of the cooling system guaranteed its function when the temperature exceeded 22 °C. The saline period began on 29th April 2010 and during twenty weeks plants were subjected to four irrigation treatments with different water sources. The irrigation treatments consisted of a control corresponding to 100% water holding capacity, Control (EC < 0.9 dS m⁻¹, leaching 15% of the applied water); NaCl solution, NaCl (EC: 4 dS m⁻¹, leaching 50%); "irrigator's water", used by a local farmers association, coming from a variety of underground and surface water sources, IW (EC: 1.2-1.8 dS m⁻¹, leaching 25%); and reclaimed water, RW (EC: 4 dS m⁻¹, leaching 50%) which came from a sewage treatment plant located in Campotejar (Murcia, Spain). On 15th September 2010 and for eight weeks, plants of NaCl, IW and RW treatments were irrigated everyday with water corresponding the to control

treatment (recovery period). The assay finished on 11th November 2010, 33 weeks from transplanting.

2.2. Water analyses

The inorganic solute content, pH and EC of the irrigation water were assessed at the beginning of the experiment. The concentrations of macronutrients (Na^+ , K^+ , P, Ca^{2+} and Mg^{2+}), micronutrients (B and S) were determined by inductively coupled plasma (ICP-ICAP 6500 DUO, Thermo, England); anions (chloride, nitrate, phosphate and sulphate) were analysed by ion chromatography with a Metrhom Chromatograph (Switzerland); pH was measured with a Cryson-507 pH meter (Crisom Instruments S.A. Barcelona, Spain); electrical conductivity (EC) and total dissolved solids (TDS) were determined using the multirange equipment, Cryson-HI8734 turbidity meter (Crisom Instruments S.A. Barcelona, Spain) and turbidity was measured with a Dinko-D-110 (Dinko Instruments S.A., Barcelona, Spain).

2.3. Measurements of growth and mineral content

After the saline and the recovery phases, plants were divided into shoots (leaves and stems) and roots and oven-dried at 80 °C until they reached a constant weight to measure the total dry biomass and to calculate root/shoot ratio. The leaf number was estimated and total leaf area (cm^2) was determined using a leaf area meter (Delta-T; Devices Ltd., Cambridge, UK). Moreover, periodic measurements of height were taken during the experiment.

Picture 2. Height of laurustinus plants at the end of the experiment

The inorganic solute content (Cl, Na and B) of leaves and roots was determined with dry mass at the end of the saline period. The concentration of Cl^- was analysed in the aqueous extracts by a chloride analyzer (Chloride Analyser Model 926, Sherwood Scientific Ltd.) and the content of Cl and B were determined by Inductively Coupled Plasma optical emission spectrometer (ICP-OES IRIS INTREPID II XDL). The absorption rate of Na, Cl and B by the root system (J) was calculated considering the total salt content expressed as mmol Na, Cl and B and the mean root weight, using the formula described by Pitman (1975): $J = (M_2 - M_1) / (WR \cdot t)$.

Picture 3. Measure of the inorganic solute content and chlorides in leaves and roots

2.4. Water relations

Stem water potential (Ψ_{stem}) was estimated throughout the experiment at midday, in leaves which were covered at least two hours before measurements according to Scholander et al. (1965), using a pressure chamber (Model 3000; Soil Moisture Equipment Co., Santa Barbara, CA, USA). Leaf stomatal conductance (g_s) and net photosynthetic rate (P_n) were measured in six plants per treatment at midday, using a gas exchange system (LI-6400; LI-COR Inc., Lincoln, NE, USA).

2.5. Statistics

In the experiment 40 plants were randomly attributed to each treatment. The data were analysed by one-way ANOVA using Statgraphics Plus for Windows. Treatment means were separated with Duncan's Multiple Range Test ($P \leq 0.05$).

3. Results

The water from RW and NaCl treatments had similar values of EC, pH and TDS (Table 1). The sodium and the chloride content in the water of the NaCl treatment was more than 15 and 18-times higher, respectively, than the control treatment, causing Nephelometrical Turbidity Units (NTU) up to 4 times higher than in the control treatment. The highest boron, calcium, potassium, magnesium, phosphorus, sulphur and sulphate values were obtained in the water of RW treatment (Table 1). The IW treatment showed intermediate values between the Control and the salinity treatments for most ions.

Table 1. Physicochemical analyses from the different irrigations treatments. Data are values from samples collected at the beginning of saline period.

Physicochemical Analyses	Treatments			
	Control	NaCl	IW	RW
pH	7.97	7.78	8.17	7.67
EC (dS m⁻¹)	0.87	4.24	1.71	4.19
TDS (mg L⁻¹)	383	1626	720	1785
Turbidity (NTU)	2.10	8.16	2.88	3.2
B (mg L⁻¹)	0.09	0.06	0.20	1.08
Ca (mg L⁻¹)	94.21	82.54	92.45	186.35
K (mg L⁻¹)	3.39	4.17	10.51	48.27
Mg (mg L⁻¹)	41.87	37.79	54.14	148.80
Na (mg L⁻¹)	52.07	801.3	187.90	662.30
P (mg L⁻¹)	0.22	<0.1	0.7	1.62
S (mg L⁻¹)	85.6	64.81	112.90	310.30
Chlorides (mg L⁻¹)	69.50	1295.90	600.70	816.80
Nitrates (mg L⁻¹)	<0.1	1.37	4.25	0.58
Sulphates (mg L⁻¹)	220.12	157.87	750.20	1044.03

After four months of applied irrigation treatments, the total dry biomass decreased in all treatments with respect to Control, without recovering at the end of the experiment (Table 2) and even NaCl tended to decrease with respect to other treatments.

Table 2. Growth and development parameters in *Viburnum tinus* plants irrigated with water from different sources and quality at the end of saline (S) and recovery (R) period. Values are means $\pm$ SEM (n=5 plants).

Measured parameters		Treatments												P				
		Control				NaCl				IW					RW			
Biomass DW (g plant ⁻¹)	S	60.86	±	5.32	a	38.85	±	1.83	b	44.77	±	1.96	b	38.40	±	4.20	b	**
	R	83.56	±	7.55	a	43.52	±	5.12	bc	51.70	±	5.17	b	54.14	±	7.62	b	***
Leaf number	S	403.50	±	31.14	b	285.50	±	29.11	a	334.20	±	20.61	ab	293.30	±	35.58	a	*
	R	501.12	±	41.27		303.91	±	35.20		360.63	±	41.46		334.29	±	32.13		ns
Total leaf area (dm ²)	S	25.87	±	4.41	a	14.35	±	0.68	b	16.96	±	0.81	b	14.59	±	1.48	b	*
	R	26.60	±	3.02		17.12	±	2.52		18.00	±	2.76		20.69	±	4.11		ns
Root/Shoot Ratio	S	0.43	±	0.04	ab	0.36	±	0.03	b	0.50	±	0.03	a	0.40	±	0.02	ab	*
	R	0.52	±	0.07	a	0.38	±	0.07	ab	0.30	±	0.02	b	0.49	±	0.03	a	*
Height	S	60.74	±	3.80	a	48.83	±	2.83	b	46.22	±	1.50	b	50.68	±	2.25	b	***
	R	64.47	±	3.66	a	51.44	±	2.66	b	53.22	±	1.44	b	51.42	±	2.21	b	***

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 $\leq$ 0.05; ** P $\leq$ 0.01; ***P $\leq$ 0.001.

The leaf number in the highest saline treatments (NaCl and RW) was lower than the Control, whereas the total leaf area was affected in all treatments (Table 2). At the end of the recovery period, these parameters did not show significant differences with respect to Control. The root/shoot ratio tended to decrease in NaCl and increase in IW. As regards the plant height, the three saline treatments consistently showed smaller plants than the Control (Table 2). Na⁺ and Cl⁻ accumulated in the leaves of the plants subjected to the highest salinity treatments (NaCl and RW), showing symptoms such as chlorosis and senescence. In addition, the highest values of B were found in roots and leaves in the RW plants (Table 3).

Table 3. Na^+ , Cl^- and B^+ concentration in leaf and root in *Viburnum tinus* plants irrigated with water from different sources and quality at the end of saline period. Values are means $\pm$ SEM (n=5)

Solutes		Treatments														P		
(mg k ⁻¹ DW)		Control				NaCl				IW				RW				
Na ⁺	Leaf	419	±	88	b	6790	±	1456	a	691	±	192	b	8939	±	971	a	***
	Root	5122	±	322	c	15100	±	920	a	4257	±	1026	c	10623	±	1290	b	***
Cl ⁻	Leaf	8320	±	463	b	19760	±	3421	a	7520	±	320	b	19840	±	2215	a	***
	Root	9760	±	744	b	16000	±	876	a	10000	±	1635	b	16480	±	1076	a	***
B ⁺	Leaf	166.78	±	4.85	b	142.08	±	2.40	c	175.43	±	4.42	b	198.44	±	10.54	a	***
	Root	130.32	±	1.59	b	116.14	±	2.13	c	125.92	±	4.66	b	142.68	±	3.07	a	***

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; * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.

In plants treated with NaCl and RW a higher rate of Na^+ and Cl^- absorption by roots was observed respect to the Control plants, especially for the Na^+ (Fig. 1). The lowest values in B absorption were found for NaCl and IW (Fig. 1).

Fig.1 Absorption rate of Na^+ (A), Cl^- (B) and B^+ (C) by roots in *Viburnum tinus* plants irrigated with water from different sources and quality at the end of saline period. Values are means of five plants.

Midday stem water potential was mainly affected by NaCl treatment in the last weeks of the saline period (Fig. 2). Throughout the experiment, the stomatal conductance and photosynthesis values were the lowest in plants that received the highest amount of salts, especially the NaCl treatment which was the only treatment

that did not recover at the end of experiment (Fig. 3). Plants of the IW treatment showed slight changes in stomatal conductance and photosynthetic rate with respect to the control (Fig. 3).

Fig.2. Evolution of stem water potential at midday in *Viburnum tinus* plants irrigated with water from different sources and quality at the end of saline period. Values are means of six plants.

Fig.3. Evolution of stomatal conductance (A) and net photosynthetic rate (B) at midday in *Viburnum tinus* plants irrigated with water from different sources and quality at the end of saline period. Values are means of six plants.

4. Discussion

There are few references to the effect of irrigation with reused water on the physiological behaviour of ornamental plants (Gori et al. 2000) although it is known that reclaimed water may contain high concentrations of salts, causing a reduction in growth and development, especially in non salt-tolerant species (Wu et al. 2001). In our experiment, in spite of the differences found in water quality, all the treatments assayed produced a significant reduction in the biomass, total leaf area and height, even in the plants irrigated with water with EC of 1.71 dS m^{-1} . In general, growth did not recover at the end of experiment due to the salts present in the substrate. In this sense, *Viburnum tinus*, a Mediterranean species adapted to environmental conditions, was sensitive to the salts (Bañón et al. 2012). However, during recovery period, the plants irrigated with NaCl and RW treatments had new leaves and increased the total leaf area to become similar to Control plants. The absorption of B by roots in plants irrigated with RW did not have negative effects on plants due to this element (Wu and Dodge 2005). Nevertheless, although similar morphological responses in plants were observed in RW and NaCl, the latter caused more effect in the photosynthetic system, reaching the lowest values of g_s and P_n . Possibly the higher contents of magnesium, sulphur, Ca^{2+} , K^+ and P in RW could have improved the nutrition with respect to plants irrigated with NaCl (Gori et al. 2000), causing a quick recovery of physiological parameters. The osmotic effect provoked in the plant water status by the salinity was similar in all treatments, as we can see from the Ψ_{stem} values. Nevertheless, there was a tendency for Ψ_{stem} to decrease at the end of the saline period as a consequence of dehydration throughout the experiment due to difficulty in taking up water from the substrate (Álvarez et al. 2012). This behaviour also occurred for g_s and P_n values.

5. Conclusions

The reduction in P_n was related to a stomatal limitation (Bacelar et al. 2007), although this observation does not exclude the possibility of metabolic damage due to

salinity, especially in NaCl and RW at the end of the saline period, which could be related to the high concentration of Cl^- and Na^+ in the leaves (Álvarez et al. 2012).

Chapter 1.3

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 reclaimed 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 Ca²⁺/Na⁺ ratios. In Laurustinus, the Ψ_{stem} did not diminish in the reclaimed wastewater-irrigated plants with respect to the control, maintaining osmotic adjustment and a high Ψ_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 reclaimed 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.

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. 2010a) 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 (Chaiyaprat 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 de Larcerra et al. 2005; Cassaniti et al. 2012a). 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. 2012a). 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, Va, USA), showed maximum/minimum average temperatures of 20/17 °C 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).

Picture 1. Placement of euonymus and laurustinus pots in the greenhouse at the beginning of the experiment

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 ($EC < 0.9 \text{ dS m}^{-1}$ indicating no restrictions or slightly restrictions according to FAO classifications); NaCl solution, NaCl ($EC: 4 \text{ dS m}^{-1}$); and reclaimed wastewater, WW ($EC: 4 \text{ dS m}^{-1}$) 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.

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

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 °C 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 (HUE^*) (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.

Picture 2. Minolta CR-10 colorimeter

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 $\text{HNO}_3:\text{HClO}_4$ (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 (Ψ_{stem}), osmotic potential (Ψ_o), osmotic potential at full turgor (Ψ_{100s}), turgor potential (Ψ_t), stomatal conductance (g_s) and net

photosynthesis (P_n) at midday were determined in five plants per treatment in laurustinus and six plants per treatment in euonymus throughout the assay (every 6-10 days).

The Ψ_{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 Ψ_{stem} measurements were taken were covered with aluminium foil for at least 2 h before measurements. Leaves from the Ψ_{stem} measurements were then frozen in liquid nitrogen (-196°C) and stored at -30°C . After thawing, the osmotic potential (Ψ_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 (Ψ_o). The osmotic potential at full turgor (Ψ_{100s}) was estimated as indicated above for Ψ_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.

Picture 3. Measure of stem water potential in the pressure chamber

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 ($P \leq 0.05$). 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).

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). Values are means $\pm$ SEM (n=5 plants).

Measured parameters			Treatments												P
			Control				NaCl				WW				
E	Total biomass	S	100	±	0.00	a	49.49	±	4.69	bA	62.87	±	8.69	bA	***
	DW (%)	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	S	100	±	0.00	a	65.24	±	5.32	b	63.99	±	7.51	b	***
	DW (%)	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	***

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; * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$

Picture 4. Height of euonymus and laurustinus plants at the end of the saline period

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).

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). Values are means $\pm$ SEM (n=19 plants for eight; n=8 plants for colour)

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	***	
	HUE	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	**	
HUE*		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	

Different lowercase letters indicate significant differences between treatments according to Duncan_{0.05} test. L*, lightness; C*, chroma; HUE*, hue angle; P, probability level; ns, not significant; * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$

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).

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. Values are means $\pm$ SEM (n=5 plants)

Solute (mg kg ⁻¹ DW)			Treatments												P
			Control				NaCl				WW				
E	Na ⁺	Shoot	4944	±	669	c	32057	±	3457	aA	20681	±	2762	bA	***
		Root	4002	±	204	b	10907	±	1102	aB	8606	±	948	aB	***
	Cl ⁻	Shoot	21120	±	2185	cA	4520	±	1786	aA	38480	±	2218	bA	***
		Root	12960	±	3113	bB	31680	±	6641	aB	15680	±	1562	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	1403	±	383	bB	11644	±	1901	a	13787	±	1172	a	***
		Root	5122	±	322	bA	15100	±	920	a	10623	±	1290	a	***
	Cl ⁻	Shoot	15200	±	1124	bA	33440	±	5370	aA	32880	±	1907	aA	**
		Root	9760	±	744	bB	16000	±	876	aB	16480	±	1076	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	***

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 $\leq$ 0.05; ** P $\leq$ 0.01; ***P $\leq$ 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. Values are means $\pm$ SEM (n=5 plants)

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	***

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; * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$

3.2. Plant water relations and gas exchange

As regards water relations in euonymus plants (Fig. 1), the stem water potential (Ψ_{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 (Ψ_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 (Ψ_{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, Ψ_{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 Ψ_t values were higher than in the control only in the WW

treatment at the end of the recovery period, while in all Ψ_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 Ψ_{100s} values between treatments at the end of recovery period (Fig. 1C).

In laurustinus plants, at the end of saline period, the Ψ_{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 Ψ_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 Ψ_{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 Ψ_{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 Ψ_t values in the WW treatment (up to 2.71 MPa) (Fig. 1E). The Ψ_{100s} continued to be lower in the saline treatments, especially in WW with values of -3.5 MPa (Fig. 1F).

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.

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).

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.

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 reclaimed 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. (2011) and 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 (Cassaniti 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 and McDonnell 1985).

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 $\text{Ca}^{2+}/\text{Na}^+$ 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 et al. 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 reclaimed 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 depend on the sensitivity of a given plant species to salt or different kinds of salts in the irrigation water used.

CHAPTER 2

Mycorrhizal euonymus plants and reclaimed water: Biomass, water status and nutritional responses

Chapter 2

Mycorrhizal euonymus plants and reclaimed water: Biomass, water status and nutritional responses.

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Abstract

The use of biological processes such as the application of mycorrhizae could be a sustainable option for alleviating the effects produced in irrigated plants using saline water. The aim of this work was to study the interactive effect between two irrigation water qualities (Control, C, with $EC < 1.2 \text{ dS m}^{-1}$, and reclaimed wastewater, RW, with $EC: 4 \text{ dS m}^{-1}$) and mycorrhizal colonization by *Glomus iranicum* var. *tenuihypharum* sp. on growth, water relations and mineral uptake of potted *Euonymus japonica* plants grown for thirty eight weeks in greenhouse conditions. A similar percentage of root mycorrhizal colonization (60%) was found for both irrigation treatments. At the end of the experiment, regardless of mycorrhizal inoculation, there was little effect on growth in RW plants although increases in Hue angle, the chlorophyll content and N, B^+ , Ca^{2+} , K^+ , Mg^{2+} , Na^+ and P concentrations in leaves were observed. The salinity level of RW led to lower values of stem water potential and gas exchange. There was no significant combined effect of mycorrhizae and irrigation water on plant growth. However, Control plants showed a slightly greater height under mycorrhizal inoculation, while stem water potential was lower in the mycorrhizal RW plants, which was related to the lower values of soil water potential at the root surface. In the absence of mycorrhizal inoculation, the content of Ca^{2+} , K^+ , P and Mg^{2+} was higher in the leaves of the RW plants. Moreover, independently of mycorrhizal application, the reclaimed wastewater did not diminish the aesthetic value of euonymus as a result of suitable irrigation management and good nutritional status of the plants. Thus, the mycorrhizal effect was more evident in the Control plants.

Keywords: Wastewater; Salinity; Gas exchange; Ornamental plants.

1. Introduction

Many countries, especially in arid and semi-arid regions have already recognised the necessity to use re-use wastewater because limitations in the availability of water are a severe constraint to development. In the case of plants, some benefits of using reclaimed water include water conservation and savings in nutrient and organic solutes (Pedrero and Alarcón 2009), however reclaimed water is of low quality, where the salt concentration of the water is enhanced (Bañón et al. 2011; Mounzer et al. 2013). Several studies have shown the environmental and agronomical interest of using reclaimed water for irrigation in different crops (Aiello et al. 2007; Boland 2008; Pedrero et al. 2012) and it has been reported that successful wastewater reuse in agriculture requires selection of salt tolerant crops, appropriate irrigation systems and water management practices (Bañón et al. 2011; Valdés et al. 2012). While Cassaniti et al. (2012) have analyzed the use of brackish water for growing ornamentals and the effects of salinity on these plants (growth reductions, leaf burn, loss of nutrient availability), little information exists concerning the advantages of using reclaimed water for growing floricultural crops.

Arbuscular mycorrhizae (AM) are the most common and widespread mycorrhizal associations, and it is estimated that this symbiosis occurs in over two-thirds of terrestrial vascular plant species (Smith and Read 2008). Under different conditions, mycorrhizae are capable of improving the establishment and growth of the plant host by transferring nutrients and increasing water absorption by the roots (Allen 1991; Sánchez-Blanco et al. 2004; Deressa and Schenk 2008). Several studies have shown that inoculation with these fungi has a greater effect when plants are subject to some kind of stress, for example under saline conditions (Ruiz-Lozano et al. 1996; Al-Karaki et al. 2001; Miransari et al. 2008). However, in general, the response of mycorrhizal plants depends on the species of plant and fungus introduced (Ruiz-Lozano et al. 1995) and the extent, duration and type of stress (Navarro et al. 2011; Mardukhi et al. 2011). Varying levels of AM colonization may also be related to the different behaviour of each AM fungal species, even in similar ecosystems (Klironomos et al. 1993) or to the influence of different environmental conditions

(Carvalho et al. 2001). Several studies investigating the role of arbuscular mycorrhizal fungi (AMF) in protecting against salt stress have demonstrated that the symbiosis often results in increased nutrient uptake, the accumulation of an osmoregulator, and increases in the photosynthetic rate and water-use efficiency, suggesting that salt-stress alleviation by AMF results from a combination of nutritional, biochemical and physiological effects. Nevertheless, salinity, not only affects the host plant but also the AMF since it can hamper the colonization capacity, spore germination and the growth of fungal hyphae. Indeed, several researchers have reported the negative effects of salinity on fungus (McMillen et al. 1998; Jahromi et al. 2008). However, a few studies have suggested that AMF colonization is not reduced in the presence of NaCl (Levy et al. 1983; Hartmond et al. 1987), and increases in AMF sporulation and colonization under salt-stress conditions have also been reported (Aliasgharzadeh et al. 2001). Whatever the case, the contribution of mycorrhizal symbiosis to agriculture is well known, although its role in helping ornamental plants to establish in saline conditions has received less attention. *Euonymus japonica* (euonymus) is a fast growing, evergreen, medium sized commercial shrub, whose aesthetic qualities has led to it being planted in streets, public recreation areas and parks. The objective of this study was to investigate the interactive effect between irrigation water quality, including a reclaimed water, and mycorrhizal colonization on the plant growth, ion uptake and water status of pot grown euonymus plants to determine whether the physiological and morphological response of the host plant depends on the physicochemical characteristics of the applied water.

2. Material and methods

2.1. Plant material and growth conditions

The experiment was realised on *Euonymus japonica* plants (n= 160) one year old, in 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). Plants were collected from a

nursery with an initial height of 40 cm and were transplanted into pots. The pots were placed in a plastic greenhouse in the CEBAS experimental farm located in Santomera (Murcia, Spain), equipped with a cooling system using a drip irrigation system. The micro-climatic conditions, recorded with a Vaisala probe (HMP45C), showed that during the experimental period the average values of the air temperature, RH and vapour pressure deficit (VPD) were around 22 °C, 64% and 1.3 KPa, respectively.

After one year of acclimation in the greenhouse, the irrigation treatments started on 18 November 2011 and consisted of a control corresponding to 100% water holding capacity, Control (EC < 1.2 dS m⁻¹, leaching 15% of the applied water), and a reclaimed wastewater, RW (EC: 4 dS m⁻¹, leaching 40%) from a sewage treatment plant located in Campotejar (Murcia, Spain). The wastewater treatment plant applies a conventional activated sludge process followed by ultraviolet application for tertiary treatment. On 7 November 2011 for each irrigation treatment, half of the pots were inoculated with 15 g of *Glomus iranicum* var. *tenuihypharum* sp. (mycorrhizal inoculant Mycogrowth) inoculum (a mixture of spores, mycorrhizal root fragments and rhizospheric soil). It was obtained by isolating the fungi under extreme saline soil conditions (Solonetz Gley, saline soil type according to FAO soil classification). The multiplication of the strain was performed as proposed by Fernández and Juárez (2011). The inoculum was applied manually, dissolved in water over the soil, around the stem.

Picture 1. Mycorrhizal application at the beginning of the experiment

The substrate of non-inoculated pots was previously sterilized by autoclaving (115 °C, 60 min) and the experimental period ended on 31 July 2012. Therefore, there were four treatments in total. One drip nozzle, 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, on climatic conditions and on plant development plant. Water consumption was measured gravimetrically throughout the experimental period and was determined from the difference in weights (weight after irrigation, when drainage stopped, and weight before irrigating again).

2.2. Water and substrate analyses

The inorganic solute content, pH and EC of the irrigation waters were assessed throughout the experiment. The samples were collected in glass bottles and stored at 5°C before being processed for chemical analyses. The pH was measured with a Cryson-507 pH-meter (Crisom Instruments S.A. Barcelona, Spain); EC was determined using the multirange equipment, Cryson-HI8734 (Crisom Instruments S.A. Barcelona, Spain); the concentrations of macronutrients (Na⁺, K⁺, P, Ca²⁺ and Mg²⁺) and micronutrients like B were determined by inductively coupled plasma optical emission spectrometer (ICP-ICAP 6500 DUO Thermo, England) and chloride was analysed by ion chromatography with a Metrhom Chromatograph (Switzerland).

Three substrate samples per treatment were collected and sent to an external analysis laboratory (Antonio Abellán Caravaca S.L. (Fitosoil) at the end of the saline period. The substrate was dried at room temperature for a week. Na⁺, Ca²⁺ and Mg²⁺ were determined by Inductively Coupled Plasma ICP-AES in a saturated soil extract and Cl⁻ was determined by ion chromatography.

2.3. Measurements of biomass, height and colour

At the end of the experimental period, the substrate was gently washed from the roots of three plants per treatment. The plants were divided into leaves, stems and roots. These were then oven-dried at 80 °C until they reached a constant weight to measure the respective dry weights (DW). Leaf number was counted directly and leaf area (cm²) was determined in the same plants, using a leaf area meter (Delta-T; Devices Ltd., Cambridge, UK). Root length was analyzed by a root system analyzer (Winrhizo LA 1600 Regent Inc., USA). Also, the height was measured throughout the experiment in ten plants per treatment.

Leaf colour was measured at the end of the experiment (week 36) with a Minolta CR-10 colorimeter, which provided the colour coordinates lightness (L*), chroma (C*) and hue angle (h°) (McGuire 1992), using eight plants per treatment.

2.4. Chlorophyll and mineral content

The chlorophyll content was assayed according to Inskeep and Bloom (1985) in leaves of four plants per treatment at the end of experiment (week 36). An extract was made from 50 mg of fresh material in 5 mL of 80% acetone in the dark at 4°C and was read at 647 and 664 nm in an Uvikon 940 spectrophotometer (Kontron Instruments AG, Zürich, Switzerland). The inorganic solute content in leaves was determined from the dry mass in four plants per treatment at the end of experiment. The concentration of Cl⁻ was analysed by chloride analyzer (Chloride Analyser Model 926, Sherwood Scientific Ltd.) in the aqueous extracts obtained after mixing 100 mg of dry vegetable powder with 40 mL of water and then shaking for 30 min and filtering. The concentrations of Na⁺, K⁺, P, N, Ca²⁺, Mg²⁺ and B⁺ were determined in a digestion extract with HNO₃:HClO₄ (2:1, v/v) by Inductively Coupled Plasma optical emission spectrometer (ICP-OES IRIS INTREPID II XDL).

2.5. Measurements of plant water status and gas exchange

The soil water potential at the root surface (Ψ_r) during the experimental period in RW treatments (with and without mycorrhizal inoculation) was estimated using the method described by Jones (1983), which assumes that $\Psi_r = 0$ for Control plants. To calculate Ψ_r for RW plants we used the following equation:

$$\Psi_r = \Psi_{RW} - (\Psi_C * g_{sRW}) / g_{sC}$$

where Ψ_{RW} and Ψ_C correspond to the mean value of stem water potential in the RW and Control treatments, respectively, and g_{sRW} and g_{sC} correspond to the mean value of stomatal conductance in the respective treatments.

Seasonal changes in stem water potential (Ψ_{stem}), stomatal conductance (g_s) and net photosynthesis (P_n) at midday were determined in six plants per treatment throughout the assay. In addition, on 17 April (week 23) the diurnal patterns of the same measures were determined from sunrise to sunset, approximately every 1.5 hours in eight plants per treatment. Ψ_{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 within 20.s of collection and pressurised at a rate of 0.02 MPa s⁻¹ (Turner, 1988). Leaves for Ψ_{stem} were taken from the north-facing side and were covered with aluminium foil for at least 2 h before measurements.

Leaf stomatal conductance (g_s) and net photosynthetic rate (P_n) were determined in attached leaves in the same plants and on the same days as the water potential was measured, using a gas exchange system (LI-6400; LI-COR Inc., Lincoln, NE, USA). Gas exchange was measured around noon, fixing the conditions of CO₂ concentration at 380 ppm, the photosynthetically active radiation (PAR) at 1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and the speed of the circulating air flow inside the system at 300

mol s^{-1} . Throughout the experiment the average values of air temperature, relative humidity and pressure deficit were around 24 °C, 41% and 1.75 KPa, respectively.

2.6. Arbuscular Mycorrhizal Colonization (%)

Mycorrhizal colonization was studied two months after inoculation and at the end of experiment, following the gridline intersect method (Giovannetti and Mosse 1980). Five plants per treatment were washed with water, cleared in 10% KOH and a detection method based on non-vital staining with trypan blue was used (Phillips and Hayman 1970). The roots samples were observed under Olympus microscope (CX21).

Picture 2. Stained mycorrhizal roots observed under microscope

2.7. Statistics

In the experiment 40 plants were randomly attributed to each treatment. The two variables (water quality and Mycorrhizal inoculation) and their interaction were analyzed for each parameter by two-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 ($P \leq 0.05$).

3. Results

The percentage of root colonization two months after inoculation and at the end of experiment was similar in both water quality treatments (C and RW) at around 60%, in spite of the higher Cl^- and Na^+ levels in the substrate of the plants irrigated with RW (Table 2). No significant effect of mycorrhizal inoculation on the chemical properties of the substrate was observed (Table 2), regardless of the water quality applied (Table 1).

Table 1. Physicochemical analyses for the irrigation treatments. Data are values from representative samples collected throughout the experiment.

Physicochemical Analyses	Irrigation water	
	Control	RW
pH	7.97	7.77
EC	0.87	4.02
Na (mg L^{-1})	52.07	586.23
Chlorides (mg L^{-1})	69.50	688.10
B (mg L^{-1})	0.09	0.97
Ca (mg L^{-1})	94.21	214.80
K (mg L^{-1})	3.39	51.15
Mg (mg L^{-1})	41.87	177.31
P (mg L^{-1})	0.22	1.62

Table 2. Effects of the different quality water (W), mycorrhizal inoculation (M) and their interaction (W x M) on the chemical properties of substrate collected from euonymus plants at the end of the experiment. Values are means (n=3)

Substrate Analyses (ppm)	Irrigation water (W)		Mycorrhizal inoculation (M)		Significance		
	Control	RW	+	-	W	M	W x M
Cl	448.18	896.23	686.52	657.9	***	ns	ns
Ca	428.35	404.70	420.29	412.75	ns	ns	ns
Mg	234.81	272.33	243.22	263.92	ns	ns	ns
Na	278.09	662.35	464.27	476.17	***	ns	ns

*, **, ***, and ns indicate the level of significance at $P \leq 0.05$, 0.01, 0.001 and the absence of significance, respectively, according to Duncan's multiple range test

In general, regardless of mycorrhizal inoculation, total, stem and leaf dry weights, leaf number and total leaf area were not affected by the RW treatment, although root dry weight was lower when RW was used (Table 3). The greatest accumulation of dry matter in relation to total plant and aerial dry matter was seen in the leaves of the RW plants and in the roots of Control plants. The root/shoot ratio decreased in the plants grown with reclaimed wastewater treatment (Table 3). Regardless of the type of water, mycorrhizal infection had no effect on plant growth and the combination of both factors (type of water and mycorrhiza) was not significant either (Table 3).

Table 3. Effects of the different irrigation water (W), mycorrhizal inoculation (M) and their interaction (W x M) on growth of euonymus plants at the end of experiment. Values are means (n=3)

Parameters	Irrigation water (W)		Mycorrhizal inoculation (M)		Significance		
	C	RW	+	-	W	M	W x M
Leaf D.W. (g plant ⁻¹)	137.23	178.34	161.43	154.14	ns	ns	ns
Stem D.W. (g plant ⁻¹)	120.96	119.78	127.72	113.01	ns	ns	ns
Root D.W. (g plant ⁻¹)	101.22	68.39	83.52	86.10	**	ns	ns
Total D.W. (g plant ⁻¹)	359.41	366.51	372.67	353.25	ns	ns	ns
Root D.W./Shoot D.W.	0.408	0.230	0.294	0.343	***	ns	ns
Total leaf number	2909	3319	3112	3116	ns	ns	ns
Total leaf area (cm ²)	16738	22999	20506	19231	ns	ns	ns
Leaf D.W./Total D.W.	37.77	48.63	43.25	43.15	***	ns	ns
Shoot D.W./Total D.W.	71.29	81.34	77.56	75.07	***	ns	ns
Root D.W. /Total D.W.	28.71	18.66	22.44	24.93	***	ns	ns

*, **, ***, and ns indicate the level of significance at $P \leq 0.05$, 0.01, 0.001 and the absence of significance, respectively, according to Duncan's multiple range test

The evolution of plant height was similar throughout the experimental period for all the treatments (Fig. 1) and three growth periods were evident; a slower period until week 17, followed by more rapid one until week 22, after which plant growth slowed again. Nevertheless, inoculated C plants showed a tendency to increase the height respect to the non-inoculated Control plants from week 27.

Fig.1 Height (a) and relative growth rate (b) of euonymus plants irrigated with water of different qualities without and with mycorrhizal inoculation during the experiment. Asterisks indicate statistically significant differences between treatments by Duncan_{0.05} test. The vertical bars indicate standard errors

At the end of the experiment, changes in colour characteristics were affected by the quality of the water, RW plants, having lower chroma and lightness values, but higher hue angle (Table 4). An increase in the chlorophyll content (Chl a, Chl b and Chl Total) was also detected in plants irrigated with RW, parameters that were statistically unaffected by mycorrhizal inoculation (table 4).

Table 4. Effects of the different irrigation water (W), mycorrhizal inoculation (M) and their interaction (W x M) on colour parameters and chlorophyll content (Chl) a, b, and total in euonymus plants at the end of the experiment. Values are means (n=8 for colour; n= 4 for chlorophyll)

Parameters	Irrigation water (W)		Mycorrhizal inoculation (M)		Significance		
	C	RW	+	-	W	M	W x M
Lightness	51.09	46.31	48.79	48.61	***	ns	ns
Chroma	27.82	20.16	24.03	25.28	***	ns	ns
HUE angle	111.95	115.72	112.56	112.30	**	ns	ns
Chl a (mg g FM⁻¹)	2.2807	2.7334	2.2238	2.1950	*	ns	ns
Chl b (mg g FM⁻¹)	0.6652	0.7617	0.9478	0.8805	*	ns	ns
Chl T (mg g FM⁻¹)	2.9459	3.4951	3.1858	3.0755	*	ns	ns

*, **, ***, and ns indicate the level of significance at $P \leq 0.05$, 0.01, 0.001 and the absence of significance, respectively, according to Duncan's multiple range test

Regardless of mycorrhizal inoculation, the use of reclaimed water had a positive influence on the leaf mineral nutrient composition (Table 5), with Na⁺, K⁺, P, N, Ca²⁺, Mg²⁺ and B⁺ showing higher levels in the plants irrigated with RW than in the Control plants. However, no significant effect was observed for the mycorrhizal inoculation factor (Table 5). The interaction between both factors was significant for Ca²⁺, K⁺ and P. The concentration of these nutrients tended to increase in mycorrhizal Control plants (C M) more than in non-mycorrhizal Control plants. In contrast, Ca²⁺, and K⁺ accumulation tended to be lower in mycorrhizal RW plants (RW M) than in non-mycorrhizal RW plants (Fig. 2).

Table 5. Effects of the different irrigation water (W), mycorrhizal inoculation (M) and their interaction (W x M) on the leaf inorganic solute content in euonymus plants at the end of experiment. Values are means (n=4)

Leaf analyses	Irrigation water (W)		Mycorrhizal inoculation (M)		Significance		
	Control	RW	+	-	W	M	W x M
B (mg Kg⁻¹)	26.83	33.19	28.82	29.80	**	ns	ns
Ca (g 100g⁻¹)	0.97	1.28	1.06	1.19	**	ns	*
K (g 100g⁻¹)	0.93	1.46	1.20	1.20	**	ns	*
Mg (g 100g⁻¹)	0.15	0.18	0.17	0.17	*	ns	ns
Na (g 100g⁻¹)	0.17	0.37	0.24	0.27	*	ns	ns
P (g 100g⁻¹)	0.14	0.18	0.16	0.15	**	ns	*
S (g 100g⁻¹)	0.12	0.14	0.13	0.14	ns	ns	ns
Fe (mg Kg⁻¹)	26.90	22.43	26.98	22.20	ns	ns	ns
Mn (mg Kg⁻¹)	20.14	22.73	19.83	23.14	ns	ns	ns
Cl (g 100g⁻¹)	1.03	1.37	1.24	1.19	ns	ns	ns
N (g 100g⁻¹)	1.20	1.42	1.29	1.26	**	ns	ns

*, **, ***, and ns indicate the level of significance at $P \leq 0.05$, 0.01, 0.001 and the absence of significance, respectively, according to Duncan's multiple range test

Fig.2 Leaf mineral content in euonymus plants irrigated with water of different qualities with and without mycorrhizal inoculation at the end of the experiment. Different lower case letters indicate significant differences between treatments according to Duncan_{0.05} test. The vertical bars indicate standard errors.

The estimated soil water potential at the root surface (Ψ_r) during the experimental period was lower for the mycorrhizal RW plants than for the non-mycorrhizal RW plants from the outset (Fig. 3b). The stem water potential was the highest in both Control treatments (both C and C M plants) (Fig. 3a). The differences in Ψ_{stem} between Control plants and RW were around 0.3 to 0.5 MPa, depending on the time, although the levels fell gradually from week 17 onwards in all treatments (Fig. 3).

Fig.3 Evolution of stem water potential (Ψ_{stem}) (a) in euonymus plants irrigated with water of different qualities without and with mycorrhizal inoculation and soil water potential at the root surface (Ψ_r) (b) in euonymus plants irrigated with wastewater treatment (RW) with mycorrhizal inoculation throughout the experiment. Asterisks indicate statistically significant differences between treatments by Duncan_{0.05} test. The vertical bars indicate standard errors

Similar behaviour was noted in g_s (Fig. 4b), whose levels fell in all treatments during the second half of the experiment. Throughout the experiment, the highest differences of g_s were found between mycorrhizal Control and non-mycorrhizal RW plants (Fig 4b).

In contrast, the values of P_n tended to be more similar among all treatments and at all times, although there was a tendency towards lower P_n values in RW non-mycorrhizal plants (Fig. 4a).

Fig.4 Photosynthesis rate (P_n) (a) and stomatal conductance (g_s) (b) of euonymus plants irrigated with water of different qualities without and with mycorrhizal inoculation at three moments of the experiment (weeks 6, 19 and 30). Different lower case letters indicate significant differences between treatments according to Duncan_{0.05} test. The vertical bars indicate standard errors

With respect to the diurnal patterns of Ψ_{stem} , g_s and P_n , in week 23 (Fig. 5), the lowest values of Ψ_{stem} at midday were for RW mycorrhizal and non-mycorrhizal plants (both RW M and RW), with similar values (-1.2 MPa) between them. This decrease was less pronounced for the Control plants, especially mycorrhizal plants, which reached values of -0.8 MPa, although a slight recovery from 16:00h was detected in all treatments (Fig. 5e, f).

Fig.5 Daily evolution of photosynthesis rate (P_n) in inoculated (a) and non-inoculated (b) plants, stomatal conductance (g_s) in inoculated (c) and non-inoculated (d) plants, and stem water potential (Ψ_{stem}) in inoculated (e) and non-inoculated (f) plants, irrigated with water of different qualities. Asterisks indicate statistically significant differences between treatments by Duncan_{0.05} test. The vertical bars indicate standard errors

During the day, the differences between Control and RW treatments in Ψ_{stem} values were greater between mycorrhizal plants of both treatments (between C M and

RW M). As regards g_s and P_n behaviour during the day, the values of non-mycorrhizal plants (both RW and C) were similar. However for the mycorrhizal plants, the differences between for both treatments (both RW M and C M) were more evident, Control plants showing higher values of both parameters between 08:00 and 10:00 h (Fig. 5). In addition, non-mycorrhizal Control plants showed a tendency to increase g_s values respect to the mycorrhizal Control plants in punctual moments before midday (Fig 5c, d). However, there were no differences of p_n values between both treatments throughout the day (Fig 5a, b).

The relationship between Ψ_{stem} and midday air temperature (T_{md}) (Fig. 6a) presented better correlations than with other environmental variables (data not shown). Increases in T_{md} were associated with decreases in Ψ_{stem} (Fig. 6a). The regression analysis between height and Ψ_{stem} pooling data throughout the experimental period indicated a clear association (Fig. 6b). For both regressions (Ψ_{stem} vs T_{md} , and height vs Ψ_{stem}), covariance analysis indicated that the relationships between mycorrhizal inoculated and non-inoculated plants within the same irrigation treatment were not statistically different, suggesting that it is possible to represent the relationships for each irrigation treatment by a single line (Fig. 6).

Fig. 6 Relationships between daily stem water potential (Ψ_{stem}) and midday air temperature (T_{md}) (a) and height and Ψ_{stem} (b) in euonymus plants irrigated with waters of different qualities without and with mycorrhizal inoculation during the experimental period. Each point is the mean of six plants. Regression equation of Ψ_{stem} vs T_{md} for Control plants (solid line): $\Psi_{\text{stem}} = -0.12 - 0.03 T_{\text{md}}$; $r = 0.68^{***}$ and for RW plants (dashed line): $\Psi_{\text{stem}} = -0.11 - 0.04 T_{\text{md}}$; $r = 0.68^{***}$. Regression equation of height vs Ψ_{stem} for Control plants (solid line): $H = 35.01 - 23.68 \Psi_{\text{stem}}$; $r = 0.77^{***}$ and for RW plants (dashed line): $H = 39.87 - 13.66 \Psi_{\text{stem}}$; $r = 0.76^{***}$

4. Discussion

Several authors have described how the colonization of plant roots by some AMF is reduced (Juniper and Abbott 2006; Sheng et al. 2008), probably as a result of the direct effect of the salt on the fungi, whereby toxic elements suppress the formation of arbuscular mycorrhizae (Tian et al. 2004; Sheng et al. 2008). In a study in *Trifolium alexandrinum* (Gharineh et al. 2009), increasing salinity from 2.2 to 10.0 dS m⁻¹ significantly decreased the percentage of root colonization from 63% to 34%. In our case, the increased Na⁺ and Cl⁻ levels in the substrate of the plants irrigated with RW (4 dS m⁻¹) (Table 2) did not affect the mycorrhizal colonization, the values obtained (60%) being similar to those obtained for the inoculated Control plants, possibly because the fungus used in this study was isolated in saline conditions (Tian et al. 2004) and/or because the salinity level was not high enough to affect root

colonization. Our results confirm that *Glomus iranicum* var. *tenuihypharum* sp. symbiosis was well established on euonymus roots regardless of the type of water used (Porrás-Soriano et al. 2009). The colonization percentage was similar to that observed in other studies (Ruiz-Lozano and Azcón 1995; Kohler et al. 2010; Aroca et al. 2013), higher than under non-saline conditions (Dell'Amico et al. 2002; Sánchez-Blanco et al. 2004).

The benefits derived from AM fungi as regards plant growth and development in saline conditions has been addressed by several authors (Feng et al. 2002; Jahromi et al. 2008; Mardukhi et al. 2011). However, AM euonymus plants had a similar biomass to non-AM plants (Table 3) (Al-Garni 2006) and only root dry weight was lower in the plants irrigated with RW. This suggests that the cell wall properties of the roots differed from those of leaves when the plants were submitted to saline reclaimed water, independently of the presence of mycorrhizae. One explanation could be that the initiation of new seminal or lateral roots is reduced, as has been seen in other ornamental species submitted to various saline levels (Fornes et al. 2007). Such a reduction can be considered as a mechanism to prevent toxic ions and heavy metals from entering the plant (Munns 2002).

The components of the reclaimed water may have improved nutrient acquisition by the leaves as seen from the differences in the contents of some elements (Table 5). It is possible that the toxic effects produced by the highest concentrations of Na^+ in these plants might be offset by the effect of other ions like magnesium, potassium, calcium, nitrogen and phosphorus, which might help to improve plant height (Sharifi et al. 2007). Also, the plants irrigated with RW produced leaves with more intense green colour, probably due to higher chlorophyll levels. Van den Driessche (1991) detected a close relationship between some nutrients (N , Mg^{2+} and Fe^{2+}) and chlorophyll content and reported that an increase in those nutrients could lead to a stimulation of the chlorophyll synthesis. Some studies have reported that improved nitrogen nutrition may help to reduce the toxic effects of Na^+ ions by reducing their uptake and that this may indirectly help in maintaining the chlorophyll content of the plant (Giri and Mukerji 2004).

AMF have been shown to have a positive influence on the content of mineral nutrients, especially phosphorus in plants grown in salt-stress conditions (Al-Karaki and Clark 1998; Giri et al. 2003) through the enhancement and/or selective uptake of some nutrients. However, curiously, in our experiment this effect was seen in the plants under control conditions, especially in the case of P, K⁺ and Ca²⁺. Improved phosphorus nutrition in AM-inoculated Control plants may affect their growth rate, compared with non-inoculated Control plants (Fig. 1). In our conditions, the effect AMF on the leaf of P, K⁺ and Ca²⁺ contents of the plants irrigated with the higher salinity level (RW) was the contrary and no additive effect was observed. Although there is evidence that mycorrhizae can mitigate the negative effects of salinity on the host plants (Evelin et al. 2009; Gharineh et al. 2009; Vicente- Sánchez et al. 2013), in our experiment the salts contained in the RW had no adverse effect on plant growth, and may even have improved the plant nutritional status. These results are not in concordance with those found in our previous works (Gómez-Bellot et al. 2013a; b) where reclaimed wastewater diminished the aesthetic value of euonymus and laurustinus plants. It might be due to the fact that the composition of the wastewater from sewage treatment plant (Campotejar) depends on the source and time of collection. Moreover, essential factors as water management strategy and the species used must be taken into account (Bañón et al. 2011; Vicente- Sánchez et al. 2013; Gómez-Bellot et al. 2013a; 2013b).

Plant water relations differed in the RW treatments, the osmotic effect due to the components of RW, especially the salts provoked leaf dehydration throughout the experiment, as seen from the Ψ_{stem} values. Nevertheless, there was a tendency for Ψ_{stem} to decrease more in the mycorrhizal RW plants (RW M) due to the greater difficulty in taking up water from the substrate (Jones 1983; Álvarez et al. 2012), which can be explained by the lower values of Ψ_r in mycorrhizal RW than in non-mycorrhizal RW (Fig. 3), reflecting the development of stronger rhizosphere resistance in mycorrhizal RW. The calculated values of Ψ_r were found to be insensitive to changes in environmental conditions. Ψ_{stem} averaged approximately from -0.8MPa to -1.4MPa in the RW treatments throughout the experiment. There was, however, no corresponding trend in Ψ_r with RW treatments, which was more

stable than Ψ_{stem} in a fluctuating environment. Good correlation was observed between Ψ_{stem} and T_{md} ($r= 0.68$) and between Ψ_{stem} and plant height ($r= 0.77$), the highest growth rate being accompanied by significant decrease in Ψ_{stem} (Fig. 1b, 3a).

As the EC in the substrate or irrigation water increases substantial reductions in g_s have been reported in many ornamental species (Lee and Van Iersel 2008; Cassaniti et al. 2012). In our experiment, in the face of an osmotic effect as a consequence of RW, plants also commonly reduced their stomatal conductance as a conservation strategy in the use of water (Navarro et al. 2011). In addition, the daily behaviour of gas exchange between both Control plants (C and C M), suggests that mycorrhizal Control plants had higher water use efficiency (Vicente-Sánchez et al. 2013). In fact, AMF are known to exert a certain control of transpiration allowing plants to maintain high levels of water use efficiency (Navarro-Ródenas et al. 2013).

5. Conclusions

Control AMF plants may have improved the nutrient supply, particularly phosphorus, and might have indirectly affected water uptake, the water potential in the cells and so, better water use efficiency. The components of reclaimed water improved the nutritional status of the plants, but the presence of AMF in the same plants had no additive effect.

CHAPTER 3

Daily patterns of water status indicators and nutrient content of *Euonymus Japonica* Thumb. plants submitted to reclaimed water

Chapter 3.1

Daily photosynthesis, water relations, and ion concentrations of euonymus irrigated with treated wastewater

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Abstract

Euonymus japonica Thunb (euonymus) plants were submitted for nine months to two irrigation treatments using water from different sources: a Control (C) water with electrical conductivity (EC) $< 1.2 \text{ dS m}^{-1}$ and reclaimed wastewater (RW) with EC 4 dS m^{-1} . At the end of the experiment, no differences in the total dry weight were observed between treatments, while the leaf dry mass increased (to the detriment of the root part in RW plants). Throughout the day, the stem water potential of the RW plants was lower than in C, while stomatal conductance (g_s) was slightly reduced in RW from 08:00 h to 12:00h, but no significant variation in photosynthesis (P_n) or energy conversion efficiency (F'_v/F'_m) in photosystem II was detected through the effect of salinity. Gas exchange and fluorescence showed a tendency to increase after midday in plants treated with RW. The photosynthetic behaviour and fluorescence of RW plants may have been related to the nitrogen and chlorophyll content of the leaves, confirming the resistance of the photosynthetic mechanism to salinity in this species in these conditions. The toxic effects produced by high concentrations of B^+ , Na^+ and Cl^- were offset by the effect of other ions like magnesium, potassium and phosphorus in plants irrigated with RW, thus improving their physiological status without decreasing their ornamental value.

Keywords: salinity; gas exchange; fluorescence; plant nutrition; ornamental plants

1. Introduction

In recent years, the increase in crop areas and associated water demands have led to a decrease in fresh water availability. In arid and semi-arid areas, where there is an increasing shortage of water, irrigation is fundamental for agricultural development (Feigin et al. 1991). Alternative sources of water, such as treated wastewater, are therefore required to satisfy the needs of crops and its use is gradually becoming a common practice worldwide (Angelakis et al. 1999).

One of the advantages of treated wastewater is that it acts as an important source of nutrients for crop production (Jimenez-Cisneros 1995; Kiziloglu et al. 2007) so that it may be possible to reduce fertilizer application when treated wastewater is used (Gori et al. 2000; Wu et al. 2001b). In addition, its use can be considered an attractive option due to the economic savings involved. Another advantage in using treated wastewater is that it can be safely used in the production of ornamental plants, which are not destined for human consumption, avoiding the health problems derived from toxicity in vegetables and fruit. However, many research projects have looked at the effects of reclaimed wastewater on the physiology of ornamental species with controversial results, probably due to the different cultivation techniques used, environmental characteristics and the different species studied (Acosta-Motos et al. 2014; Bañón et al. 2011; Gori et al. 2000; Schuch 2005).

The need to obtain ornamental plants of a high quality entails the proper management of irrigation (Weber et al. 1996) and a careful evaluation of its effect on plants. The chemical composition of treated wastewater varies according to the treatment process, source of waters, location and time of year (Niu and Cabrera 2010a). Although some water reclamation treatment facilities offer periodic laboratory test results, the data are often incomplete for assessing irrigation quality because they are oriented to the “human impact factor” rather than performance (Duncan et al. 2009). As a consequence, it is necessary to take precautions before reusing wastewater (Angelakis et al. 1999).

One of the main factors that determine the suitability of low quality water for irrigation is its salinity. Among the many effects, salinity generates the decrease in the

osmotic potential of the medium which, as a consequence, increases the difficulty of the plant to take water from the medium (Neumann 1997). In saline conditions, reductions in the levels of CO₂ assimilation have been related, among others, to partial stomatal closure, decreases in the photosynthetic pigments and changes in the ion concentration of leaves (Demetriou et al. 2007). Furthermore, such physiological responses in plants are also subject to the daily changes in environmental factors, such as atmospheric temperature, light or humidity.

From this point of view, the study of the daily patterns of leaf water relations and gas exchange activity is a good physiological approximation for analyzing the optimum water use by plants (Hsiao 1993) and can provide basic information on plant responses to irrigation. Moreover, there are recent studies which use other less common methods, such as infrared thermography to indirectly determine the plant water status. The physiological basis of this methodology is the decrease in energy dissipation caused by transpiration due to the stomatal closure in plants under water stress, the consequence of which is an increase in leaf temperature (Costa et al. 2012; Jackson et al. 1981; Jones et al. 2002).

Euonymus japonica Thunb. (euonymus) is a popular shrub from Japan, which grows naturally in coastal zones where salt accumulates in soils and is widely used as an ornamental plant. The aim of this work was the daily characterization of the water status of euonymus plants irrigated with good quality water and treated wastewater with a high salinity level, in order to determine the physiological behaviour in this plant. We studied the daily and seasonal gas exchange parameters, water relations, and canopy temperature as well as leaf ion accumulation, photosynthetic pigments and plant growth.

2. Material and methods

2.1. Plant material and growth conditions

The experiment was conducted in one year old *Euonymus japonica* plants (n= 80), in 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 in the CEBAS experimental farm located in Santomera (Murcia, Spain), equipped with a cooling system. The micro-climatic conditions recorded with a Vaisala probe (HMP45C) during the experimental period showed air temperature, RH and vapour pressure deficit (VPD) values of around 22 °C, 64% and 1.3 KPa, respectively. The pots were randomly placed in two independent irrigation lines.

The treatments were started on 18 November 2011 and for 36 weeks the plants were irrigated with water from two different sources: Control, C (to 100% water holding capacity, electric conductivity, EC < 1.2 dS m⁻¹, leaching 15% of the applied water) and reclaimed wastewater, RW (EC: 4 dS m⁻¹, leaching 40% of the applied water in order to avoid excessive accumulation of salts in the substrate) from a sewage treatment plant in Campotejar (Murcia, Spain). The wastewater treatment plant applies a conventional activated sludge process followed by ultraviolet application for the tertiary treatment. The experimental period ended on 31 July 2012. The height of all the plants at the beginning of the experiment were similar, around 40-45 cm.

Picture 1. Rows of euonymus pots (A) and a Vaisala probe in the greenhouse

One drip nozzle, 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. Water consumption was measured gravimetrically throughout the experimental period and was determined from the difference in weights (weight after irrigation, when drainage stopped, and weight before rewatering).

2.2. *Water analyses*

The inorganic solute content, pH and EC of the irrigation waters were assessed in January 2012, eight weeks after beginning the treatments. The samples were collected in glass bottles and stored at 5 °C before being processed for chemical analysis. The pH was measured with a Cryson-507 pH-meter (Crisom Instruments S.A. Barcelona, Spain); EC and total dissolved solids (TDS) were determined using the multirange equipment, Cryson-HI8734 (Crisom Instruments S.A. Barcelona, Spain); the concentrations of macronutrients sodium, potassium, phosphorous, calcium, and magnesium, and the micronutrient boron were determined by inductively coupled plasma optical emission spectrometer (ICP-ICAP 6500 DUO Thermo, England). Chlorides were analysed by ion chromatography with a Metrhom Chromatograph (Switzerland).

2.3. *Growth parameters and mineral content*

At the end of the experiment, the substrate was gently washed from the roots of three plants per treatment. Then, the plants were divided into shoots (leaves and stems) and roots. These were then oven-dried at 80 °C until they reached a constant weight to measure the respective dry weights (DW). Leaf number was counted directly and leaf area (cm²) was determined in the same plants, using a leaf area meter (Delta-T; Devices Ltd., Cambridge, UK). The height was also determined in ten plants per treatment.

On 17 April, 22 weeks after beginning the treatments, the inorganic solute content of leaves was determined with dry mass in four plants per treatment. The concentration of Cl⁻ was analysed by chloride analyzer (Chloride Analyser Model 926,

Sherwood Scientific Ltd.) in the aqueous extracts obtained when mixing 100 mg of powdered dry vegetable with 40 mL of water before shaking for 30 min and filtering. The concentrations of Na^+ , K^+ , P, N, Ca^{2+} , Mg^{2+} and B^+ were determined in a digestion extract with $\text{HNO}_3:\text{HClO}_4$ (2:1, v/v) by Inductively Coupled Plasma optical emission spectrometer (ICP-OES IRIS INTREPID II XDL). Total nitrogen (N) was determined in four plants per treatment. For every plant, approximately 0.1 mg of dry leaf were weighed and ground before analysing the N content in a Flash EA 1112 series thermo elementary analyzer by pure oxygen combustion and nitrogen separation. A thermal conductivity detector provided signals proportional to the total nitrogen concentration.

2.4. Chlorophyll content, fluorescence and colour parameters in leaves

On 17 April 2012, 22 weeks after beginning the treatments, the chlorophyll content was assayed according to Inskeep and Bloom (1985) in the leaves of four plants per treatment. The extraction was made from 50 mg of fresh material in 5 mL of 80% acetone in the dark at 4 °C. The extract was read at 647 and 664 nm in an Uvikon 940 spectrophotometer (Kontron Instruments AG, Zürich, Switzerland). Chlorophyll fluorescence parameters were measured with the Li-Cor 6400 equipped with a fluorometer (6400-40; Li-Cor Inc). The energy conversion efficiency in photosystem II (F'_v/F'_m) was determined during the day in the same plants in which gas exchange was measured. The maximal photochemical efficiency of photosystem (F_v/F_m) on the adaxial leaf surface was also measured once at predawn.

Twenty two weeks after beginning the treatments, leaf colour was measured with a Minolta CR-10 colorimeter, which provided the colour coordinates lightness (L^*), chroma (C^*) and HUE angle (h°) (McGuire 1992), using eight plants per treatment.

2.5. Water relations

Twenty two weeks after beginning the treatments, the diurnal patterns of stomatal conductance (g_s), net photosynthesis (P_n) and stem water potential (Ψ_{stem}) were determined from sunrise to sunset, approximately every 1.5 hours in five plants per treatment. Leaf stomatal conductance and net photosynthesis were determined in leaves exposed to sunlight using a gas exchange system (LI-6400; LI-COR Inc., Lincoln, NE, USA) fixing the conditions of CO_2 concentration at 380 ppm, the photosynthetically active radiation (PAR) at $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$ and the speed of the circulating air flow inside the system at 300 mmol s^{-1} .

Stem water potential was estimated immediately in the leaves in which g_s and P_n were measured, according to Scholander et al. (1965) using a pressure chamber (Model 3000; Soil Moisture Equipment Co., Santa Barbara, CA, USA), placing the leaves in the chamber within 20s of collection and at a pressure of 0.02 MPa s^{-1} (Turner, 1988). Leaves for measuring Ψ_{stem} were taken from the north facing side and were covered with aluminium foil for at least two hours before measurements.

In addition, seasonal changes in g_s and P_n at midday were determined in six leaves per treatment once a week, as described above. The osmotic potential at full turgor (Ψ_{100s}) was also estimated throughout the experiment using excised leaves with their petioles placed in distilled water overnight to reach full saturation. Then, these leaves were frozen in liquid nitrogen (-196°C) and stored at -30°C . After thawing, the osmotic potential at full turgor (Ψ_{100s}) was measured in the extracted sap using a WESCOR 5520 vapour pressure osmometer (Wescor Inc., Logan, UT, USA), according to Gucci et al. (1991).

2.6. Leaf hydraulic conductance and transpiration

Twenty two weeks after beginning the treatments, the leaf hydraulic conductance (K_s) was calculated in four plants per treatment using the formula:

$$K_s = E / (\Psi_{\text{leaf}} - \Psi_{\text{stem}})$$

where E is the transpiration calculated as the difference between evapotranspiration (ET) and evaporation. The ET was determined by recording the weight losses in four pots per treatment, and the evaporation was determined in the same way in a representative plant from which all the leaves had been detached. Ψ_{leaf} is the leaf water potential and Ψ_{stem} is the stem water potential.

Picture 2. Measure of the weight of plants in the scale

2.7. Canopy temperature

Twenty two weeks after beginning the treatments, approximately every 1.5 hours from sunrise to sunset, the canopy temperature from eight plants per treatment was measured manually with a thermal imager (ThermaCam FLIR-e50 System, Inc., UK), which consisted of a 240 x 180 pixel line scan imager operating in the wavebands 7.5–13 μm , with a noise equivalent temperature difference (NETD) of 0.05 $^{\circ}\text{C}$ at 30 $^{\circ}\text{C}$ and an accuracy of 2 $^{\circ}\text{C}$ or 2% of the reading. Infrared (IR) images were taken from the sunlit side of the canopy (Jones et al. 2002). Background temperature was determined as the temperature of a crumpled sheet of aluminium foil in a similar position to the leaves of interest with the emissivity set at 1.0 (Jones et al. 2002). The emissivity for measurements of euonymus leaves/canopies was set at 0.96 (Grant et al. 2006; Leinonen et al. 2006). Images were taken at a distance of ~ 0.7 m from the canopy of interest and later processed with ThermaCam Explorer software (FLIR QuickReport), as previously described in the literature (Grant et al.

2006; Leinonen et al. 2006). Using the IR image software, sunlit portions of leaves were selected based the corresponding visible images which were corrected for spatial calibration drift by subtracting a corresponding reference image of an isothermal surface (the lens cap), as suggested in the literature (Jones et al. 2002; Grant et al. 2006).

Picture 3: thermal imager

2.8. Data analyses and statistics

Eighty plants were randomly distributed to two irrigation treatments (40 plants per 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 ($P \leq 0.05$).

3. Results

3.1. Water analyses

Eight weeks after beginning treatments, the chemical properties of the irrigation waters were analyzed (Table 1). Although the control and RW waters

showed similar pH values, the EC value of RW water was about four times higher than the EC value of control. Also, higher values of boron, calcium, potassium, magnesium, and phosphorus values were observed in the RW water. The Na⁺ and Cl⁻ values in this water were about 11 and 10 times higher, respectively, than in the control water (Table 1).

Table 1. Physicochemical analyses for the irrigation treatments. Data are values from samples collected eight weeks after applying the treatments.

Physicochemical Analyses	C	RW
pH	7.97	7.77
EC	0.87	4.02
Cl (mg L ⁻¹)	69.5	688.1
Na (mg L ⁻¹)	52.07	586.2
B (mg L ⁻¹)	0.09	0.97
Ca (mg L ⁻¹)	94.21	214.8
K (mg L ⁻¹)	3.39	51.15
Mg (mg L ⁻¹)	41.87	177.3
P (mg L ⁻¹)	0.22	1.62

3.2. Measurements of growth and mineral content

At the end of the experiment, in spite of there being no differences in the total dry weight (DW) between treatments, the leaf DW of euonymus plants irrigated with RW was statistically higher, while the root DW was lower than in C plants (Table 2). Therefore, the leaf DW/total DW ratio increased while the root/shoot ratio and root DW/total DW decreased in RW plants with respect to values in the C plants. In contrast, total leaf number and total leaf area showed no difference between irrigation treatments. As regards the height, the C plants were shorter than RW plants (Table 2).

Table 2. Biomass parameters at the end of experiment in euonymus plants irrigated with water from different sources. Values are means $\pm$ SEM (n=3 plants for biomass and n=10 plants for height).

Growth parameters	C	RW
Leaf DW (g plant ⁻¹)	129.27 $\pm$ 33.06 *	179.00 $\pm$ 12.13
Root DW (g plant ⁻¹)	104.64 $\pm$ 13.21 *	67.56 $\pm$ 5.27
Total DW (g plant ⁻¹)	346.89 $\pm$ 70.04	359.61 $\pm$ 24.86
Root/ Shoot ratio	0.455 $\pm$ 0.056 *	0.231 $\pm$ 0.008
Total leaf number	2910.4 $\pm$ 1049.5	3321.4 $\pm$ 653.0
Total leaf area (cm ²)	15053 $\pm$ 4677	23409 $\pm$ 3197
Leaf DW/Total DW	36.51 $\pm$ 2.05 *	49.79 $\pm$ 0.45
Root DW/Total DW	31.08 $\pm$ 2.62 *	18.78 $\pm$ 0.51
Height (cm)	57.80 $\pm$ 1.70 *	62.50 $\pm$ 2.66

Values within a row followed by * indicate statistically significant differences at confidence 95% between treatments, according to Duncan's multiple range test.

Twenty two weeks after beginning the treatments, an increase in the Na⁺, Cl⁻ and B⁺ content of leaves was observed in RW, although Mg²⁺, K⁺ and P were also accumulated. Moreover, the total nitrogen concentration was higher in RW plants than in the control plants (Table 3).

Table 3. Leaf mineral content in euonymus plants irrigated with water from different sources, twenty two weeks after applying treatments. Values are means $\pm$ SEM (n=4 plants).

Leaf analyses	C	RW
B (mg Kg⁻¹)	25.91 $\pm$ 0.70 *	31.63 $\pm$ 1.85
Ca (g 100g⁻¹)	0.86 $\pm$ 0.06	1.09 $\pm$ 0.12
Mg (g 100g⁻¹)	0.15 $\pm$ 0.01 *	0.18 $\pm$ 0.01
Na (g 100g⁻¹)	0.15 $\pm$ 0.04 *	0.33 $\pm$ 0.06
Cl (g 100g⁻¹)	1.02 $\pm$ 0.07 *	1.35 $\pm$ 0.01
K (g 100g⁻¹)	0.79 $\pm$ 0.06 *	1.23 $\pm$ 0.09
P (g 100g⁻¹)	0.11 $\pm$ 0.01 *	0.15 $\pm$ 0.01
Zn (mg Kg⁻¹)	24.41 $\pm$ 9.01	21.44 $\pm$ 1.53
Fe (mg Kg⁻¹)	27.39 $\pm$ 7.70	23.42 $\pm$ 2.14
Mn (mg Kg⁻¹)	20.20 $\pm$ 1.41	23.28 $\pm$ 1.81
Cu (mg Kg⁻¹)	1.50 $\pm$ 0.48	1.77 $\pm$ 0.37
N (mg Kg⁻¹)	1.02 $\pm$ 0.09*	1.36 $\pm$ 0.10

Values within a row followed by * indicate statistically significant differences at confidence 95% between treatments, according to Duncan's multiple range test.

3.3. Chlorophyll content, fluorescence and colour parameters in leaves

Twenty two weeks after beginning the treatments, the leaf chlorophyll content (a, b and total) was higher in RW plants than in the control plants (Table 4). As regards colour parameters, the lightness and chroma were lower and the HUE angle was higher in the RW plants than in the C plants (Table 4). The result was a more intense green colour in leaves of the RW plants than in the control plants. No visual damage, such as chlorosis or necrosis to plants was observed and the aesthetic value of the plants was not diminished.

Picture 4. Control and RW plants at the end of the experiment

Table 4. Colour parameters and chlorophyll content (Chl a, b, and total) and leaf hydraulic conductance (K_s) in euonymus plants irrigated with water from different sources, twenty two weeks after applying treatments. Values are means $\pm$ SEM (n=8 plants for colour parameters and n=4 plants for leaf hydraulic conductance).

Parameters	C	RW
Lightness	47.74 $\pm$ 0.43 *	44.56 $\pm$ 0.59
Chroma	25.69 $\pm$ 0.64 *	20.31 $\pm$ 0.86
HUE angle	115.08 $\pm$ 0.54 *	117.54 $\pm$ 0.66
Chl a (mg g_{DW}⁻¹)	2.12 $\pm$ 0.14 *	2.59 $\pm$ 0.15
Chl b (mg g_{DW}⁻¹)	0.72 $\pm$ 0.05 *	0.87 $\pm$ 0.05
Chl T (mg g_{DW}⁻¹)	2.84 $\pm$ 0.18 *	3.46 $\pm$ 0.20
K_s (mmol m⁻² MPa⁻¹ s⁻¹)	2.245 $\pm$ 0.313 *	1.501 $\pm$ 0.272

Values within a row followed by * indicate statistically significant differences at confidence 95% between treatments, according to Duncan's multiple range test.

Twenty two weeks after beginning the treatments, the F'_v/F'_m fluorescence decreased in all plants as the day progressed, with no statistical differences between treatments until 14:00 h, when the RW plants began to show higher F'_v/F'_m values which remained higher than in C plants until the end of the solar light (Fig. 1a). The maximum efficiency of PSII in dark-adapted leaves (F_v/F_m) remained around 0.8 in all plants.

3.4. *Plant water status and gas exchange*

The stomatal conductance values in C plants were statistically higher than in RW plants between 08:00 h and 12:00 h. In addition, a decrease in stomatal conductance was observed in the all plants between 08:00 h and 10:00 h (Fig. 1b). From the midday, there were no significant differences between treatments in g_s and the RW plants showing a tendency to recover their values with respect to the control plants. Similar behaviour was found for the maximum photosynthesis rate, which showed a tendency to decrease as the morning progressed, but with no significant differences between treatments (Fig. 1c). The stem water potential decreased in both control and RW plants, reaching the lowest values between 14:00 h and 16:00 h. However, the Ψ_{stem} in the RW plants was almost always significantly lower than in the C plants during the day (with differences of 0.2 to 0.4 MPa between treatments) (Fig. 1d).

Fig. 1 Daily evolution of fluorescence, F_v/F_m (A), stomatal conductance, g_s (B), net photosynthetic rate, P_n (C) and stem water potential, Ψ_{stem} (D), in euonymus plants irrigated with water from different sources twenty two weeks after beginning the treatments. Values are mean of five plants. The vertical bars indicate standard errors. Asterisks indicate statistically significant differences by Duncan_{0.05} test.

Throughout the experiment, g_s and P_n were similar for both treatments, except on a few occasions when the values in RW plants fell below those of C plants (Fig. 2a, b). The osmotic potential at full turgor (Ψ_{100s}) was lower in RW plants than in C plants from week 18 (Fig. 2c).

Fig. 2 Stomatal conductance, g_s (A), net photosynthetic rate, P_n (B) and osmotic potential at full turgor, Ψ_{100s} (C) at midday in euonymus plants irrigated with water from different sources throughout the experiment. Values are mean of six plants. The vertical bars indicate standard errors. Asterisks indicate statistically significant differences by Duncan_{0.05} test

3.5. Leaf hydraulic conductance, canopy temperature and transpiration

Twenty two weeks after beginning the treatments, the leaf hydraulic conductance was lower in RW plants than in C plants (Table 4). The canopy

temperature throughout the day was slightly higher in the RW plants, with maximum differences between treatments between 08:00 h and 12:00 h (Fig. 3a). Gravimetric transpiration of both treatments increased in the central hours of the day. However, the transpiration rate was higher in C plants than in RW plants during practically the whole day (Fig. 3b).

Fig. 3 Daily canopy temperature, T_c (A) and gravimetric transpiration (B) in euonymus plants irrigated with water from different sources twenty two weeks after beginning the treatments. Values from temperature are mean of eight plants. The vertical bars indicate standard errors. Asterisks indicate statistically significant differences by Duncan_{0.05} test

4. Discussion

The effect of using treated wastewater on the growth of ornamental species has been studied by many authors (Bañón et al. 2011; Gori et al. 2008; Lubello et al. 2004; Wu et al. 2001b) with different results. In euonymus plants, the reclaimed wastewater did not affect the total dry biomass at the end of experiment (Table 2). However, leaf DW increased in these plants probably because the nutrients synthesized were destined to the leaves, at the expense of the roots, whose DW decreased. This decrease in root dry mass could be explained by a mechanism to

avoid the entrance of toxic ions. Similar results were found in *Abutilon* 'Kentish Belle' (Gori et al. 2000), *Phlomis purpurea* (Álvarez et al. 2012) and in *Polygala myrtifolia* (Bañón et al. 2011), although other authors have reported that shoot growth is more sensitive to salinity than the root part of the ornamental plants, such as *Lantana camara*, *Delosperma cooperi*, and *Teucrium chamaedrys* (Bañón et al. 2011; Niu and Rodriguez 2006a). Thus, the excess salinity of reclaimed water may affect each part of the plant differently (Álvarez et al. 2012). In our previous study (Gómez-Bellot et al. 2013b) euonymus plants irrigated with reclaimed wastewater (from a sewage treatment plant in Campotejar, EC= 4 dS m⁻¹ and leaching of 30%) showed decreased growth and a lower aesthetic value. However, in this case, our results are more in accordance with Miyamoto et al. (2004) who reported that euonymus is able to support 6-8 dS m⁻¹, since the total growth in our plants was not negatively affected by salinity. Similar results were observed in cultivars of calla lilies (Veatch-Blohm et al. 2012).

The decrease in stem water potential (Fig. 1d) and in leaf hydraulic conductivity (Table 4) of plants irrigated with RW reflected their greater difficulty in absorbing water during the day than the control plants, as a consequence of higher salt accumulation in the substrate (Álvarez et al. 2012). As regards gas exchange, the most dramatic response to salinity is a decrease in stomata aperture (Munns and Tester 2008), and as a consequence, stomatal conductance decreases. Although photosynthesis could also be the growth-limiting factor (Sánchez-Blanco et al., 2002), many species are able to maintain the photosynthesis rate unaltered despite reduced stomatal conductance (James et al. 2002) as occurred in our case (Fig. 1c). In addition, all the plants in our experiment showed decreased g_s values between 08:00 h and 10:00 h as well as lower F'_v/F'_m values (Fig. 1a, b) between 06:30 h and 10:00 h, probably due to limited stomatal aperture, since the temperature in the greenhouse increased during this period (Cameron et al. 2008). The same behaviour was found by Petkova et al. (2007). Plants irrigated with reclaimed wastewater showed higher F'_v/F'_m values than the control plants from 14:00 h and the F_v/F_m values indicated the absence of damage to the photosynthetic apparatus (Percival 2005; Sixto et al. 2006).

As regards seasonal gas exchange, the g_s and P_n values (Fig. 2a,b) hardly showed any statistical differences between treatments during the experiment, and RW plants were able to maintain osmotic adjustment (Fig. 2c). It is well known that osmotic adjustment involves the net accumulation of solutes in a cell in response to salinity (Ghoulam et al. 2002). Consequently, the osmotic potential decreases, which, in turn, attracts water into the cell and enables turgor to be maintained (Bradley et al., 1991).

On the other hand, the canopy temperature in RW plants (Fig. 3a) responded to the high temperature in the greenhouse and reflected the difficulty of plants to take water from the substrate during the day, as it was evidenced by the stem water potential behavior in RW plants. The canopy temperature values were closely related to the stomatal opening, even though the g_s values of euonymus plants were not excessively high in our conditions. As the plant transpires, the evaporation of water consumes heat energy, which decreases the leaf temperature and results in a cooling effect. The decrease of transpiration in RW plants led to an increase in the canopy temperature, due to a reduction in the rate of heat removal (Colaizzi et al. 2012). Thus, the measurement of leaf temperature by thermal imaging can be regarded as a reliable way to detect changes in the physiological status of plants in response to abiotic stresses such as salinity (Sirault et al. 2009).

Toxic ions like B^+ , Cl^- and Na^+ were accumulated in leaves of the RW plants (Table 3) as a consequence of the water stream from the substrate to aerial tissues. Normally, The uptake of beneficial nutrients such as Ca^{2+} and Mg^{2+} , which play an important role in cell structure, development and plant metabolism, is often reduced in salt-sensitive plants grown in saline environments (Almansouri et al. 1999; Chaparazedh et al. 2003). However, in our experiment, Mg^{2+} , K^+ , P and total nitrogen were accumulated in leaves and Ca^{2+} was not reduced, because of the greater amounts of these ions supplied with the wastewater. Similar results have been observed in *Petunia hybrida* and *Calceolaria hybrida* (Fornes et al. 2007). In addition, leaf Cl^- concentration in RW plants was relatively low compared with other horticultural crops irrigated with low quality water (Niu et al. 2010b; Sun et al. 2013). Therefore, the salts present in the reclaimed wastewater did not interfere in the

absorption of Mg^{2+} , K^+ , P, Ca^{2+} and total nitrogen, whose availability for the plant was maintained.

The increase in HUE angle in RW plants produced leaves with a more intense green colour, which was related with the higher chlorophyll (Table 4) and nitrogen content of these plants. Furthermore, the increase in fluorescence after midday and higher chlorophyll and nitrogen content of the RW plants could also be related with photosynthetic behaviour (Evans et al. 2001; Jiang et al. 2000; Reich et al. 1995) and with a higher recovery capacity of these plants after midday. Salinity stimulates chlorophyll degradation and affects chlorophyll synthesis (Santos 2004). However, the above results indicate that Cl^- and Na^+ were not concentrated enough in the leaves to cause photochemical damage. In contrast, the beneficial components from reclaimed wastewater could increase the chlorophyll content and consequently helped maintain P_n , during the growth. Salinity derived from reclaimed wastewater may reduce growth and leaf expansion as a result of osmotic effects or toxicity, as we observed in a previous study in euonymus (Gómez-Bellot et al. 2013b). However, the physiological response to reclaimed wastewater throughout the day suggested that euonymus is resistant to these saline conditions while the nutrients provided by the wastewater probably conferred benefits on the physiological function of the plant.

5. Conclusions

In this experiment, the plants showed no toxicity symptoms or loss of growth, and their aesthetic value was maintained. This response could be explained by: 1) the age of the plants and hence, a long acclimatization period in the greenhouse before the reclaimed wastewater treatment was imposed, 2) the higher leaching percentage than in the earlier experiment and 3) the appreciable amounts of Mg^{2+} , Ca^{2+} , K^+ and P contained in the wastewater which could offset the toxic effects produced by high B^+ , Na^+ and Cl^- concentrations, which in turn were lower in these conditions.

Chapter 3.2

Evaluation of the sensitivity of thermal imaging and infrared thermometry to detect water status changes in *Euonymus japonica* plants irrigated with saline reclaimed water

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Abstract

To assess the potential of thermal imaging and infrared thermometry for monitoring the effect of salinity in *Euonymus japonica* (euonymus) plants an experiment was developed with the following irrigation treatments: Control ($EC < 0.9 \text{ dS m}^{-1}$) and reclaimed water, RW ($EC \approx 4 \text{ dS m}^{-1}$). The experiment was conducted for a period of more than eight months in a plastic greenhouse. The diurnal course of canopy temperature (T_c) and the difference between canopy and air temperature ($T_c - T_a$) of both sunlit and shaded side was influenced by high concentration of salts in saline reclaimed water. The negative relationship between T_c and stomatal conductance (g_s), and between $T_c - T_a$ and g_s in April was significant during most of the day, and more specifically at midday. Regarding the effect of canopy side, better regression coefficients were obtained between T_c and g_s , and between $T_c - T_a$ and g_s on the sunlit side of the canopy than on the shaded side. However, except for CWSI on the sunlit side of the canopy, few differences between treatments during the day were reported for thermal indices. From April onwards, canopy temperatures were higher in RW plants than in the control, indicating the dependence of canopy temperature on salinity. Comparison of the sensitivity of discrete (stem water potential and g_s) and continuous (T_c and thermal-derived parameters measured by infrared thermometers) indicators of saline stress, showed that T_c and $T_c - T_a$ were the most suitable plant-based indicators for precise irrigation scheduling.

Keywords: Infrared thermography; infrared thermometers; ornamental plants; salinity; stomatal conductance; treated wastewater.

1. Introduction

Euonymus japonica (euonymus) is a fast-growing, woody evergreen shrub that is used to add colour to the landscape along the Mediterranean coast of Spain, an area where water scarcity is a reality and there is a constant search for new alternatives to optimize the use of irrigation water. Reclaimed water is a valuable resource for irrigation water, since, besides acting as an alternative water resource, its use decreases the potential for environmental contamination and puts the nutrients it contains to good use, providing fertilizers to plants (Angelakis et al. 1999; Adrover et al. 2008). For this reason reclaimed water is being widely studied in many developing countries (Pedrero et al. 2010; Norton-Brandao et al. 2013), especially for use in agricultural plants (Reboll et al. 2000; Paranychianakis et al. 2004; Mounzer et al. 2013; Pedrero et al. 2013) and, to a lesser extent, in ornamental plants (Bañón et al. 2011; Valdés et al. 2012; Cassaniti et al. 2013; Gómez-Bellot et al. 2013a, 2013b). However, poor water management with reclaimed water can cause problems of salinity that affect the plant and induce soil degradation. The use of reclaimed water in ornamental plants has a number of disadvantages, including the effects of salinity may have on plants due to an excess of ions, nutritional imbalances, the potential toxicity of heavy metals and obstruction of the irrigation systems. Some of these responses are reductions in the growth and size of the plants, and leaf burn. Also different behaviors in ornamental plant water relations when reclaimed water for irrigation was applied have been provided (Acosta-Motos et al. 2014; Gómez-Bellot et al. 2013a).

Studies have reported that canopy temperature or thermal indices may also be useful indicators of an osmotic effect due to salt stress (Kluitenberg and Biggar 1992; Azevedo-Neto et al. 2004). As the osmotic stress increases, the transpiration rate decreases and leaf temperature increases. Thus, the differences in temperature between control and salt stressed plants widen (Halim et al. 1990; Kluitenberg and Biggar 1992; Sirault et al. 2009). However, leaf temperature depends on humidity, air temperature and incident radiation. To normalize the remotely sensed measurements for the effect of these environmental factors in the greenhouse, temperature variables

and indices such as crop water stress index (CWSI) and the stomatal conductance index (I_G) have been proposed. These indices take into account reference temperatures of maximum and minimum transpiration (Jones 1999; Leinonen et al. 2006). Higher CWSI values indicate greater plant stress and the opposite in the case of I_G (Costa et al. 2013). On the other hand, other authors have considered the difference between canopy temperature (T_c) and air temperature (T_a) as a normalization index to study the water status of the plants (Jackson et al. 1977): the greater the plant abiotic stress, the more pronounced the differences between T_c and T_a (Jones et al. 2009).

In recent decades, infrared thermometry and infrared thermal imaging have come to be regarded as good tools for detecting stomatal closure and indirectly assessing plant water status (Jones 2004; Costa et al. 2013). Infrared thermometers (IRTs) are infrared temperature sensors that provide a non-contact means of measuring the surface temperature of the plants. They can be used to continuously detect plant leaf temperatures. They are fast and cheap, and can be installed permanently in the field. However, depending on the architecture of the plant, the recorded temperature is not only the canopy temperature but a composite of vegetation and background temperatures (Jackson et al. 1981). Thermal imaging is a technique capable of producing a visible image from an infrared radiation emitted by objects according to their thermal condition. It is a more delicate and expensive technique than infrared thermometry. However, the temperatures obtained are very precise and, by processing of the thermal images, plant material temperature can be distinguished from the background temperature (Leinonen and Jones 2004; Möller et al. 2007).

Many studies have reported the use of thermal imaging and/or infrared thermometry to detect abiotic stresses in diverse field crops: grapevine (Baluja et al. 2012; Fuentes et al. 2012), citrus (García-Tejero et al. 2011; Ballester et al. 2013), persimmon trees (Ballester et al. 2013), almond trees (García Tejero et al. 2012), bean (Durigon and van Lier 2013), cotton (Padhi et al. 2012), maize (Zandonadi et al. 2005), oak (Grant et al. 2010), strawberries (Grant et al. 2012). However, although results of greenhouse experiments using different ornamental plants point to the

potential of using thermal imaging for early selection of physiologically superior lines in breeding programmes (Reynolds et al. 1999; Lopes and Reynolds 2010), very few studies have been conducted to diagnose plant water status in ornamental plants using these techniques (Grant et al. 2011).

For all these reasons, the main objective of this study was to assess the potential of thermal imaging and infrared thermometry for monitoring water status changes of euonymus plants irrigated with two types of water from different saline levels (one a reclaimed water with an EC of 4 dS m^{-1} and other control with $\text{EC} < 0.9 \text{ dS m}^{-1}$). The specific aims were (i) to assess the daily and seasonal impact of the water quality on the plant water status, based on their canopy temperature obtained with a hand-operated thermographic camera, and (ii) to compare the feasibility of using non-destructive thermal measurements as an alternative to using methods based on discrete measurements of conventional plant water indicators. Also, a comparison was made between thermal imaging and infrared thermometry for measuring canopy temperature.

2. Material and methods

2.1. Plant material and growth conditions

One year old euonymus plants ($n=80$) were planted in 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 experiment was conducted from 18 November 2011 (DOY 322) to 31 July 2012 (DOY 212) in a plastic greenhouse equipped with a cooling system at the CEBAS experimental farm located in Santomera (Murcia, Spain). The micro-climatic conditions were recorded continuously with a Vaisala probe (HMP45C) throughout the experimental period. The average temperature, relative humidity and vapour pressure deficit (VPD) were 22°C , 64% and 1.3 kPa , respectively.

Two irrigation treatments were imposed during the experimental period: Control ($EC < 0.9 \text{ dS m}^{-1}$, leaching 15% of the applied water), and reclaimed water, RW ($EC \approx 4 \text{ dS m}^{-1}$, leaching 40%) from a sewage treatment plant located in Campotejar (Murcia, Spain). The reclaimed water treatment plant applies a conventional activated sludge process followed by ultraviolet application for tertiary treatment. Plants were irrigated daily to 100% water-holding capacity, the duration of each irrigation episode depending on the season, climatic conditions and plant development. Water consumption was measured gravimetrically throughout the experimental period and was determined from the difference in weights (weight after irrigation, when drainage stopped, and weight before irrigating again). One drip nozzle, delivering 2 L h^{-1} per pot, was connected to two spaghetti tubes, one on each side of every pot.

2.2. Water quality

The inorganic solute content, pH and EC of the irrigation waters were assessed in January 2012, 8 weeks after beginning the treatments (Table 1). The samples were collected in glass bottles and stored at 5°C before being processed for chemical analysis. The pH was measured with a Cryson-507 pH-meter (Crisom Instruments S.A. Barcelona, Spain); EC was determined using the multirange equipment, Cryson-HI8734 (Crisom Instruments S.A. Barcelona, Spain); the concentrations of macronutrients (Na^{+} , K^{+} , P^{+} , Ca^{2+} and Mg^{2+}) and micronutrients including B^{+} were determined by inductively coupled plasma optical emission spectrometer (ICP-ICAP 6500 DUO Thermo, England) and chloride was analysed by ion chromatography with a Metrhom Chromatograph (Switzerland).

Table 1. Physicochemical analyses for both irrigation treatments: Control (C) and reclaimed water (RW). Data are values from samples collected on January 2012.

Physicochemical		
Analyses		
	C	RW
pH	7.97	7.77
EC	0.87	4.02
Na (mg L ⁻¹)	52.07	586.20
Chlorides (mg L ⁻¹)	69.50	688.10
B (mg L ⁻¹)	0.09	0.97
Ca (mg L ⁻¹)	94.21	214.80
K (mg L ⁻¹)	3.39	51.15
Mg (mg L ⁻¹)	41.87	177.30
P (mg L ⁻¹)	0.22	1.62

2.3. Canopy temperature and thermal indices

Thermal images were obtained with a thermal imager (ThermaCam FLIR-e50 System, Inc., UK), which consisted of a 240 x 180 pixel line scan imager operating in the 7.5–13 μm region, with a noise equivalent temperature difference (NETD) of 0.05°C at 30°C and an accuracy of 2°C or 2% of the reading. This camera supplies a combination of high resolution infrared and visible spectrum images facilitating the identification of leaves in the images. The background temperature required for calculating object temperatures was estimated as the radiative temperature of a crumpled sheet of aluminium foil in a similar position to the leaves of interest with the emissivity set at 1.0 (Jones et al. 2002). Emissivity for leaf measurements was set at 0.96 (Jones 2004; Grant et al. 2006; Leinonen et al. 2006). Images were taken at a distance of $\approx 0.7\text{m}$ from the canopy of interest. Thermal images were processed with ThermaCam Explorer software (FLIR QuickReport).

Picture 1. Thermogram taken by thermal imager

Infrared (IR) images were taken periodically from the sunlit side of the canopy (Jones et al. 2002) in four plants per irrigation treatment at midday. In addition, diurnal variations in the same measurements were determined on two days during the experiment (DOYs 107 and 193) approximately every 1.5 hours from 6.30 h until 18.30 h in eight plants per treatment from the sunlit and shaded side of the plant. To facilitate understanding of the work only the results of DOY 107 (17 April) are given.

Moreover, from the beginning of the experiment, four plants per treatment were monitored to capture the canopy temperature (T_c) using infrared thermometers SI-111 (Apogee Instruments) connected to a datalogger (model CR1000 with AM16/32 multiplexer, Campbell Scientific Ltd., Logan, USA) programmed to report 30 min means. A value of 0.96 was assumed for the emissivity to calculate T_c .

Picture 2. Infrared thermometers to measure canopy temperature

Two thermal indices were calculated: the stomatal conductance index (I_G) and the crop water stress index (CWSI). The index $I_G = (T_{dry} - T_c)/(T_c - T_{wet})$ is theoretically proportional to stomatal conductance of water vapour (Jones et al. 2002). The $CWSI = (T_{dry} - T_c)/(T_{dry} - T_{wet})$ (Idso 1982) commonly varies between 0 and 1. Values close to 0 indicate a fully transpiring leaf/crop (no stress); whereas values close to 1 indicate a non-transpiring leaf/crop (maximum stress). Wet reference leaf temperatures (T_{wet}) were obtained by regularly spraying both sides of the leaves with water to maintain their moisture level. Dry reference leaf temperatures (T_{dry}) were obtained by covering both sides of the leaves with petroleum jelly (Vaseline). T_{wet} and T_{dry} were measured just before IR images were taken.

2.4. Water relations and stomatal conductance

Seasonal changes in stem water potential (Ψ_{stem}) and stomatal conductance (g_s) were measured at midday immediately after thermal imaging measurements. We measured six plants per treatment throughout the assay. The osmotic potential at full turgor (Ψ_{100s}) was also estimated throughout the experiment using excised leaves with their petioles placed in distilled water overnight to reach full saturation. Then, these leaves were frozen in liquid nitrogen (-196°C) and stored at -30°C . After thawing, the osmotic potential at full turgor (Ψ_{100s}) was measured in the extracted sap

using a WESCOR 5520 vapour pressure osmometer (Wescor Inc., Logan, UT, USA) according to Gucci et al. (1991).

In addition, diurnal variations of g_s and Ψ_{stem} were determined from predawn for Ψ_{stem} and from 6.30 h for g_s to sunset at 1.5 hourly intervals on two cloudless days (DOY 107 and 193) in five plants per treatment. For the same reason as mentioned above only results of DOY 107 (17 April) are shown.

Stem water potential (Ψ_{stem}) was estimated according to the method described by Scholander et al. (1965), using a pressure chamber (Model 3000; Soil Moisture Equipment Co., Santa Barbara, CA, USA) in which leaves were placed within 20s of collection and pressurised at a rate of 0.02 MPa s^{-1} (Turner 1988). Leaves for Ψ_{stem} measurements were taken from the north-facing side and were covered with aluminium foil for at least 2 h before measurements.

Picture 3. Leaves covered with aluminium foil

Leaf stomatal conductance (g_s) was measured on a similar number and type of leaves as use for Ψ_{stem} , using a portable infrared gas analyser (Li-Cor 6400, Li-Cor, Lincoln, NE, USA) equipped with a fluorescence chamber. The measurements were made at $1000 \mu\text{mol m}^{-2} \text{ s}^{-1}$ to ensure light saturation, with 10% blue light. Cuvette CO_2 concentration was set at $380 \mu\text{mol CO}_2 \text{ mol air}^{-1}$ and air flow rate at 300 mmol s^{-1} .

2.5. Indicator sensitivity

To compare the sensitivity of both continuous (canopy temperature and thermal-derived parameters measured with infrared thermometers) and discrete (Ψ_{stem} and g_s) plant water status indicators, it is not meaningful to compare absolute values but values relative to those of the control plants (Naor and Cohen 2003). For this, the signal intensity of an indicator is defined as the relative value (Control/RW or RW/Control), while variability or noise is the coefficient of variation of the mean. Thus, the signal:noise ratio integrates both the indicator strength and its variability, giving the potential of the indicator (Goldhamer and Fereres 2001).

2.6. Data analyses and statistics

Eighty plants were randomly distributed to two irrigation treatments (40 plants per treatment). A one-way ANOVA was performed and means were separated by LSD_{0.05} range test. Relationships between parameters were fitted to linear regressions using SPSS v. 14.0 software (SPSS Inc. 1996) and Pearson's correlation analysis was used to test for any relationship between canopy temperature and physiological parameters.

3. Results

3.1. Climate parameters

Air temperature (T_a) inside the greenhouse could be clearly divided into two periods (Fig. 1), the first lasting until the end of April ($\approx$ DOY 120), with a mean air temperatures of 14°C, and a second warmer period until the end of the experiment with a mean air temperatures of around 24°C. Vapour pressure deficit (VPD) during the experiment showed the same trend as T_a , although less pronounced, reaching maximum values (2.72 kPa) at the end of June (DOY 177) (Fig. 1).

Fig. 1. Daily mean air vapour pressure deficit (VPD, solid line) and daily mean air temperature (T_a , dotted line) during the experimental period.

3.2. Diurnal course of stem water potential (ψ_{stem}), stomatal conductance (g_s) and canopy temperature (T_c)

Diurnal pattern of stem water potential (ψ_{stem}) in April showed maximum values at predawn, followed by a decrease in the morning, regardless of the irrigation treatment (Table 2). ψ_{stem} and g_s values of RW plants were lower than the control values, although in both cases the maximum differences between treatments (RW and C) were more evident before 14.00 h (Table 2).

Table 2. Diurnal pattern of vapour pressure deficit (VPD), stem water potential (Ψ_{stem}) and stomatal conductance (g_s) for euonymus plants irrigated with control (C) and reclaimed water (RW) on DOY 107 (17 April). Values are means $\pm$ SEM (n=5 plants).

Solar time (h)	VPD (kPa)	Ψ_{stem} (MPa)		g_s (mmol m ⁻² s ⁻¹)	
		C	RW	C	RW
04.30	0.99	-0.60 $\pm$ 0.03 a	-0.75 $\pm$ 0.05 b	n.m.	n.m.
06.30	1.03	-0.56 $\pm$ 0.05 a	-0.71 $\pm$ 0.02 b	6.53 $\pm$ 1.03 a	11.77 $\pm$ 7.26 a
08.00	1.46	-0.92 $\pm$ 0.13 a	-1.21 $\pm$ 0.06 b	53.74 $\pm$ 11.39 a	37.81 $\pm$ 6.17 b
09.00	1.70	-0.88 $\pm$ 0.04 a	-1.20 $\pm$ 0.03 b	37.03 $\pm$ 7.95 a	20.91 $\pm$ 3.64 b
10.00	1.97	-0.76 $\pm$ 0.08 a	-1.08 $\pm$ 0.08 b	32.33 $\pm$ 7.52 a	21.49 $\pm$ 3.71 b
12.00	1.88	-0.85 $\pm$ 0.07 a	-1.04 $\pm$ 0.02 b	34.51 $\pm$ 4.93 a	21.77 $\pm$ 4.82 b
14.00	1.96	-1.09 $\pm$ 0.07 a	-1.11 $\pm$ 0.04 a	28.41 $\pm$ 4.24 a	37.31 $\pm$ 6.07 a
16.00	1.85	-1.01 $\pm$ 0.09 a	-1.10 $\pm$ 0.10 a	32.19 $\pm$ 3.13 a	32.37 $\pm$ 3.95 a
17.00	1.77	-0.96 $\pm$ 0.08 a	-1.19 $\pm$ 0.08 b	33.44 $\pm$ 8.00 a	28.18 $\pm$ 3.25 a
18.30	1.46	-0.84 $\pm$ 0.04 a	-1.00 $\pm$ 0.03 b	4.03 $\pm$ 2.23 a	3.04 $\pm$ 0.44 a

Means within a row without a common lowercase letter are significantly different. n.m., not measured

On the sunlit side, the canopy temperature (T_c) of RW plants was higher than that of the control from 8.00 h to 12.00 h (Fig. 2A). Similar behaviour was observed for the diurnal time course of $T_c - T_a$ (Fig. 2C). CWSI values of RW plants tended to be higher than those of the control plants for most of the day (Fig. 2E), reaching values up to 2.4 times greater in the case of CWSI in RW plants in the morning. In contrast, the I_G values of RW plants were only significantly lower than those of the control plants at 8.00 h (Fig. 2G). On the shaded side, the trend of T_c and $T_c - T_a$ was similar to that on the sunlit side, although with slightly lower values and with no differences between treatments at midday (Fig. 2B, D). The thermal indices (CWSI and I_G) in RW plants were not influenced by high concentration of salts on this side (Fig. 2F, H).

Fig. 2. Diurnal course on DOY 107 (17 April) of (A) canopy temperature, (C) differences between canopy and air temperature ($T_c - T_a$), (E) crop water stress index (CWSI) and (G) I_G index on the sunlit side and, (B) canopy temperature, (D) differences between canopy and air temperature ($T_c - T_a$), (F) crop water stress index (CWSI) and (H) I_G index on the shaded side in euonymus plants irrigated with control (C) and reclaimed water (RW). Values are mean of eight plants per treatment. Asterisks indicate statistically significant differences between treatments by LSD_{0.05} test.

Linear regressions between T_c , $T_c - T_a$ and the thermal indices (CWSI and I_G), and g_s during the course of the day, are shown in Table 3.

On the sunlit side, the negative relationships between T_c , $T_c - T_a$ and CWSI, and g_s were significant throughout the day except at 18.30 h when the g_s values of the plants were very low. Similar behaviour, although positive, was shown in the relationship between I_G and g_s . However, the best coefficient of determination in almost all linear regressions was obtained at around midday with values of $R^2=0.80$, $P<0.001$; $R^2=0.78$, $P<0.001$; $R^2=0.65$, $P<0.001$ and $R^2=0.71$, $P<0.001$ for T_c , $T_c - T_a$, CWSI and I_G , respectively (Table 3). Similar behaviour was observed for the shaded side, although, in general, coefficients of correlations were lower on this side (Table 3).

Table 3. Coefficients of determination (R^2) for linear regression and sign for Pearson's correlations (s) found between canopy temperature (T_c), difference between canopy and air temperature (T_c-T_a), crop water stress index (CWSI) and I_G index, and stomatal conductance (g_s) of plants from sunlit and shaded canopy, regardless of irrigation treatments, at different times of DOY 107. $n = 10$.

	SUNLIT			SHADED		
	Sign (r)	R^2	P	Sign (r)	R^2	P
T_c versus g_s						
6.30	-	0.42	*	-	0.36	*
9.00	-	0.62	**	-	0.52	*
10.00	-	0.61	**	-	0.58	**
12.00	-	0.80	***	-	0.68	**
14.00	-	0.45	*	-	0.44	*
16.00	-	0.63	**	-	0.52	*
18.30	+	0.06	n.s.	-	0.02	n.s.
T_c-T_a versus g_s						
6.30	-	0.41	*	-	0.37	*
9.00	-	0.55	**	-	0.39	*
10.00	-	0.61	**	-	0.59	**
12.00	-	0.78	***	-	0.63	**
14.00	-	0.42	*	-	0.42	*
16.00	-	0.59	**	-	0.56	**
18.30	+	0.05	n.s.	-	0.01	n.s.
CWSI versus g_s						
6.30	-	0.41	*	-	0.37	*
9.00	-	0.53	*	-	0.44	*
10.00	-	0.62	**	-	0.40	*
12.00	-	0.65	**	-	0.48	*
14.00	-	0.40	*	-	0.40	*
16.00	-	0.53	*	-	0.48	*
18.30	+	0.06	n.s.	+	0.09	n.s.
I_G versus g_s						
6.30	+	0.37	*	+	0.39	*
9.00	+	0.67	**	+	0.55	**
10.00	+	0.54	**	+	0.48	*
12.00	+	0.71	**	+	0.69	**
14.00	+	0.60	**	+	0.42	*
16.00	+	0.56	**	+	0.55	**
18.30	-	0.01	n.s.	-	0.09	n.s.

P, probability level; n.s., not significant; * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$

3.3. Seasonal course of water relations, stomatal conductance (g_s) and canopy temperature (T_c)

Coinciding with the first climatic period, Ψ_{stem} values of control plants were high and fairly constant until mid-April ($\approx$ DOY 107) with mean values of -0.70 MPa, but fell during the course of the summer, reaching lowest values in July (-1.01 MPa) (Table 4) when T_a was maximum. Similar trends, although in reverse, were observed for T_c which registered mean value of 22.85°C in the first period (January –April) and 33.96°C in the second period (May-July) (Table 4).

Salt concentrations in reclaimed water caused a reduction in Ψ_{stem} and g_s in RW plants, with respect to control. The stem water potential values of RW plants remained significantly lower than in the control throughout the experiment, the differences between treatments reaching 0.5 MPa at the end of the trial, whereas the differences in g_s between treatments were not so pronounced as those of Ψ_{stem} (Table 4). The osmotic potential at full turgor (Ψ_{100s}) decreased in RW plants with respect to control plants from DOY 107 (Fig. 3), pointing to their osmotic adjustment.

Thermal images detected differences in water status between control and RW plants. Canopy temperature and $T_c - T_a$ in RW plants tended to be above the control values, temperatures being higher from May to the end of the experiment (Table 4). Maximum differences in T_c , between treatments were observed on DOY 193 when RW plants were almost 3.5°C hotter than the control. Thermal indices took longer to show the differences between irrigation treatments. Only from DOY 158 onwards were I_G and CWSI values of RW plants significantly lower and higher ($P < 0.001$), respectively, than those of control plants (Table 4).

Fig. 3. Osmotic potential at full turgor (Ψ_{100s}) at different times of experimental period for euonymus plants irrigated with control (closed columns) and reclaimed water (open columns). Bars are mean of six plants per treatment. Means without a common lowercase letter are significantly different.

Table 4. Stem water potential (Ψ_{stem}), stomatal conductance (g_s), canopy temperature (T_c) and difference between canopy and air temperature ($T_c - T_a$), at midday for euonymus plants irrigated with control (C) and treated wastewater (RW) during the experimental period. Values are means $\pm$ SEM (n=6 plants for Ψ_{stem} and g_s ; n=4 plants for T_c , $T_c - T_a$, I_G and CWSI).

DOY	Ψ_{stem} (MPa)		g_s (mmol m ⁻² s ⁻¹)		T_c (°C)		$T_c - T_a$ (°C)		I_G		CWSI	
	C	RW	C	RW	C	RW	C	RW	C	RW	C	RW
3	-0.58 $\pm$ 0.03 a	-0.99 $\pm$ 0.10 b	37.30 $\pm$ 4.82 a	25.63 $\pm$ 5.44 a	23.4 $\pm$ 1.1 a	25.3 $\pm$ 0.2 a	6.0 $\pm$ 0.5 a	7.8 $\pm$ 0.3 a	2.17 $\pm$ 0.51 a	1.71 $\pm$ 0.61 a	0.24 $\pm$ 0.10 a	0.36 $\pm$ 0.20 a
39	-0.80 $\pm$ 0.05 a	-0.94 $\pm$ 0.06 b	56.35 $\pm$ 2.26 a	40.50 $\pm$ 3.77 b	20.0 $\pm$ 1.5 a	21.1 $\pm$ 2.2 a	1.8 $\pm$ 0.5 a	2.8 $\pm$ 0.4 a	3.82 $\pm$ 0.46 a	3.45 $\pm$ 0.58 a	0.17 $\pm$ 0.02 a	0.21 $\pm$ 0.01 a
53	-0.63 $\pm$ 0.04 a	-0.80 $\pm$ 0.02 b	36.50 $\pm$ 2.44 a	28.50 $\pm$ 3.06 b	23.7 $\pm$ 1.0 a	24.8 $\pm$ 1.3 a	5.0 $\pm$ 0.9 a	6.1 $\pm$ 1.0 a	2.41 $\pm$ 0.34 a	1.79 $\pm$ 0.21 a	0.25 $\pm$ 0.07 a	0.27 $\pm$ 0.01 a
74	-0.63 $\pm$ 0.03 a	-0.91 $\pm$ 0.04 b	15.77 $\pm$ 4.34 a	12.79 $\pm$ 3.15 a	25.0 $\pm$ 0.5 a	25.3 $\pm$ 0.8 a	4.8 $\pm$ 0.5 a	5.1 $\pm$ 0.9 a	1.82 $\pm$ 0.25 a	1.95 $\pm$ 0.17 a	0.23 $\pm$ 0.05 a	0.30 $\pm$ 0.02 a
107	-0.85 $\pm$ 0.07 a	-1.04 $\pm$ 0.02 b	34.51 $\pm$ 4.53 a	21.77 $\pm$ 4.82 b	22.2 $\pm$ 0.7 b	25.4 $\pm$ 0.9 a	1.5 $\pm$ 0.4 b	4.3 $\pm$ 0.5 a	1.95 $\pm$ 0.37 a	1.15 $\pm$ 0.42 a	0.28 $\pm$ 0.09 a	0.40 $\pm$ 0.05 a
158	-0.98 $\pm$ 0.01 a	-1.38 $\pm$ 0.03 b	30.21 $\pm$ 4.13 a	20.99 $\pm$ 0.71 b	36.2 $\pm$ 0.7 b	39.4 $\pm$ 0.6 a	7.5 $\pm$ 0.9 b	10.7 $\pm$ 0.7 a	1.31 $\pm$ 0.23 a	0.66 $\pm$ 0.05 b	0.44 $\pm$ 0.04 b	0.60 $\pm$ 0.06 a
184	-1.01 $\pm$ 0.07 a	-1.55 $\pm$ 0.05 b	15.20 $\pm$ 3.05 a	9.94 $\pm$ 2.55 a	33.7 $\pm$ 0.4 b	36.7 $\pm$ 0.4 a	9.5 $\pm$ 0.7 b	11.2 $\pm$ 0.5 a	0.97 $\pm$ 0.28 a	0.24 $\pm$ 0.06 b	0.53 $\pm$ 0.07 b	0.82 $\pm$ 0.04 a
193	-0.78 $\pm$ 0.01 a	-1.28 $\pm$ 0.12 b	20.01 $\pm$ 2.26 a	15.82 $\pm$ 0.56 a	32.0 $\pm$ 1.0 b	35.5 $\pm$ 0.3 a	7.0 $\pm$ 1.0 b	10.5 $\pm$ 0.3 a	2.23 $\pm$ 0.27 a	1.01 $\pm$ 0.49 b	0.32 $\pm$ 0.02 b	0.50 $\pm$ 0.02 a

Means within a row without a common lowercase letter are significantly different.

The average T_c , T_c-T_a and CWSI values in both treatments correlated negatively with average g_s and Ψ_{stem} , while average I_G value correlated positively with g_s and Ψ_{stem} (Table 5). However, the best coefficients of determination were obtained between T_c and T_c-T_a with Ψ_{stem} with values of $R^2=0.63$, $P<0.001$ and $R^2=0.54$, $P<0.001$, respectively (Table 5).

Table 5. Slope (a), intercept (b) and coefficient of determination (R^2) of linear equations between canopy temperature (T_c), difference between canopy and air temperature (T_c-T_a), crop water stress index (CWSI) and I_G index, with stomatal conductance (g_s) and stem water potential (Ψ_{stem}) regardless of irrigation treatments throughout the experiment. $n=64$.

	a	b	R^2	Sign
T_c vs g_s	-1.47	66.26	0.45	***
T_c-T_a vs g_s	-2.35	42.71	0.44	***
CWSI vs g_s	-42.48	42.08	0.37	***
I_G vs g_s	7.50	12.22	0.38	***
T_c vs Ψ_{stem}	-0.05	0.30	0.63	***
T_c-T_a vs Ψ_{stem}	-0.06	-0.54	0.54	***
CWSI vs Ψ_{stem}	-0.86	-0.72	0.36	***
I_G vs Ψ_{stem}	0.19	-1.38	0.44	***

P, probability level; *** $P \leq 0.001$

3.4. Comparison of methods used to measure canopy temperature

The diurnal course of canopy temperatures obtained using infrared thermometers were significantly related to Ψ_{stem} and g_s made in April (data not shown). In fact, canopy temperatures measured manually with a hand-held thermal imager were closely correlated with the T_c measured continuously with infrared thermometers ($R^2=0.96$, $P<0.001$; data not shown). Throughout the experimental period, this relationship was highly significant, with a coefficient of determination $R^2=0.88$ and a slope close to unity (Fig. 4).

Fig. 4. Relationship between canopy temperature measured by hand-operated thermographic camera (T_c TC) and canopy temperature measured by infrared thermometers (T_c IRT) of euonymus plants regardless irrigation treatments during the experimental period. Each point is the mean of four replications.

3.5. Comparison of the sensitivity of continuously measured canopy temperature-derived parameters with discrete measurements of g_s and ψ_{stem}

Plant-based abiotic stress indicators need to take into consideration the changes which occur in plant water status during a short period of time to optimize irrigation scheduling. For this reason, we studied the signal intensity, noise and signal:noise ratio for some indicators during the first month of the experiment. More specifically we analysed ψ_{stem} , g_s and indicators derived from the canopy temperature measured continuously with infrared thermometers. Data in Table 6 indicated that the signal for all the indicators cited, except that of $T_{c, min}$ and $(T_c - T_a)_{min}$, was above unity

due to the high saline level of the RW treatment. The highest signals were reached for daily mean T_c-T_a and daily midday T_c-T_a . The low noise of T_c and parameters derived from it produced the highest mean signal:noise ratio, which was 7 and 10 times higher than that of Ψ_{stem} and g_s , respectively, in the case of $T_{c,\text{midday}}$ (Table 6), indicating that thermal indicators were more sensitive than g_s and Ψ_{stem} for detecting the high salt content imposed in our experimental conditions.

Table 6. Indicators mean signal intensity, mean noise, and mean signal:noise during the first month of the reclaimed water imposed.

Indicators	Mean signal	Mean noise	Mean signal:noise
Ψ_{stem}	1.73	0.16	8.51
g_s	2.00	0.36	5.58
$T_{c,\text{min}}$	0.98	0.01	33.97
$T_{c,\text{max}}$	1.13	0.04	30.75
$T_{c,\text{midday}}$	1.12	0.02	61.12
ΔT	1.22	0.05	31.11
$T_{c,\text{m}}$	1.09	0.03	30.66
$(T_c-T_a)_{\text{min}}$	0.85	0.13	6.70
$(T_c-T_a)_{\text{max}}$	2.09	0.13	21.47
$(T_c-T_a)_{\text{midday}}$	3.50	0.12	36.04
$\Delta (T_c-T_a)$	1.47	0.13	14.67
$(T_c-T_a)_m$	3.22	0.16	19.15

Ψ_{stem} = stem water potential, g_s = stomatal conductance, $T_{c,\text{min}}$ = daily minimum canopy temperature, $T_{c,\text{max}}$ = daily maximum canopy temperature, $T_{c,\text{m}}$ = daily mean canopy temperature, $T_{c,\text{midday}}$ = daily midday canopy temperature, ΔT = differences between $T_{c,\text{min}}$ and $T_{c,\text{max}}$, $(T_c-T_a)_{\text{min}}$ = daily minimum differences between canopy and air temperatures, $(T_c-T_a)_{\text{max}}$ = daily maximum differences between canopy and air temperatures, $(T_c-T_a)_m$ = daily mean differences between canopy and air temperatures, $(T_c-T_a)_{\text{midday}}$ = daily midday differences between canopy and air temperatures, $\Delta(T_c-T_a)$ = differences between $(T_c-T_a)_{\text{max}}$ and $(T_c-T_a)_{\text{min}}$.

4. Discussion

As seen in previous studies in euonymus plants, the effect due to the salts from the reclaimed water induced an alteration in plant water relations (Gómez-Bellot et al. 2013 a,b). The osmotic effect due to the components of the reclaimed water, especially the salts, provoked dehydration in the leaves as consequence to the greater difficulty in taking up water from the substrate (Álvarez et al. 2012) which was reflected when considering the seasonal pattern of Ψ_{stem} and g_s values of these plants throughout the experimental period (Table 4). Reductions in stomatal conductance, rather than merely being a negative consequence of high concentration of salts, could represent an adaptive mechanism to cope with excessive salts avoiding an excessive loss of water from the transpiration process (Munns and Tester 2008; Navarro et al. 2008; Gómez-Bellot et al. 2013b).

According to Halim et al. (1990), Kluitenberg and Biggar (1992) and Sirault et al. (2009) stomatal closure of RW plants caused by reclaimed water induced an increase of canopy foliage temperature (Table 4). Furthermore, the fact that the growth of plants in the two treatments was similar and in both cases the plants were compact with a large number of leaves with no evidence of leaf wilting, folding, rolling or falling caused by the salts of the reclaimed water (data not shown) avoided the problem of taking background temperatures (Fuchs 1990; Jackson et al. 1981).

The diurnal course of canopy temperature and $T_c - T_a$ of both sunlit and shaded side was influenced by reclaimed water effect, especially in the morning, when the maximum differences of Ψ_{stem} values between treatments were reached (Fig. 2; Table 2). In this sense, our results showed that the negative relationship between T_c and g_s in April was significant on both sides for most of the day, especially at midday (Table 3). Furthermore, the midday dip of T_c coincided with a slight decrease of VPD suggesting the leaf temperature is highly dependent on atmospheric conditions (Grant et al. 2010).

Regarding the effect of the canopy side, we recommended using the thermal images obtained on the sunlit side of the euonymus canopy rather than those from the shaded side of the canopy, because of the higher coefficients of determination

obtained between T_c and $T_c - T_a$, and g_s (Table 3). This agrees with Ballester et al. (2013), who reported that images of citrus leaves most directly exposed to solar radiation detected plant water stress better than frontal images. In fact, some researchers consider that a viewing angle similar to the solar azimuth is the most suitable, since leaves orientated towards the sun show a greater sensitivity to temperature than the leaves from more than one orientation (Jones 2004). In contrast, Pou et al. (2014) suggested that the water status of vines was better estimated from images taken from the shaded side than from the sunlit side of the canopy because of the lower temperature variations recorded for the shaded side of the canopy. In our study, the fact that thermal images were analysed manually allowed sunlit leaves and shaded leaves to be distinguished, as reported Leinonen and Jones (2004), thus avoiding this variability.

Thermal indices (CWSI and I_G) were not as sensitive such as T_c or $T_c - T_a$ in the diagnosis of osmotic effect since there were little statistically significant differences between treatments during the day (DOY 107), and only on the sunlit side at certain times (Fig. 2). In this sense, De Lorenzi et al. (1993), Jones (1999) and Blonquist et al. (2009) reported that under humid or overcast conditions, temperature differences between a leaf and the reference surfaces are quite small, so that CWSI and I_G are unreliable indices. Other studies reported that the spatial variability of irradiance and air temperature within a greenhouse due to shading by other plant parts or the greenhouse structure prevents the use of thermal indices (Kaukoranta et al. 2005; Grant et al. 2006).

Throughout the experimental, differences of up 3 °C were recorded between control and RW plants (Table 4), when Ψ_{stem} of RW plants was below ≈ -1 MPa, indicating a dependence of canopy temperature on salinity. This agrees with Ballester et al. (2013), who reported the feasibility of using canopy temperature for plant water status detection under moderate stress levels in citrus trees; although with differences of temperature between stress and non-stress plants that were lower than ours. In other small-leafed plants high temperature differences were observed, but only under severe stress (Sepulcre-Cantó et al. 2009; García-Tejero et al. 2011).

Although coefficients of determination of the relationships between thermal parameters and g_s at midday during the experiment were highly significant (Table 5), they decreased considerably with respect those obtained on DOY 107. This agrees with Jones et al. 2009, who reported that the magnitude of canopy temperature is not constant over a period of days with different environmental conditions, so it is necessary to normalize T_c in relation to references to account for changing meteorology. In the case of $T_c - T_a$, its weaker coefficient of correlation with respect to that obtained on DOY 107 suggests that other factors such as seasonal changes in sink-source relationships or leaf senescence are influential (Grant et al. 2010; Costa et al. 2012; Hackl et al. 2012).

Whatever the case, for irrigation scheduling, it is better to use the concept of signal intensity (SI), normalizing the obtained values with respect to values obtained in control conditions (Goldhamer and Fereres 2001; Naor and Cohen 2003; Ortuño et al. 2010). Furthermore, taking into account the proposals of Naor and Cohen (2003) for comparing the sensitivity of different plant-based indicators for detecting abiotic stresses, it might be borne in mind that the strength of an indicator signal should be seen in the context of its variability (noise) (Goldhamer and Fereres 2001). The sensitivity of an indicator will depend on the signal:noise ratio. In our work, we have compared discrete indicators (Ψ_{stem} and g_s) with continuous indicators (T_c and $T_c - T_a$ parameters obtained with IRTs) (Table 6). We did not consider canopy temperature measured by thermal imaging as a discrete measurement because the high correlation and the slope close to unity between the temperature obtained with thermometers and thermal imaging suggested that both temperatures were similar (Fig. 4). Our results showed that T_c and $T_c - T_a$ -derived parameters were the most suitable indicators for detecting salt stress since the signal:noise ratio was higher. This agrees with Ballester et al. (2013) who obtained a signal:noise ratio for T_c that was around 5 times greater than for g_s in persimmon trees. In addition, the fact that the highest signal:noise ratio values were those obtained for T_c and $T_c - T_a$ at midday agrees with the assumption that the best moment to take canopy temperature is at this moment.

5. Conclusions

Both daily and seasonal canopy temperatures and differences between canopy and air temperatures measured at midday and on the sunlit side of the canopy provide a useful means of documenting the presence of high salts concentrations in euonymus plants. Furthermore, the higher signal:noise ratio of canopy temperature and $T_c - T_a$ compared with other eco-physiological indicators, such as stem water potential and stomatal conductance, makes them the most suitable plant-based salt stress indicators for irrigation scheduling. Thermal indices (CWSI and I_G) were not highly sensitive to saline reclaimed wastewater under greenhouse conditions. Furthermore the high correlation and the slope close to unity between the temperature obtained with thermometers and thermal imaging demonstrated the possibility of using either of both techniques for euonymus plants under greenhouse conditions.

CHAPTER 4

**Protective effects of arbuscular mycorrhizae on soil and
laurustinus plants irrigated with treated wastewater under
field conditions**

Chapter 4

Protective effects of *Glomus iranicum* var. *tenuihypharum* on soil and *Viburnum tinus* plants irrigated with treated wastewater under field conditions.

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Abstract

Currently irrigation using recycled water (RW) is increasing, especially in semiarid environments, but a potential problem of using RW is its elevated salt levels. The application of arbuscular mycorrhizal fungi (AMF) could be a suitable option to mitigate the negative effects produced by the salinity. In this work, during forty weeks, the combined effect of *Glomus iranicum* var. *tenuihypharum* and two types of water: Control, C, with $EC < 1.2 \text{ dS m}^{-1}$ and Reclaimed Water; RW1 with $EC: 4 \text{ dS m}^{-1}$ during a first saline period and RW2 with $EC: 6 \text{ dS m}^{-1}$ during a second saline period was evaluated for laurustinus plants. Chemical properties of soil as well as physiological behavior, leaf nutrition and aesthetic value of plants growing in soil were evaluated. Due to the high salinity from treated wastewater at 6 dS m^{-1} , laurustinus plants decreased their stem water potential values and, to a lesser extent, the stomatal conductance. Also the visual quality of the plants was diminished. Under mycorrhizal inoculation, AMF satisfactorily colonized the laurustinus roots and enhanced the structure of the soil by increasing the glomalin and carbon contents. Furthermore, AMF decreased toxic ions, stimulated flowering and improved the water status of the plants irrigated with both types of reclaimed water. AMF also had a positive effect in well watered plants, as a consequence of a stimulation of physiological parameters. Effective AMF associations that avoid excessive salinity could provide treated wastewater reuse options, especially when the plants grow in soils.

Keywords: reclaimed water; salinity; *Glomus iranicum*; soil structure; glomalin content, ornamental plants.

1. Introduction

The Mediterranean area, both in the agricultural and urban sectors, needs to improve its efficiency of water use. Recycled water produced from treated wastewater can be used in many non-potable applications and can help to reduce the overall demand for fresh water (Anderson 2005; Qadir et al. 2007). Reclaimed water usually contains a high concentration of nutrients such as N, P, and K^+ that produce a direct benefit for crops and also allow the accumulation of organic matter (Pedrero et al. 2013). However, depending upon its source and treatment, reclaimed water may have high salt content, heavy metals or pathogenic organisms. In general, as salinity increases in the treated wastewater used for irrigation, the probability for cropping problems increases, which may have measurable adverse effect on plants, soils and systems. The major difference in evaluating the suitability of waters for irrigation of agricultural crops and waters for irrigating landscape plants is that the former is based on harvested crop yield, while the latter is based on aesthetic quality or appearance (Fox et al. 2005; Cassaniti et al. 2012b). In any case, it is important to select plants which are able to tolerate salt stress in order to allow the use of low quality water without damaging the development of plants, since salt tolerance varies considerably among the different genotypes of ornamentals used in landscaping. Apart from plant characteristics, the degree and duration of salt stress as well as soil composition also need to be taken into consideration as they can influence the severity of plant damage by saline irrigation water (Chaves et al. 2009; Cassaniti et al. 2012b). In fact, the characteristics of soil such as structure, physicochemical properties, biologic activity, possible organic and inorganic amendments and possible presence of pollutants (heavy metals and others) may influence the growth and survival of the plant in the soil (Clemente et al. 2005) and, as a consequence, the response of the plant to salinity .

Many researchers have shown that arbuscular mycorrhizal fungi (AMF) have a positive influence on cultivation systems (Hamel and Plenchette 2007). They not only improve plant growth through increased nutrient uptake, but they have also 'non – nutritional' effects in stabilizing soil aggregates, in preventing erosion, and in

alleviating the negative effects induced by salinity (Evelin et al. 2009; Dudhane et al. 2011; Tallat and Shawky 2011; Cekic et al. 2012). AMF are able to develop several mechanisms to enhance the salt tolerance of host plants such as increased nutrient acquisition, maintenance of the K/Na ratio, biochemical changes, physiological changes such as water status and photosynthetic efficiency and molecular or structural changes. (Sheng et al. 2008; Evelin et al. 2009; Hajiboland et al. 2010; Abdel- Fattah and Asrar 2012; Cekic et al. 2012).

Salinity may reduce the colonization capacity and the growth of fungal hyphae (Jahromi et al. 2008). However, the success of AMF development depends on the characteristics of soil, type of arbuscular fungi introduced, species of plant selected and their interaction, as well as the degree, duration and type of stress. Some authors have demonstrated that mycorrhizal inoculation can alleviate the negative effects produced by saline irrigation in ornamental and wild plants (Giri and Mukerji 2004; Giri et al. 2007; Kumar et al. 2010; Navarro et al. 2012).

Viburnum tinus L. (laurustinus) is a perennial shrub, autochthonous to the Iberian Peninsula, which is used for biodiversity conservation and has a great economic and ecological importance. According to Bañón et al. (2012), laurustinus plants are moderately sensitive to salinity at 6 dS m⁻² while according to Fox et al. (2005) this species can withstand 2.5-3 dS m⁻¹ without losing its aesthetic value. Also, in our previous experiment, we evaluated the effect of reclaimed water in pot- grown laurustinus plants in greenhouse conditions, in which the aesthetic value and growth decreased as consequence of salinity (Gómez-Bellot et al. 2013a).

In the present study, in order to check the effectiveness of *Glomus iranicum* var. *tenuihypharum* in field conditions, we evaluated the combined effect of arbuscular fungi inoculation and two kinds of reclaimed water (RW) with different electrical conductivity on the ion acquisition, water status, gas exchange and aesthetic value of *Viburnum tinus* plants, as well as the effect of RW on mycorrhizal root colonization and soil properties.

2. Material and methods

2.1. *Plant material and growth conditions*

The experiment was performed in a one-year-old laurustinus (*Viburnum tinus* L.) (n=80) at the experimental farm of CEBAS- CSIC in Santomera (Murcia, Spain) (38°06'N, 1°02'W, elevation 110 m), using a planting pattern of approximately 1 m x 1 m. The soil, classified as Lithic xeric haploxeroll, is stony (33%, w/w) and shallow, with a clay-loam texture. Analytical data showed a high lime content, low organic matter content and low cationic exchange capacity.

Every 15 min, climatic data were recorded by an automatic weather station located next to the plot. The average maximum and minimum values of air temperature and RH were 24 °C and 13 °C, and 84% and 35%, respectively. The annual reference evapotranspiration (ET_o) determined by the FAO, Penman-Monteith equation (Allen et al., 1998) was 1091 mm, with a maximum of 194.98 mm in July. During the experiment the total rainfall was 185 mm, most of it occurring in spring and autumn.

On April 2012, laurustinus plants were collected from a nursery with an initial height of 10-15 cm and were transplanted into the experimental plot. The soil were amended initially with 2 g L⁻¹ of Osmocote Plus (14:13:13 N, P, K plus microelements) and every three-four months, a Hoagland solution (standard nutrient solution) were supplied through the drip irrigation system.

Picture 1. Experimental plot after transplanting laurustinus plants

On 8 March 2013 for each irrigation treatment expected, half of the pots were inoculated (3 kg ha^{-1}) with *Glomus iranicum* var. *tenuihypharum* inoculum (a mixture of spores, mycorrhizal root fragments and rhizospheric soil). It was obtained by isolating the fungi under extreme saline soil conditions (Solonetz Gley, saline soil type according to FAO soil classification). The multiplication of the strain was performed as proposed by Fernández and Juárez (2011). The inoculum was applied throughout the drip irrigation. The remaining plants were not inoculated.

On 5 April 2013, after one year of acclimation, the first saline period started with two irrigation treatments which consisted of a Control ($\text{EC} < 0.9 \text{ dS m}^{-1}$) and a reclaimed water, RW1 ($\text{EC}: 4 \text{ dS m}^{-1}$) from a sewage treatment plant located in Campotejar (Murcia, Spain). The wastewater treatment plant applies a conventional activated sludge process followed by ultraviolet application for tertiary treatment. Therefore, there were four treatments in total: Control and RW treatments with and without mycorrhizal inoculation.

On 20 June 2013, fifteen weeks after inoculation, due to hardly any differences were observed in physiological behaviour of the plants, a second saline period started by replacing RW1 at 4 dS m^{-1} for a new reclaimed water treatment, RW2 ($\text{EC}: 6 \text{ dS m}^{-1}$) from another sewage treatment plant located in Mazarrón (Murcia, Spain). Therefore, the treatments of the second saline period consisted of the same Control water ($\text{EC} < 0.9 \text{ dS m}^{-1}$) and a reclaimed water, RW2 ($\text{EC}: 6 \text{ dS m}^{-1}$). The second

saline period ended on 18 December 2013, forty weeks after the mycorrhizal inoculation.

Picture 2. Irrigation tanks (A) and laurustinus plants at the beginning of the first saline period (B)

Plants were irrigated so that the stem water potential of the Control plants did not fall below -0.9 MPa. Therefore, in order to maintain this potential, the quantity of water applied depended on the season, climatic conditions and plant development. Irrigation was carried out twice a day using a drip irrigation system with one lateral pipe per plant row and one emitter (each delivering 3 L h^{-1}) per plant. The quantity of water applied was controlled every week using in-line water meters. The volumetric water content (θ_v) of the top 20 cm of the soil profile was measured every week in seven plants per treatment throughout the experiment by time domain reflectometry (TDR) (Model 1502C, Tektronix Inc., Beaverton, OR), as described by Moreno et al. (1996).

2.2. Water analyses

The inorganic solute content, pH and EC of the irrigation waters were assessed on May and June 2013 (At the beginning of the first and second saline period, respectively). The samples were collected in glass bottles and stored at 5°C before being processed for chemical analyses. The pH was measured with a Cryson-507 pH-meter (Crisom Instruments S.A. Barcelona, Spain); EC was determined using

the multirange equipment, Cryson-HI8734 (Crisom Instruments S.A. Barcelona, Spain); the concentrations of macronutrients (Na^+ , K^+ , P, Ca^{2+} and Mg^{2+}) and micronutrients like B were determined by inductively coupled plasma optical emission spectrometer (ICP-ICAP 6500 DUO Thermo, England) and chloride was analysed by ion chromatography with a Metrhom Chromatograph (Switzerland).

2.3. Percentage of mycorrhizal colonization and enzymatic mycorrhizal activity

At the end of the first saline period and second saline period, young root samples with the surrounding rhizosphere soil were collected at a depth of 20 – 25 cm to assess the symbiotic development. Three root samples per treatment were used with at least three replicates per root. The percentage of mycorrhizal root colonization was estimated following the gridline intersect method (Giovannetti & Mosse 1980) under a microscope (100 x magnification) after clearing 166 washed roots (i) in 10% KOH for 10 minutes at 90 °C, (ii) in HCl 2N for 10 minutes and (iii) 167 staining with 0.05% trypan blue dissolved in lactic acid (v/v) (Phillips and Hayman, 1970).

Picture 3. Stained roots

At the end of the second saline period, the enzymatic activity of AMF were estimated in the same three young roots samples collected, by staining for succinate dehydrogenase (SDH) (Smith and Gianinazzi- Pearson, 1990) and fungal alkaline phosphatase (ALP) (Tisserant et al., 1993) activities, in order to measure living or

functional mycorrhizal infection. To determine the SDH activity, the root samples were incubated at room temperature overnight in an NBT-succinate solution (MacDonald and Lewis, 1978). Our NBT (Nitro blue tetrazodium) solution consisted of 5 mL of NBT (4 mg mL^{-1}), 5 mL of Tris-HCl buffer (0.2 mol L^{-1} ; pH 7.4), 2 mL of sodium succinate (2.5 mol L^{-1}), 2 mL of MgCl_2 (5 mmol L^{-1}) and 6 mL of distilled water. To determine the ALP activity, the roots samples were incubated at room temperature overnight in 20 mL of Naphthyl acid phosphate (1 mg mL^{-1}), 20 mL of Fast Blue RR salt (1 mg mL^{-1}), 18 mL of Tris-citric acid (0.05 mol L^{-1} ; pH 9.2), 1 mL of MgCl_2 (0.5 mg mL^{-1}) and 1 mL of MnCl_2 (0.8 mg mL^{-1}). After incubation, roots were cleared in sodium hypochlorite solution (containing 3% and 1% active chlorine for SDH and ALP, respectively) during 5 minutes and washed with distilled water. The percentage of mycorrhizal activity (SDH and ALP) was estimated following the gridline intersect method (Giovannetti and Mosse 1980) under a microscope (100 x magnification).

2.4. Measurement of glomalin and mineral content in soil

At the end of the first and second saline period three soil samples per treatment were collected. Soil samples were dried, sieved to 1–2 mm and extracted with 2 mL of extractant. EEG was extracted with 20 mM citrate, pH 7.0 at 121°C for 30 min. Samples were then subjected to 121°C for 30 min and centrifuged to 3000 rpm during 15 min. The protein in the supernatant was determined by the Bradford dye-binding assay with bovine serum albumin as standard (Wright et al., 1996). Concentration of glomalin was extrapolated to mg g^{-1} of soil particles by correcting for the dry weight of coarse fragments $>0.25 \text{ mm}$ included in the weight of aggregates and for the volume of extractant.

At the end of the first and second saline period, the mineral content of three soil samples per treatment were determined by Inductively Coupled Plasma optical emission spectrometer (ICP-OES IRIS INTREPID II XDL). The soil was dried at room temperature for a week ground and sieved through a 2 mm nylon mesh before analysis. The macronutrients concentrations were determined in a digestion extract with $\text{HNO}_3\text{:HClO}_4$ (2:1, v/v) by Inductively Coupled Plasma optical emission

spectrometer (ICP-OES IRIS INTREPID II XDL). 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 sample powder with 40 mL of water before shaking for 30 min and filtering.

The total nitrogen (N_T), total carbon (C_T) and organic carbon (C_{org}) concentrations of the soil were measured from the soil samples collected with an elemental analyser Flash EA 1112 Series- Leco Truspec. The organic matter content (OM) of the soil was determined by multiplying C_{org} by 1.72. Available- P was also analysed colorimetrically as molybdovanadophosphoric acid (Watanabe and Olsen, 1965).

2.5. Leaf mineral content and relative chlorophyll content

At the end of the first and second saline period, the mineral content of leaves was determined in four plants per treatment as indicated above for the mineral content of soil. The leaf samples were oven-dried at 80 °C and ground before analyses. Throughout the experiment, the relative chlorophyll content was determined in seven leaves per treatment using a Minolta SPAD-502 chlorophyll meter (Konica Minolta Sensing Inc., Osaka, Japan).

2.6. Measurements of plant water status and gas exchange

Seasonal changes in stem water potential (Ψ_{stem}), osmotic potential at full turgor ($\Psi_{100\text{s}}$), stomatal conductance (g_s) and net photosynthesis (P_n) at midday were determined in seven plants per treatment throughout the whole experimental period.

Stem water potential 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 within 20s of collection and pressurised at a rate of 0.02 MPa s^{-1} (Turner 1988). Leaves for Ψ_{stem} were taken from the north-facing side and were covered with aluminium foil for at least 2 h before measurements. The osmotic potential at full turgor ($\Psi_{100\text{s}}$) was estimated using excised leaves with their

petioles placed in distilled water overnight to reach full saturation. Then, these leaves were frozen in liquid nitrogen ($-196\text{ }^{\circ}\text{C}$) and stored at $-30\text{ }^{\circ}\text{C}$. After thawing, the osmotic potential at full turgor (Ψ_{100s}) was measured in the extracted sap using a WESCOR 5520 vapour pressure osmometer (Wescor Inc., Logan, UT, USA), according to Gucci et al., (1991).

Leaf stomatal conductance (g_s) and net photosynthetic rate (P_n) were determined in attached leaves in the same plants and on the same days as the stem water potential was measured, using a gas exchange system (LI-6400; LI-COR Inc., Lincoln, NE, USA). Gas exchange was measured around noon, fixing the conditions of CO_2 concentration at 380 ppm, the photosynthetically active radiation (PAR) at $1500\text{ }\mu\text{mol m}^{-2}\text{ s}^{-1}$ and the speed of the circulating air flow inside the system at 300 mol s^{-1} . Throughout the experiment the average values of air temperature, relative humidity and pressure deficit were around $22\text{ }^{\circ}\text{C}$, 42% and 1.74 KPa, respectively.

Picture 4. Measure of gas exchange and stem water potential

2.7. Ornamental parameters

At the end of the second saline period, all the plants were visually evaluated as follows: (1) PIC, percentage of plants in ideal condition; (2) PAC, percentage of plants in acceptable condition; (3) PDB, percentage of plants with dry branches; and (4) DP,

percentage of dry plants. The number of flowers per plant was counted at the end of the second saline period.

2.8. Statistics

In the experiment 20 plants were randomly attributed to each treatment. The data from Table 2 were analysed by one-way ANOVA using Statgraphics Plus for Windows 5.1 software. For the rest of data, both variables (water quality and mycorrhizal inoculation) and their interaction were analyzed for each parameter by two-way ANOVA. 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 ($P \leq 0.05$).

3. Results

3.1. Water analyses

At the beginning of the first saline period, the physicochemical properties of the irrigation waters were analysed (Table 1). The sodium, chloride and boron content of the RW1 (4 dS m⁻¹) was about 10, 9 and 3-times higher, respectively, than the corresponding values of the Control water. The Control water showed the highest P values while the highest Ca²⁺ and Mg²⁺ values were observed in the RW water.

At the second saline period, the sodium and chloride content of the RW2 (6 dS m⁻¹) was about 28 and 16-times higher, respectively, than those observed for Control water. RW at 6 dS m⁻¹ also showed higher B⁺, Ca²⁺ and K⁺ content and lower P⁺ than Control water (Table 1).

Table 1. Physicochemical analysis for the irrigation treatments. Data are values from samples collected at the beginning of each saline period

	Control	RW1 (4 dS m ⁻¹)	RW2 (6 dS m ⁻¹)
pH	8.25	7.78	7.80
EC (dS m⁻¹)	0.92	3.87	6.06
B (mg L⁻¹)	0.23	0.75	1.33
Ca (mg L⁻¹)	102.70	143.00	217.32
K (mg L⁻¹)	45.88	40.12	118.41
Mg (mg L⁻¹)	39.73	104.60	37.56
Na (mg L⁻¹)	46.92	475.50	1331.00
P (mg L⁻¹)	15.74	2.29	5.63
Cl (mg L⁻¹)	61.98	544.80	1006.28

3.2. Mycorrhizal colonization and establishment

At the end of the first and second saline period, the inoculated plants for Control and RW treatments presented a higher percentage of root colonization than non-inoculated plants. Nevertheless, for non-inoculated plants, these percentages were relatively high, probably due to a native AMF proliferation (Table 2). On the other hand, only AMF colonization in inoculated RW plants decreased at the end of the second saline period with respect to the end of the first saline period.

At the end of the second saline period, nearly all the total AMF inoculum present in roots remained alive (SDH staining) and active (ALP staining) (Table 2). The percentage of SDH activity was the highest in inoculated Control plants, followed by inoculated RW plants. Similar behavior was observed for ALP activity. The values of both parameters were similar between them.

Table 2. Percentage of final root colonization at the end of the first (I) and second saline period (II). Percentage of final succinate dehydrogenase (SDH) and fungal alkaline phosphatase (ALP) activities at the end of the second saline period (II). Values are means $\pm$ SEM (n=3 plants)

Mycorrhizal inoculation	Treatments				P
	C	C M	RW	RW M	
% Colonization (I)	24.0 $\pm$ 1.0 bB	83.5 $\pm$ 4.5 a	27.1 $\pm$ 2.0 bB	81.0 $\pm$ 1.5 aA	***
% Colonization (II)	49.5 $\pm$ 0.5 cA	90.4 $\pm$ 2.7 a	33.9 $\pm$ 1.2 dA	75.9 $\pm$ 0.9 bB	***
% SDH (II)	44.4 $\pm$ 4.1 b	82.5 $\pm$ 2.6 a	31.0 $\pm$ 3.2 b	75.3 $\pm$ 3.1 a	*
% ALP (II)	45.3 $\pm$ 2.6 b	73.7 $\pm$ 0.6 a	29.9 $\pm$ 1.6 c	67.8 $\pm$ 4.2 a	*

Means within a row without a common 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; * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$

3.3. Glomalin and mineral content in soil

Results from soil analyses were similar for both periods; therefore, the results presented in this work corresponded to the second saline period. Regardless of mycorrhizal inoculation, the easily extractable glomalin (EEG) in the soil decreased under RW irrigation and was significantly increased by mycorrhizal inoculation effect (Table 3). In addition, there was an interaction between both factors (W x M) (Fig. 1a); the EEG content in the soil increased in inoculated Control treatment respect to the non-inoculated Control, while the lowest EEG content was observed in non-inoculated RW, increasing these values when AMF were inoculated in plants irrigated with RW (Fig. 1a).

The total nitrogen (N_T), total carbon (C_T), C_{org} , OM and C/N ratio in soil did not show differences by the effect of the irrigation water. As regards mycorrhizal inoculation, the OM and C_{org} was higher in inoculated plants than in non-inoculated plants (Table 3). Although N_T and C_T had a slight trend to increase by the effect of AMF, they did not show statistical differences, either for C/N ratio. On contrary, the available-P content in soil was higher for Control than in RW treatment, while the mycorrhizal presence did not affect the Available-P content for both irrigation treatments (Table 3).

Table 3. Effects of the irrigation water (W) and mycorrhizal inoculation (M) on the chemical properties of soil and the easily extractable glomalin of soil (EEG) at the end of the second saline period. Values are means (n=3 samples)

Parameters in soil	Irrigation water (W)		Mycorrhizal inoculation (M)		Significance		
	Control	RW	+	-	W	M	W x M
Available-P (mg Kg ⁻¹ soil)	15.89	11.31	13.34	13.61	**	ns	ns
N _T (g 100g ⁻¹)	0.15	0.13	0.15	0.13	ns	ns	ns
C _T (g 100g ⁻¹)	7.15	7.71	7.62	7.24	ns	ns	ns
C _{org} (g 100g ⁻¹)	1.36	1.36	1.42	1.31	ns	*	ns
OM (%)	2.34	2.34	2.43	2.25	ns	*	ns
C/N	9.49	10.28	9.81	9.96	ns	ns	ns
EEG (mg g ⁻¹ soil)	38.26	22.43	38.14	22.55	***	***	***

*, **, ***, and ns indicate the level of significance at $P \leq 0.05$, 0.01, 0.001 and the absence of significance, respectively, according to Duncan's multiple range test

3.4. Leaf mineral content and relative chlorophyll content.

At the end of the first saline period, leaf analyses hardly presented any significant differences between treatments. Therefore, the results presented in this work corresponded to the second saline period. As a result of irrigating with reclaimed water, Na⁺, Cl⁻, Ca²⁺, K⁺ and B⁺ accumulated in leaves while Mg²⁺ and P content decreased (Table 4). The opposite occurred under mycorrhizal inoculation, in which a decrease of Cl⁻, Na⁺ and K⁺ leaf content as well as an increase of Mg²⁺ and P was observed in inoculated plants (Table 4).

Table 4. Effects of irrigation water (W) and mycorrhizal inoculation (M) on the mineral content of leaves at the end of the second saline period. Values are means (n=4 plants)

Leaf analyses (mg Kg ⁻¹ DW)	Irrigation water (W)		Mycorrhizal inoculation (M)		Significance		
	Control	RW	+	-	W	M	W x M
Cl	4475.0	12200.0	7850.0	8825.0	***	*	ns
Na	201.8	1770.6	742.6	1229.8	***	***	***
Ca	12584.4	14995.0	13600.0	13979.4	***	ns	**
Mg	4651.1	4152.0	4570.5	4232.6	***	***	***
P	1745.5	1458.8	1702.1	1502.1	***	***	ns
K	12020.0	15202.5	13027.5	14195	***	***	ns
B	196.5	201.5	201.63	205.4	*	ns	***

*, **, ***, and ns indicate the level of significance at $P \leq 0.05$, 0.01, 0.001 and the absence of significance, respectively, according to Duncan's multiple range test

A significant interaction between both factors was observed for Na⁺, Ca²⁺, Mg²⁺ and B⁺ (Fig.1b); the leaf concentration of Mg²⁺ and B⁺ was higher in mycorrhizal Control plants than in non-inoculated Control plants, while Na⁺, Ca²⁺ and B⁺ decreased in inoculated RW plants respect to non-inoculated RW plants (Fig. 1b). From the end of the first saline period, the relative chlorophyll content showed significant differences between treatments (Fig. 2d), mycorrhizal Control plants reaching the highest values during both saline periods, while the lowest values corresponded to non mycorrhizal RW plants.

Fig.1 Easily extractable glomalin content (EEG) in soil (a) and leaf mineral content (b) of laurustinus plants irrigated with water of different qualities without and with mycorrhizal inoculation at the end of the second saline period. Values are mean of three soil samples for EEG and four plants for leaf mineral content. Different lower case letters indicate significant differences between treatments according to Duncan_{0.05} test. The vertical bars indicate standard errors

3.5. Measurements of plant water status and gas exchange

During the first saline period, the stem water potential (Ψ_{stem}) showed very similar values for all treatments (Fig. 2a). Nevertheless, from the beginning of the second saline period, non-inoculated RW plants showed the lowest values and inoculated RW plants had intermediate values, week 15 coinciding with a high temperature and evaporative demand. At the end of the experiment, the inoculated RW plants reached similar values of Ψ_{stem} than Control plants (Fig. 2a) with no significant differences between them.

Fig.2 Stem water potential (Ψ_{stem}) (a), osmotic potential at full turgor (Ψ_{100s}) (b), volumetric water content in soil (θ_v) (c) and relative chlorophyll content in leaves (d) of laurustinus plants irrigated with water of different qualities without and with mycorrhizal inoculation during the experiment. Values are mean of seven plants. Asterisks indicate statistically significant differences between treatments by Duncan_{0.05} test. The vertical bars indicate standard errors. Dash lines separate both saline periods

As regards osmotic potential at full turgor, a decrease was observed in both RW treatments (inoculated and non-inoculated) with respect to both Control treatments during the experiment, reaching values up to -2.54 MPa for the non-inoculated RW treatment compared to -1.85 MPa for the Control treatment (Fig. 2b). This parameter was hardly statistically affected by mycorrhizal inoculation. In addition,

the volumetric water content increased in soil irrigated with treated wastewater with respect to the soil irrigated with Control water throughout the experiment (Fig 2c).

Throughout practically all of both saline periods, the highest g_s values were found in mycorrhizal inoculated plants (C M and RW M), this parameter being the lowest in non-inoculated RW plants (Fig 3a). In contrast, throughout the whole experiment, the values of P_n tended to be more similar among all treatments than those observed for the stomatal conductance (Fig 3b).

Fig.3 Stomatal conductance, g_s (a) and net photosynthetic rate, P_n (b) at midday of laurustinus plants irrigated with water of different qualities without and with mycorrhizal inoculation during the experiment. Values are mean of seven plants. The vertical bars indicate standard errors. Asterisks indicate statistically significant differences by Duncan_{0.05} test. Dash lines separate both saline periods

A decrease in both g_s and P_n values were observed for all treatments in week 15, as a consequence of the high temperature registered in these days. In spite of non-significant differences between all treatments, mycorrhizal inoculation slightly increased the values of P_n in C and RW plants.

3.6. Ornamental parameters

At the end of the saline period, inoculated Control plants showed 100 % of PIC, followed by non-inoculated Control plants, with 90 % of PIC (Fig. 4a). The lowest PIC values were found in non-inoculated RW plants, (35 % of PIC and 5 % of DP). On the other hand, AMF produced 65 % of PIC, 30 % of PAC and 5 % of DP in RW plants. The number of flowers was also increased by the mycorrhizal inoculation, reaching in the inoculated Control plants the highest number of inflorescences per plant (Fig. 4b).

Fig.4 Percentage of plants according to the visual characteristics (a) and number of inflorescences per plant (b) of *laurustinus* plants irrigated with water of different qualities without and with mycorrhizal inoculation at the end of the second saline period. Values are mean of ten plants for number of inflorescences. PIC, percentage of plants in ideal conditions PAC, percentage of plants in acceptable conditions; PDB, percentage of plants with dry branches; and DP, percentage of dry plants

Picture 5. Plant in ideal conditions (A), plant in acceptable conditions (B), plant with dry branches (C), and dry plant (D)

4. Discussion

The establishment of the AM symbiosis begins with the colonization of a compatible root by the hyphae produced by AM fungal soil propagules, asexual spores or mycorrhizal roots. After host colonization, the fungal mycelium grows out of the root exploring the soil in search of mineral nutrients, and it can also colonize other susceptible roots (Breuninger and Requena 2004). In our case, our results confirm that *Glomus iranicum* var. *tenuihypharum* symbiosis was well established on laurustinus roots regardless of the type of water used (Table 2) (Porrás-Soriano et al. 2009, Nicolás et al. 2014). On the other hand, both non- inoculated plants presented relatively high levels of root colonization, probably due to a native AMF proliferation. Similar results were observed by Vicente-Sánchez et al. (2013) and Nicolás et al. (2014) in lettuce and grapevine, respectively. This behaviour is very common in most of the terrestrial plants species since the roots naturally form mutualistic associations with arbuscular mycorrhizal fungi (AMF), making this the most common known plant–

fungal interaction (Smith and Read 2008). In addition, only AMF colonization in inoculated RW plants decreased slightly at the end of the second saline period with respect to the end of the first saline period. Therefore, Na^+ and Cl^- content present in the soil of the plants irrigated with RW could affect negatively the mycorrhizal colonization. Many authors have reported fungal colonization of plant roots is reduced in the presence of NaCl (Tian et al. 2004; Juniper and Abbott 2006; Giri et al. 2007; Sheng et al. 2008). However, in our case, *Glomus iranicum* var. *tenuihypharum* was isolated under extreme saline soil conditions, being resistant to salinity (Fernández and Juárez 2011). As a result, in spite of the high EC level of treated wastewater (6 dS m^{-1}), this colonization percentage at the end of the second saline period remained at a fairly high level.

It is known that inoculating plants often improves the P nutrition of host plants growing in soils with low levels of soluble P forms as AMF facilitates the mobility of P^+ in the soil (Evelin et al. 2009). The enzyme fungal alkaline phosphatase (ALP), identified in AMF, is involved in the processes of phosphorus acquisition in mycorrhizal plants (Gianinazzi et al. 1992). In fact, ALP activity plays an important role in the nutrient acquisition, not only by facilitating P acquisition, but also increasing photosynthesis and improving plant growth (Amaya-Carpio et al. 2009; Borde et al. 2011). In our experiment, the available-P content in soil was statistically unaffected by mycorrhizal inoculation (Table 3) and lower for RW treatment respect to Control, due to the low P content dissolved in treated wastewater (Table 1). However, in concordance with Watanabe and Olsen (1965), our soil presented very high levels of available-P for all treatments. The increase of leaf P content in inoculated RW and C plants demonstrates the high fungal ADH and ALP activity in inoculated roots.

Fungal activity enhanced the phosphorus as well as magnesium nutrition of leaves and alleviated the adverse effect of RW suppressing toxic ions such as Cl and Na (Table 4) (Talaat and Shawky 2014). Many authors suggested that in mycorrhizal plants, Na may be kept inside root cell vacuoles and intraradical fungal hyphae and may not be allocated to the shoots (Cantrell and Linderman 2001; Mardukhi et al. 2011). Therefore, as observed in the leaf interaction analyses, AMF limited the Na and Cl^- transport towards the leaves, guaranteeing a better functionality of

chloroplasts and a better photosynthetic efficiency of inoculated plants, as shown by Rabie (2005) and Zuccarini et al (2008) in horticultural crops. On the contrary, from interaction results (Table 4), mycorrhizal inoculation decreased leaf Ca^{2+} content and did not increase leaf Mg content of RW plants. These results suggested that, AMF activated other different mechanisms than those used to decrease toxic ions (Fig.1 b). If we consider reclaimed water as a source of nutrients, it may be because the improved nutrient uptake is less obvious when mycorrhizal inoculation is combined with fertilizer application (Nidchaporn et al. 2005; Zuccarini et al. 2008).

On the other hand, the high content of easily extractable glomalin (EEG) observed in both C and RW inoculated soils (Fig. 1a) could be related to the higher content of C_{org} , OM and the tendency of N_T to increase in inoculated soils with respect to non-inoculated soils (Zhu and Miller 2003). In fact, glomalin is a glycoprotein produced by AMF which protects hyphae from the losses of nutrients and water. Once hyphae finish the transport of nutrient, the glomalin inside of their cells is released and it accumulates in the soil (Grümberg et al. 2010). This glomalin in the soil indicates a high percentage of carbon and nitrogen edaphic (Treseder and Turner 2007). Its contribution to organic matter, carbon and nitrogen reservoirs is even more significant than the microbial biomass uptake, due to its recalcitrant behaviour in soil, which permits its accumulation, creating stable aggregates (Comis 2002). Thus, this protein helps to improve the nutrient uptake of the roots from the soil and it could be playing a key role in the structural stability of soil (Rillig 2004).

The plants subjected to RW presented a decrease in stem water potential from the second saline period, as a result of the increase of EC in soil (Fig. 2a). The results from volumetric content in soil (Fig. 2c) suggested that toxic ions like Na^+ and Cl^- dissolved in treated wastewater caused a high difficulty of roots in absorbing water from the soil (Munns 2002; Niu and Cabrera 2010a). However, the mycorrhizal inoculation improved the water status and slightly improved the stomatal conductance of inoculated RW plants respect to the non-inoculated plants (Fig. 3a). Similar results were observed for the same inoculum, *Glomus iranicum* var. *tenuihypharum* (Vicente-Sánchez et al. 2013; Nicolás et al. 2014) and for ornamental inoculated plants such as *Arbutus unedo* (Navarro et al. 2011) with the beneficial effects of AMF becoming

more visible at the end of the second saline period, when the level of salinity increased (Mardukhi et al. 2011).

In spite of *laurustinus* has been classified as a salt-sensitive plant (Azza et al. 2007), in our case, the results from osmotic potential at full turgor (Fig. 2b), according to Gómez-Bellot et al. (2013a) indicated that *laurustinus* plants irrigated with RW were able to develop osmotic adjustment permitting the enhancement of water status and obtaining values of photosynthesis close to Control plants throughout the experiment. Although non statistical differences between treatments, mycorrhizal inoculation slightly increased P_n values, especially from Control plants (Fig. 3b), which was probably due to AMF increasing the Mg uptake which helped to maintain the high chlorophyll content in leaves (Fig. 1b). According to Evelin et al. (2009), by maintaining a high Mg/Na ratio in leaves, the chlorophyll concentration increases and hence the photosynthetic efficiency and plant growth improve. In our study, under mycorrhizal inoculation, the effect of Na uptake is probably suppressed by Mg^{2+} in leaves (Giri et al. 2003). Therefore, the levels of photosynthetic capacity (estimated by chlorophyll content) improved.

At the end of the second saline period, there was a clear accumulation of toxic ions in RW plants which led to leaf chlorosis due to salts reducing the chlorophyll content in leaves (Fig. 2d). As a consequence, the lowest percentage of plants in ideal conditions was found in non-inoculated RW plants, (35 % of PIC and 5% of DP). Nevertheless, as a result of this, *laurustinus* plants improved their aesthetic value when AMF was inoculated (Fig. 4a). On the other hand, AMF increased the number of flowers per plant (Fig. 4b). A similar increase in flowers due to mycorrhizal inoculation has been previously described for many ornamental plants (Perner et al. 2007; Navarro et al. 2012), due to an increase in K^+ and P concentration in flowers. Among the different functions of both elements, P and K^+ are associated with bud production and flowers development (Krizek and Fletcher 2005; Gaur and Adholeya 2005). In our case, although there is no data on the mineral flower content, the flower stimulation could be related to the increase of leaf P^+ in inoculated plants or a possible hormonal effect induced by the presence of AMF (Perner et al. 2007).

5. Conclusions

The accumulation of toxic ions in the plant after thirty six weeks irrigating with RW, first at 4 dS m⁻¹ and then at 6 dS m⁻¹ led to a high percentage of plants with chlorotic leaves. In spite of the development of native AMF, the results suggested that the inoculation of *Glomus iranicum* var. *tenuihypharum* improved the aesthetic value of the laurustinus plants in both irrigation conditions, by ameliorating to a certain extent the negative effects of reclaimed water with high salinity. Furthermore, AMF also had a positive effect in well watered plants, as a consequence of a stimulation of physiological parameters. Effective AMF associations that avoid excessive salinity will provide wastewater reuse options with economic and environmental benefits, especially when the plants are grown in soils.

GENERAL CONCLUSIONS

1. The chemical properties of the waters applied (NaCl solution and reclaimed wastewater both with $EC=4 \text{ dS m}^{-1}$) had different physiological effects on euonymus and laurustinus plants, especially as regards the capacity to recover from salinity, possibly the higher contents of magnesium, sulphur, calcium, potassium and phosphorous in RW than in NaCl solution improve the nutrition in laurustinus plants, although the aesthetic value and growth decrease in both species.
2. The higher leaf content of magnesium, potassium and phosphorous from plants irrigated with reclaimed wastewater might counteract the effects of toxic ions in euonymus, producing plants with a more intense green colour, higher chlorophyll content and higher height. However, as a consequence of the accumulation of toxic ions in the plant, an osmotic effect was reflected in the daily physiological behaviour of this species.
3. In order to obtain plants irrigated with reclaimed waters with an acceptable ornamental value, it is important to attend not only to the species of plant selected but also the composition of the reclaimed wastewater, as well as its source and treatment. In spite of the high salt content, the reclaimed wastewater could content beneficial nutrients which might improve the nutritional status of the plants.
4. Both techniques (thermographic camera and infrared thermometers) for measuring canopy temperature were good indicators of water status in euonymus plants for both irrigated conditions (Control and reclaimed wastewater). Significant correlations were found between stomatal conductance and canopy temperature.
5. Different growing conditions (pot and soil) affect the response of plants when are inoculated with AMF. Inoculated plants growing in soil are able to explore more volume of the surrounding soil and capture more organic nutrients than in containers.
6. Mycorrhizal inoculation in the plant was more effective when the irrigation water applied had a higher concentration of toxic ions and lower nutrients such as calcium,

magnesium and phosphorus. Nevertheless, its effectiveness not only depends on the intensity and duration of salinity level but also on other factors such as the species used, type of AMF selected and their association of both, the characteristics of soil or substrate and the water regimen applied. *Euonymus* growing in pots under greenhouse conditions behaved differently respect to *laurustinus* growing in soil under field conditions when they were inoculated with AMF.

7. In the case of *laurustinus*, the reclaimed wastewater (4 and 6 dS m⁻¹) caused an osmotic stress and diminished the aesthetic value of plants. However, AMF satisfactorily colonized the roots and presented a high percentage of enzymatic activity. AMF enhanced the structure of soil by increasing the glomaline and C_{org} content, increased beneficial nutrients and decreased toxic ions in leaves, stimulated flowering and enhanced the water status of the plants irrigated with reclaimed water with 6 dS m⁻¹. In addition, AMF not only ameliorated the negative effects of reclaimed water, but also had a positive effect in well watered plants.

8. Mycorrhizal inoculation had a clear beneficial effect in both soil and plants which led to high quality plants, suggesting that effective AMF associations that avoid excessive salinity could provide wastewater reuse options, especially when the plants grow in soils.

REFERENCES

- AATSE (Australian Academy of Technological Sciences and Engineering). 2004. Water recycling in Australia. AATSE, Victoria, Australia.
- Abdel-Fattah, G.M., Asrar, A.A. 2012. Arbuscular mycorrhizal fungal application to improve growth and tolerance of wheat (*Triticum aestivum* L.) plants grown in saline soil. *Acta Physiol. Plant* 34:267-277.
- Acosta-Motos, J.R., Álvarez, S., Barba-Espín, G., Hernández, J.A., Sánchez-Blanco, M.J. 2014a. Salts and nutrients presented in regenerated waters induce changes in water relations, antioxidative metabolism, ion accumulation and restricted ion uptake in *Myrtus communis* L. *Plants. Plant Physiol. Bioch.* 85: 41-50
- Acosta-Motos, J.R., Álvarez, S., Hernández, J.A., Sánchez-Blanco, M.J. 2014b. Irrigation of *Myrtus communis* plants with reclaimed water: morphological and physiological responses to different levels of salinity. *J. Hortic. Sci. Biotech.* in press.
- Adrover, M., Forss, A., Ramon, G., Vadell, J., Moya, G., Taberner, A. 2008. Selection of woody species for wastewater enhancement and restoration of riparian woodlands. *J Environ. Biol.* 29:357–361.
- Aiello, R., Cirelli, G.L., Consoni, S. 2007. Effects of reclaimed wastewater irrigation on soil and tomato fruits: a case study in Sicily (Italy). *Agr. Water Manage.* 93:65-72.
- Alarcón, J.J., Sánchez-Blanco, M.J., Bolarín, M.C., Torrecillas, A. 1994. Growth and osmotic adjustment of two tomato cultivars during and after saline stress. *Plant Soil* 166:75-82.
- Al-Garni, S.M.S. 2006. Increasing NaCl-salt tolerance of a halophytic plant *Phragmites australis* by mycorrhizal symbiosis. *Am. Eurasian. J. Agric. Environ. Sci.* 1:119–126.
- Aliasgharzadeh, N., Saleh Rastin, N., Towfighi, H., Alizadeh, A. 2001. Occurance of arbuscular mycorrhizal fungi in saline soils of the Tabriz plain of Iran in relation to some physical and chemical properties of soil. *Mycorrhiza* 11:119–122.
- Al-Karaki, G.N., Clark R.B. 1998. Growth, mineral acquisition, and water use by mycorrhizal wheat grown under water stress. *J. Plant. Nutr.* 21:263–176.

- Al-Karaki, G.N., Hammad, R., Rusan, M. 2001. Response of two tomato cultivars differing in salt tolerance to inoculation with mycorrhizal fungi under salt stress. *Mycorrhiza* 11:43–47.
- Allen, M.F. 1991. *The Ecology of Mycorrhizae*. University Press, Cambridge, UK, 196p.
- Allen, R.G., Pereira, L.S., Raes, D., Smith, M. 1998. Crop evapotranspiration. Guidelines for computing crop water requirements. FAO Irrigation and Drainage Paper 56, FAO, Rome, Italy.
- Almansouri, M., Kinet, J.M., Lutts, S. 2001. Effect of salt and osmotic stresses on germination in durum wheat (*Triticum durum* Desf.). *Plant Soil* 231:243-254.
- Álvarez, S., Gómez-Bellot, M.J., Castillo, M., Bañón, S., Sánchez-Blanco, M.J. 2012. Osmotic and saline effect on growth, water relations, and ion uptake and translocation in *Phlomis purpurea* plants. *Environ. Exp. Bot.* 78:138-145.
- Álvarez, S., Sánchez-Blanco, M.J., 2014. Long-term effect of salinity on plant quality, water relations, photosynthetic parameters and ion distribution in *Callistemon citrinus*. *Plant Biol.* 16:757-764.
- Amaya-Carpio, L., Davies, F.T., Fox, T., He, C. 2009. Arbuscular mycorrhizal fungi and organic fertilizer influence photosynthesis, root phosphatase activity, nutrition, and growth of *Ipomoea carnea* ssp. *fistulosa*. *Photosynthetica* 47:1-10.
- Anderson, J.M. 2005. Integrating Recycled Water into Urban Water Supply Solutions. In: Khan SJ, Muston MH, Schäfer AI. (eds) *Integrated Concepts in Water Recycling*, University of Wollongong, Australia, pp 32-40.
- Angelakis, A.N., Marekos, M.H.F., Bontoux, L., Asano, T. 1999. The status of wastewater reuse practice in the Mediterranean basin: need for guidelines. *Wat. Res.* 33:2201-2217.
- Aroca, R., Ruíz-Lozano, J.M., Zamarreño, A., Paz, A., García-Mina, J.M., Pozo, M.J., López-Ráez, J.A. 2013. Arbuscular mycorrhizal symbiosis influences strigolactone production under salinity and alleviates salt stress in lettuce plants. *J. Plant. Physiol.* 170:47-55.

- Ashraf, M., Harris, P.J. 2004. Potential biochemical indicators of salinity tolerance in plants. *Plant Sci.* 166:3–16.
- Ashraf, M., Athar, H.R., Harris, P.J.C., Kwon, T.R. 2008. Some prospective strategies for improving crop salt tolerance. *Adv. Agron.* 97:45-110.
- Aucejo, A., Ferrer, J., Gabaldón, P., Marzal, P., Seco, A. 1997. Toxicity in citrus plantations in Villareal, Spain. *Water Air Soil Poll.* 94:349-360.
- Azevedo-Neto, A.D., Prisco, J.T., Enéas-Filho, J., Lacerda, C.F., Silva, J.V., Costa, P.H.A., Gomes-Filho, E. 2004. Effects of salt stress on plant growth, stomatal response and solute accumulation of different maize genotypes. *Braz. J. Plant Physiol.* 16:31–38.
- Azza, A., Fatma, E., Favahat, M. 2007. Responses of ornamental plants woody trees to salinity world. *J. Agr. Sci.* 3:386–395.
- Bacelar, E.A., Moutinho-Pereira, J.M., Gonçalves, B.C., Ferreira, H.F., Correia, C.M. 2007. Changes in growth, gas exchange, xylem hydraulic properties and water use efficiency of three olive cultivars under contrasting water availability regimes. *Environ. Exp. Bot.* 60:183-192.
- Ballester, C., Jiménez-Bello, M.A., Castel, J.R., Intrigliolo, D.S. 2013. Usefulness of thermography for plant water stress detection in citrus and persimmon trees. *Agric. For Meteorol.* 168:120–129.
- Baluja, J., Diago, M.P., Balda, P., Zorer, R., Meggio, F., Morales, F., Tardáguila, J. 2012. Assessment of vineyard water status variability by thermal and multispectral imagery using an unmanned aerial vehicle (UAV). *Irrig. Sci.* 30:511–522.
- Bansal, R.L., Nayyar, V.K., Takkar, P.N. 1992. Accumulation and bioavailability of Zn, Cu, Mn and Fe in soils polluted with industrial wastewater. *J. Indian Soc. Soil. Sci.* 40:796-799.
- Bañón, S., Miralles, J., Ochoa, J., Franco, J.A. Sánchez-Blanco, M.J. 2011. Effects of diluted and undiluted treated wastewater on the growth, physiological aspects and visual quality of potted lantana and polygala plants. *Sci. Hortic.* 129:869-876.

- Bañón, S., Miralles, J., Ochoa, J., Sánchez-Blanco, M.J. 2012. The effect of salinity and high boron non growth, photosynthetic activity and mineral contents of two ornamental shrubs. Hort. Sci. 39:188-194.
- Barar, M.S., Mahli, S.S., Singh, A.P., Aroroa, C.L., Gill, K.S. 2000. Sewer water irrigation effects on some potentially toxic trace elements in soil and potato plants in northwestern India. Can. J. Soil. Sci. 80:465-471.
- Bennett, W.F. 1993. Nutrient deficiencies and toxicities in crop plants. Minnesota: APS Press.
- Bernstein, N., Asher, B.T., Haya, F., Pini, S., Ilona, R., Amram, C., Marina, I. 2006. Application of treated wastewater for cultivation of roses (*Rosa hybrida*) in soil-less culture. Sci. Hort. 108:185–193.
- Blonquist, J.M., Norman, J.M., Bugbee, B. 2009. Automated measurement of canopy stomatal conductance based on infrared temperature. Agric. For Meteorol. 149:2183–2197.
- Blum, A. 1988. Plant breeding for stress environments. CRC Press: Boca Raton, FL.
- Bohnert, H.J., Jensen, R.G. 1996. Metabolic engineering for increased salt tolerance: the next step. Aust. J. Plant Physiol. 23:661–667.
- Boland, A.M. 2008. Management of saline/or recycled water for irrigated horticulture. Acta Hort. 792:123–134.
- Borde, M., Dudhane, M., Jite, P. 2011. Growth photosynthetic activity and antioxidant responses of mycorrhizal and non-mycorrhizal bajra (*Pennisetum glauca*) crop under salinity stress condition. Crop Prot. 30:265–271.
- Bradley, P.M., Morris, J.T. 1991. The influence of salinity on the kinetics of NH_4^+ uptake in *Spartina alterniflora*. Oecologia 85:375–380.
- Bressler, E., McNeal, B.I., Carter, D.L. 1982. Saline and sodic soil. Springer, New York.
- Breuninger, M., Requena, N. 2004. Recognition events in AM symbiosis: analysis of fungal gene expression at the early appressorium stage. Fungal Genet Biol 41:794–804.
- Brown, J.J., Glenn, E.P., Fitzsimmons, K.M., Smith, S.E. 1999. Halophytes for the treatment of saline aquaculture effluent. Aquaculture 175:255-268.

- Cameron, R., Harrison-Murray, R., Fordham, M., Wilkinson, S., Davies, W., Atkinson, C., Else, M. 2008. Regulated irrigation of woody ornamentals to improve plant quality and precondition against drought stress. *Ann. Appl. Biol.* 153:49-61.
- Cantrell, I.C., Linderman, R.G. 2001. Preinoculation of lettuce and onion with VA mycorrhizal fungi reduces deleterious effects of soil salinity. *Plant Soil* 233:269–281.
- Carter, C.T., Grieve, C.M., Poss, J.A., Suarez, D.L. 2005. Production and ion uptake of *Celosia argentea* irrigated with saline wastewaters. *Sci. Horti.* 106:381–394.
- Carter, C.T. and C.M. Grieve. 2008. Mineral nutrition, growth, and germination of *Antirrhinum majus* L. (snapdragon) when produced under increasingly saline conditions. *HortScience* 43:710–718.
- Carvalho, L.M., Cacador, I., Martins-Loucao, M.A. 2001. Temporal and spatial variation of arbuscular mycorrhizas in salt marsh plants of the Tagus estuary (Portugal). *Mycorrhiza* 11:303–309.
- Cassaniti, C., Leonardi, C., Flowers, T.J. 2009. The effect of sodium chloride on ornamental shrubs. *Sci. Hortic.-Amsterdam* 122:586–593.
- Cassaniti, C., Romano, D., Hop, M.E.C.M., Flowers, T.J. 2012a. Growing floricultural crops with brackish water. *Environ. Exp. Bot.* In press.
- Cassaniti, C., Romano, D., Flowers, T.J. 2012b. The response of ornamental plants to saline irrigation water. In: García-Garizábal I, Abrahao R. (eds) *Irrigation: Types, Sources and Problems/Book 2*. Intech, Rijeka, Croatia, pp 131–158.
- Çekiç, F.Ö., Ünyayar, F., Ortaş, İ. 2012. Effects of arbuscular mycorrhizal inoculation on biochemical parameters in *Capsicum annuum* grown under long term salt stress. *Turk. J. Bot.* 36:63-72.
- Cervilla, L.M., Blasco, B., Ríos, J.J., Rosales, M.A., Rubio-Wilhelmi, M.M., Sánchez-Rodríguez, E., Romero, R., Ruiz, J.M. 2009. Response of nitrogen metabolism to boron toxicity in tomato plants. *Plant Biol.* 5:671-677.
- Chaiprapat, S., Sdoodee, S., 2007. Effects of wastewater recycling from natural rubber smoked sheet production on economic crops in southern Thailand. *Resour. Conserv. Recy.* 51:577–590.

- Chaparzadeh, N., Khavari-Nejad, R.A., Navari –Izzo, F., Izzo, A. 2003. Water relations and ionic balance in *Calendula officinalis* L. under saline conditions. *Agrochimica* 47:69-79.
- Chapman, H.D. 1968. The mineral nutrition of citrus, in: Reuther LD, Batchebor and Webber HJ, eds., *The Citrus Industry*, pp 127-274.
- Chaves, M.M., Flexas, J., Pinheiro, C. 2009. Photosynthesis under drought and salt stress. Regulation mechanisms from whole plant to cell. *Ann. Bot.-London* 103:551-560.
- Chaves, M.M., Miguel, C.J. Madeira, S.N.J. 2011. Chapter 3 Recent advances in photosynthesis under drought and salinity. In: Tukan, I. (Ed), *Advances in botanical research*. Academic Press 57:49-104.
- Clemente, R., Walker, D.J., Bernal, M.P. 2005. Uptake of heavy metals by *Brassica juncea* grown in a contaminated soil in Aznalcollar (Spain): the effect of soil amendments, *Environ. Pollut.* 138:46–58.
- Colaizzi, P.D., Evett, S.R., O’Shaughnessy, S.A., Howell, T.A. 2012. Using plant canopy temperature to improve irrigated crop management. In: *Proc. 24th Annual Central Plains Irrigation Conf.* Colby, KS.
- Colmer, T.D., Flowers, T.J., Munns, R. 2006. Use of wild relatives to improve salt tolerance in wheat. *J. Exp. Bot.* 57:1059-1078.
- Comis, D. 2002. Glomalin: Hiding place for a third of the world’s stored soil carbon. *Agricultural Research Magazine*.
- Costa, J.M., Ortuño, M.F., Lopes, C.M., Chaves, M.M. 2012. Grapevine varieties exhibiting differences in stomatal response to water deficits. *Funct. Plant Biol.* 39:179–189.
- Costa, J.M., Grant, O.M., Chaves, M.M. 2013. Thermography to explore plant–environment interactions. *J Exp Bot* 64: 3937-3949.
- Delgado Díaz, S. 2003. Tendencias en la reutilización de aguas. El caso de Tenerife. *Tecnología del Agua*. 236:30-42.
- Dell’Amico, J., Torrecillas, A., Rodriguez, P., Morte, A., Sanchez-Blanco, M. 2002. Responses of tomato plants associated with the arbuscular mycorrhizal fungus *Glomus clarum* during drought and recovery. *J. Agr. Sci.* 138 :387–393.

- De Lorenzi, F., Stanghellini, C., Pitacco, A. 1993. Water shortage sensing through infrared canopy temperature: timely detection is imperative. *Acta Horti* 335:373–380.
- Demetriou, G., Neonaki, C., Navakoudis, E., Kotzabasis, K. 2007. Salt stress impact on the molecular structure and function of the photosynthetic apparatus-the protective role of polyamines. *BBA-Bioenergetics* 1767:272–280.
- Deressa, T.G., Schenk, M.K. 2008. Contribution of roots and hyphae to phosphorus uptake of mycorrhizal onion (*Allium cepa* L.). A mechanistic modeling approach. *J. Plant Nutr. Soil Sci.* 17:810-820.
- Donoghue, M.J., Baldwin, B.G., Li, J., Winkworth, C. 2004. *Viburnum* phylogeny based on chloroplast trnK intron and nuclear ribosomal ITS DNA sequences. *Syst. Bot.* 29:188–198.
- Dudhane, M., Borde, M., Jite, P. 2011. Effect of arbuscular mycorrhizal fungi on growth and antioxidant Activity in *Gmelina arborea* Roxb. under salt stress condition. *Not Sci Biol* 3:71–78.
- Duncan, R.R., Carrow, R.N., Huck, M.T. 2009. Turfgrass and landscape irrigation water quality: Assessment and management. p. 13–38. CRC Press, Boca Raton, FL.
- Durigon, A., van Lier Q.D. 2013. Canopy temperature versus soil water pressure head for the prediction of crop water stress. *Agric Water Manage* 127:1–6.
- Elbana, M., Puig-Bargues, J., Pujol, J., de Cartagena, F.R. 2010. Preliminary planning for reclaimed water reuse for agricultural irrigation in the province of Girona, Catalonia (Spain). *Desalin. Water Treat.* 22:47-55.
- Evans, J.R., Poorter, H. 2001 Photosynthetic acclimation of plants to growth irradiance: the relative importance of specific leaf area and nitrogen partitioning in maximizing carbon gain. *Plant Cell. Environ.* 24:755-768.
- Evelin, H., Kapoor, R., Giri, B. 2009. Arbuscular mycorrhizal fungi in alleviation of salt stress: a review. *Ann Bot* 104:1263–1280.
- Feigin, A., Ravina, I., Shalhevet, J. 1991. Irrigation with treated sewage effluent. In: Management for environmental protection. Advanced series in agricultural sciences. Springer-Verlag. p:18-31.

- Feitosa de Larcera, C., Cambraia, J., Oliva, M.A., Ruiz, H.A., 2005. Changes in growth and in solute concentrations in sorghum leaves and roots during salt stress recovery. *Environ. Exp. Bot.* 54:69-76.
- Feng, G., Zhang, F.S., Li, X.L., Tian, C.Y., Tang, C., Rengel, Z.: 2002. Improved tolerance of maize plants to salt stress by arbuscular mycorrhiza is related to higher accumulation of soluble sugars in roots. *Mycorrhiza* 12:185–190.
- Fernández, F. La simbiosis micorrízica arbuscular, En: El manejo efectivo de la simbiosis micorrízica, una vía hacia la agricultura sostenible. Estudio de caso: El Caribe. Primera Edición, La Habana:Ediciones INCA, 2003, p. 13-48.
- Fernández, F., Juárez, J. 2011. Procedimiento de obtención de un agente micorrizógeno. Solicitud de Patente Invención no 201130566. OEPM. 17 p.
- Flexas, J., Bota, J., Loreto, F., Cornic, G. Sharkey, T.D. 2004. Diffusive and metabolic limitations to photosynthesis under drought and salinity in C-3 plants. *Plant Biology* 6:269- 279.
- Flexas, F., Ribas-Carbo, M., Bota, J., Galmes, J., Henkle, M., Martinez-Canellas, S., Medrano, H. 2006. Decreased activity during water stress is not induced by decreased relative water content but related to conditions of low stomatal conductance and chloroplast CO₂ concentration, *New Phytol.* 172:73-82.
- Flowers, T.J., Colmer, T.D. 2008. Salinity tolerance of halophytes. *New Phytol.* 179:945–963.
- Fornes, F. Belda, R.M., Carrión, C. Noguera, V., García-Agustín, P., Abad, M. 2007. Pre-conditioning ornamental plants to drought by mean of saline water irrigation as related to salinity tolerance. *Sci. Hortic.* 113:52-59.
- Fox L.J., Grose, J.N., Appleton, B.L., Donohue, S.J. 2005. Evaluation of treated effluent as an irrigation source for landscape plants. *J Environ Hortic* 23:174–178.
- Franco, J.A., Martínez-Sánchez, J.J., Fernández, J.A., Bañón, S., 2006. Selection and nursery production of ornamental plants for landscaping and xerogardening in semi-arid environments. *J. Hortic. Sci. Biotech.* 81:3-17.

- Franco, J.A., Bañón, S., Vicente, M.J., Miralles, J., Martínez-Sánchez, J.J. 2011. Root development in horticultural plants grown under abiotic stress conditions– a review. *J. Hortic. Sci. Biotech.* 86:543–556.
- Friedman, H., Bernstein, N., Bruner, M., Rot, I., Ben-Noon, Z., Zuriel, A., Zuriel, R., Finkelstein, S., Umiel, N., Hagiladi, A. 2007. Application of secondary-treated effluents for cultivation of sunflower (*Helianthus annuus* L.) and celosia (*Celosia argentea* L.) as cut flowers. *Scientia Hort.* 115:62–69.
- Fuchs, M. 1990. Infrared measurement of canopy temperature and detection of plant water stress. *Theor Appl Climatol* 42:253–261.
- Fuentes, S., De Bei R., Pech, J., Tyerman, S. 2012. Computational water stress indices obtained from thermal image analysis of grapevine canopies. *Irrig Sci* 30:523–536.
- Galmes, J., Medrano, H., Flexas, J. 2007. Photosynthesis and photoinhibition in response to drought in a pubescent (var. *minor*) and a glabrous (var. *palaui*) variety of *Digitalis minor*. *Environ. Exp. Bot.* 60:105-111.
- García-Tejero, I., Durán-Zuazo, V.H., Muriel-Fernández, J.L., Jiménez-Bocanegra, J.A. 2011. Linking canopy temperature and trunk diameter fluctuations with other physiological water status tools for water stress management in citrus orchards. *Funct Plant Biol.* 38:106–117.
- García-Tejero, I., Durán-Zuazo, V.H., Arriaga, J., Hernández, A., Vélez, L.M., Muriel-Fernández, J.L. 2012. Approach to assess infrared thermal imaging of almond trees under water-stress conditions. *Fruits* 67:463–474.
- Gaur, A., Adholeya, A. 2005. Diverse response of five ornamental plant species to mixed indigenous and single isolate arbuscular mycorrhizal inocula in marginal soil amended with organic matter. *J Plant Nutr.* 28:707–723.
- Gharineh, M.H., Nadian, H., Fathi, G., Siadat, A., Maadi, B. 2009. Role of arbuscular mycorrhizae in development of salt-tolerance of *Trifolium alexandrinum* plants under salinity stress. *J. Food Agric. Environ.* 7:432–437.
- Ghoulam, C.F., Ahmed, F., Khalid, F. 2002. Effects of salt stress on growth, inorganic ions and proline accumulation in relation to osmotic adjustment in five sugar beet cultivars. *Environ. Exp. Bot.* 47:139–150.

- Gianinazzi, S., Gianinazzi-Pearson, V., Tisserant, B., Lemoine, M.C. 1992. Protein activities as potential markers of functional endomycorrhizas in plants. In: Read, D. H. Lewis, A. H. Fitter and I. Alexander (eds) *Mycorrhizas in Ecosystems*, CAB International: Oxon, U.K. pp. 333-339.
- Gilman, E.F. 1999. *Euonymus japonica*. Cooperative Extension Service, Institute of Food and Agricultural Science 204:1-3.
- Giovannetti, M., Mosse, B. 1980. An evaluation of techniques for measuring vesicular–arbuscular mycorrhizal infection in roots. *New Phytol.* 84:489–499.
- Giri, B., Kapoor, R., Mukerji, K.G. 2003. Influence of arbuscular mycorrhizal fungi and salinity on growth, biomass, and mineral nutrition of *Acacia auriculiformis*. *Biol. Fert. Soils* 38:170–175.
- Giri, B., Mukerji, K.G. 2004. Mycorrhizal inoculant alleviates salt stress in *Sesbania aegyptiaca* and *Sesbania grandiflora* under field conditions: evidence for reduced sodium and improved magnesium uptake. *Mycorrhiza* 14:304–312.
- Giri, B., Kapoor, R., Mukerji, K.G. 2007. Improved tolerance of *Acacia nilotica* to salt stress by arbuscular mycorrhiza, *Glomus fasciculatum* may be partly related to elevated K/Na ratios in root and shoot tissues. *Microb. Ecol.* 54:753–760.
- Goldhamer, D.A., Fereres, E. 2001. Irrigation scheduling protocols using continuously recorded trunk diameter measurements. *Irrig. Sci.* 20:115–125.
- Goldhamer, D.A., Fereres, E., Salinas, M. 2003. Can almond trees directly dictate their irrigations needs? *Calif. Agric.* 57:138–144.
- Gómez-Bellot, M.J., Álvarez, S., Bañón, S., Ortuño, M.F., Sánchez-Blanco, M.J. 2013a. Physiological mechanisms involved in the recovery of euonymus and laurustinus subjected to saline waters. *Agric. Water Manage.* 128:131–139.
- Gómez-Bellot, M.J., Álvarez, S., Castillo, M., Bañón, B., Ortuño, M.F. Sánchez-Blanco, M.J. 2013b. Water relations, nutrient content and developmental responses of *Euonymus* plants irrigated with water of different degrees of salinity and quality. *J. Plant. Res.* 126:567–576.
- Gorham, J.W., McDonnell, E. 1985. Some mechanism of salt tolerance in crop plants. *Plant Soil* 89:15–40.

- Gori, R., Ferrini, F., Nicese, F.P., Lubello, C. 2000. Effect of reclaimed wastewater on the growth and nutrient content of three landscape shrubs. *J. Environ. Hort.* 18:108-114.
- Gori, R., Lubello, C., Ferrini, F., Nicese, F.P., Coppini, E. 2008. Reuse of industrial wastewater for the irrigation of ornamental plants. *Water. Sci. Technol.* 57:883–889.
- Grant, O.M., Chaves, M.M., Jones, H.G. 2006. Optimizing thermal imaging as a technique for detecting stomatal closure induced by drought stress under greenhouse conditions. *Physiol. Plant.* 127:507–518.
- Grant, O.M., Davies, M.J., Longbottom, H., Atkinson, C.J. 2009. Irrigation scheduling and irrigation systems: optimising irrigation efficiency for container ornamental shrubs. *Irrig. Sci.* 27:139–153.
- Grant, O.M., Davies, M.J., Jamesa, C.M., Johnson, A.W., Leinonen, I., Simpson, DW. 2012. Thermal imaging and carbon isotope composition indicate variation amongst strawberry (*Fragaria xananassa*) cultivars in stomatal conductance and water use efficiency. *Environ. Exp. Bot.* 76:7–15.
- Greenway, M., Munns, R. 1980. Mechanisms of salt tolerance in nonhalophytes. *Ann. Rev. Plant Physiol.* 31:149- 190.
- Grieve, C.M., Poss, J.A., Amrhein, C. 2006. Response of *Matthiola incana* to irrigation with saline wastewaters. *HortScience* 41:119–123.
- Grunenfelder, L., Hiller, L.K., Richard Knowles, N. 2006. Color indices for the assessment of chlorophyll development and greening of fresh market potatoes. *Postharvest Biol. Tec.* 40:73-81.
- Grüenberg, B., Conforto, C., Rovea, A., Boxler, M., March, G., Luna, C., Meriles, J., Vargas Gil, S. 2010. La glomalina y su relación con la productividad del cultivo de maíz. *Informaciones Agronómicas del Cono Sur.* 47:23-25.
- Grzesiak, M.T. 2004. Effect of drought stress on photosynthetic apparatus and productivity of triticale and maize genotypes differing in drought tolerance. PhD Thesis, Agricultural University, Krakow (in Polish).

- Gucci, R., Xilyannis, C., Flores, 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.
- Hackl, H., Baresel, J.P., Mistele, B., Hu, Y., Schmidhalter, U. 2012. A comparison of plant temperatures as measured by thermal imaging and infrared thermometry. *J. Agron. Crop Sci.* 198:415–429.
- Hajiboland, R., Aliasgharzadeh, N., Laiegh, S.F., Poschenrieder, C. 2010. Colonization with arbuscular mycorrhizal fungi improves salinity tolerance of tomato (*Solanum lycopersicum* L.) plants. *Plant Soil* 331:313–327.
- Halim, R.A., Buxton, D.R., Hattendorf, M.J., Carlson, R.E. 1990. Crop water–stress index and forage quality relationships in alfalfa. *Agron. J.* 82:906–909.
- Hamel, C., Plenchette, C. 2007. Mycorrhizae in crop production. Haworth Food and Agricultural Products. Haworth Press, Binghamton, p 366.
- Hartmond, U., Schaesberg, N.V., Graham, J.H., Syvertsen, J.P. 1987. Salinity and flooding stress effects on mycorrhizal and non mycorrhizal citrus rootstock seedlings. *Plant Soil.* 104:37-43.
- Heiskanen, J. 2005. Foliar colour as an indicator of foliar chlorophyll and nitrogen concentration and growth in Norway spruce seedlings. *Scan. J. Forest Res.* 20:329-336.
- Hochstrat, R., Wintgens, T., Melin, T., Jeffrey, P. 2005. Wastewater reclamation and reuse in Europe: a model-based potential estimation. *Water Sci. Technol.* 5:67–75.
- Hodge, A., Campbell, C.D., Fitter, A.H. 2001. An arbuscular mycorrhizal fungus accelerates decomposition and acquires nitrogen directly from organic material. *Nature* 413:297–299.
- Hsiao, T.C. 1993. Effects of drought and elevated CO₂ on plant water use efficiency and productivity, p. 435–465. In: M.D. Jackson, and C.R. Black (eds) *Global environmental change. Interacting stresses on plants in a changing climate.* NATO ASI series. Springer, New York.
- Hu, Y., Schmidhalter, U. 2005. Drought and salinity: a comparison of their effects on mineral nutrition of plants. *J. Plant. Nutr. Soil Sci.* 168:541-549.

- Iannelli, M.A., Pietrini, F., Fiore, L., Petrilli, L., Massacci, A. 2002. Antioxidant response to cadmium in *Phragmites australis* plants. *Plant Physiol. Biochem.* 40: 977-982.
- Idso, S.B. 1982. Non-water-stressed baselines: a key to measuring and interpreting plant water stress. *Agric. Meteorol.* 27:59–70.
- Inskeep, W.P., Bloom, P.R. 1985. Extinction coefficients of chlorophyll *a* and *b* in N,N-dimethylformamide and 80% acetone. *Plant Physiol.* 77:483–485.
- Jackson, R.D., Reginato, R.J., Idso, S.B. 1977. Wheat canopy temperature: a practical tool for evaluating water requirements. *Water Resour. Res.* 13:651–656.
- Jackson, R.D., Idso, D.B., Reginato, R.J., Pinter Jr P.J. 1981. Canopy temperature as a crop water stress indicator. *Water Resour. Res.* 17:1133-1138.
- Jahromi, F., Aroca, R., Porcel, R., Ruiz-Lozano, J.M. 2008. Influence of salinity on the in vitro development of *Glomus intraradices* and on the in vivo physiological and molecular responses of mycorrhizal lettuce plants. *Microb. Ecol.* 55:45-53.
- James, R.A., Rivelli, A.R. Munns, R., Von Caemmerer, S. 2002. Factors affecting CO₂ assimilation, leaf injury and growth in salt-stressed durum wheat. *Funct. Plant Biol.* 29:1393–1403.
- Jiang, H., Xu, D.-Q. 2000. Physiological basis of the difference in net photosynthetic rate of leaves between two maize strains. *Photosynthetica* 38:199-204.
- Jimenez-Cisneros, B. 1995. Wastewater reused to increase soil productivity. *J. Water Sci. Technol.* 32:173–180.
- Jones, H.G. 1983. Estimation of an effective soil water potential at the root surface of transpiring plants. *Plant, Cell Environ.* 6:671–674.
- Jones, H.G., Stoll, M., Santos, T., Sousa, C., Chaves, M.M., Grant, O.M. 2002. Use of infrared thermography for monitoring stomatal closure in the field: application to grapevine. *J. Exp. Bot.* 53:2249–2260.
- Jones, H.G. 2004. Application of thermal imaging and infrared sensing in plant physiology and ecophysiology. *Adv. Bot. Res.* 41:107–163.
- Jones, H.G., Serraj, R., Loveys, B.R., Xiong L, Wheaton, A., Price, A.H. 2009. Thermal infrared imaging of crop canopies for the remote diagnosis and

- quantification of plant responses to water stress in the field. *Funct. Plant Biol.* 36:978–979.
- Jordan, F.L., Yoklic, M., Morino, K., Brown, P., Seaman, R., Glenn, E.P. 2009. Consumptive water use and stomatal conductance of *Atriplex lentiformis* irrigated with industrial brine in a desert irrigation district. *Agr. Forest Meteorol.* 149:899-912.
- Juniper, S., Abbott, L.K. 2006. Soil salinity delays germination and limits growth of hyphae from propagules of arbuscular mycorrhizal fungi. *Mycorrhiza* 5:371–379.
- Kalavrouziotis, I.K., Kanatas, P.I., Papadopoulos A.H, Bladenopoulou, S, Koukoulakis, P.H, Leotsinides, M.N. 2005. Effects of municipal reclaimed wastewater on the macro and microelement status of the soil and plants. *Fresenius Environ. Bull.* 14:1050-1057.
- Katerji, N., van Hoorn, J.W., Hamdy, A., Mastrorilli, M. 2003. Salinity effect on crop development and yield, analysis of salt tolerance according to several classification methods. *Agr. Water Manage.* 62:37-66.
- Kaukoranta, T., Murto, J., Takala, J., Tahvonen, R. 2005. Detection of water deficit in greenhouse cucumber by infrared thermography and reference surfaces. *Sci. Hortic.* 106:447–463.
- Keren, R., Bingham, F.T. 1985. Boron in water, soils, and plants. *Adv. soil sci.* 1:229-276.
- Khan, M.A., Gul, B., Weber, D.J., 2001. Effect of salinity on the growth and ion content of *Salicornia rubra* Commun. *Soil Sci. Plan.* 32, 2965-2977.
- Kiziloglu, F.M., Turan, M., Sahin, U., Angin, I., Anapali, O., Okuroglu, M. 2007. Effects of wastewater irrigation on soil and cabbage-plant (*Brassica oleracea* var. capitata cv. yalova-1) chemical properties. *J. Plant Nutr. Soil. Sci.* 170:166–172.
- Klironomos, J.N., Moutoglis, P., Kendrick, B., Widden, P. 1993. A comparison of spatial heterogeneity of vesicular-arbuscular mycorrhizal fungi in two maple-forest soils. *Can. J. Botany* 71:1472–1480.

- Kluitenberg, G.J., Biggar, J.W. 1992. Canopy temperature as a measure of salinity stress on sorghum. *Irrig. Sci.* 13:115–121.
- Kohler, J., Caravaca, F., Roldán, A. 2010. An AM fungus and a PGPR intensify the adverse effects of salinity on the stability of rhizosphere soil aggregates of *Lactuca sativa*. *Soil Biol. Biochem.* 42:429–434.
- Krizek, B.A., Fletcher, J.C. 2005. Molecular mechanisms of flower development: an armchair guide. *Nat. Rev. Genet.* 6:688–698.
- Kumar, A., Sharma, S., Mishra, S. 2010. Influence of arbuscular mycorrhizal (AM) fungi and salinity on seedling growth, solute accumulation and mycorrhizal dependency of *Jatropha curcas* L. *J. Plant Growth Regul.* 29:297–306.
- Lawlor, D.W., Cornic, G. Photosynthetic carbon assimilation and associated metabolism in relation to water deficits in higher plants. *Plant Cell Environ.* 2002:25:275–94.
- Lee, K.M., van Iersel, M.S. 2008. Sodium chloride effects on the growth, morphology and physiology of chrysanthemum (*Chrysanthemum* × *morifolium*). *HortScience* 43: 1888–1891.
- Leinonen, I., Jones, H.G. 2004. Combining thermal and visible imagery for estimating canopy temperature and identifying plant stress. *J. Exp. Bot.* 55:1243–1231.
- Leinonen, I., Grant, O.M., Tagliavia, C.P.P, Chaves, M.M, Jones, H.G. 2006. Estimating stomatal conductance with thermal imagery. *Plant Cell. Environ.* 29:1508–1518.
- Levy, Y., Syvertsen, J.P., Nemec, S. 1983. Effects of drought stress and vesicular-arbuscular mycorrhizae on citrus transpiration and hydraulic conductivity of roots. *New Phytol.* 93:61–66.
- Lopes, M.S., Reynolds, M.P. 2010. Partitioning of assimilates to deeper roots is associated with cooler canopies and increased yield under drought in wheat. *Funct. Plant Biol.* 37:147–156.
- Lubello, C., Gori, R., Nicesse, F.P., Ferrini, P. 2004. Municipal-treated wastewater reuse for plant nurseries irrigation. *Water Res.* 38:2939–2947.
- Mabberley, D.J. 1997. The plant-book: a portable dictionary of the vascular plants. Cambridge: Cambridge University Press.

- MacDonald, R.M., Lewis, M. 1978. The occurrence of some acid phosphatases and dehydrogenases in the vesicular-arbuscular mycorrhizal fungus *Glomus mosseae*. New Phytol. 80:135-141.
- MAGRAMA, 2011. Superficie y producción de flor cortada y planta ornamental. Anuario de estadística. www.magrama.es
- Mardukhi, B., Rejali, F., Daei, G., Ardakani, M.R., Malakouti, M.J., Miransari, M. 2011. Arbuscular mycorrhizas enhance nutrient uptake in different wheat genotypes at high salinity levels under field and greenhouse conditions. C. R. Biol. 334:564–571.
- Maria, D., Tadeusz, B. 2005. Growth parameters and photosynthetic pigments in leaf segments of *Zea mays* exposed to cadmium, as related to protection mechanisms. Plant Physiol. 162:1013-1021.
- Mathur, N., Singh, J., Bohra, S., Vyas, A. 2007. Arbuscular mycorrhizal status of medicinal halophytes in saline areas of Indian Thar Desert. Int. J. Soil Sci. 2:119-127.
- McGuire, R.G. 1992. Reporting of objective colour measurements. HortScience 27:1254-1255.
- McMillen, B.G., Juniper, S., Abbott, L.K. 1998. Inhibition of hyphal growth of a vesicular-arbuscular mycorrhizal fungus in soil containing sodium chloride limits the spread of infection from spores. Soil Biol. Biochem. 30:1639–1646.
- Meloni, D.A., Gulotta, M.R., Martínez, C.A. 2008. Salinity tolerance in *Schinopsis quebracho* colorado: seed germination, growth, ion relations and metabolic responses. J. Arid Environ. 72:1785–1792.
- Ministry of Agriculture, Fish and Food, 2008. National Irrigation Plan. Madrid, 486 pp.
- Miralles, J., Valdés, R., Franco, F., Bañón, S. Sánchez-Blanco, M.J. 2011. Irrigation of hydrangea with saline reclaimed wastewater: effects of fresh water flushing. Acta Hort. 1000 : 229-236.
- Miralles, J., Valdés, R., Martínez-Sánchez, J.J., Bañón, S. 2012. Pot-in-pot reduces salinity, chloride uptake, and maintains aesthetic value in *Euonymus japonicus* Thunb. under saline irrigation. HortScience 47:607–613.

- Miransari, M., Bahrami, H.A., Rejali, F., Malakouti, M.J. 2008. Using arbuscular mycorrhiza to alleviate the stress of soil compaction on wheat (*Triticum aestivum* L.) growth. *Soil Biol. Biochem.* 40:1197-1206.
- Miyamoto, S., Martinez, I., Padilla, M., Portillo, A. 2004. Landscape Plant Lists for Salt Tolerance Assessment. Texas A&M Univ. Agri. Research Center at El Paso and El Paso Water Utilities. p 12.
- Morales, M.A., Sánchez-Blanco, M.J., Olmos, E., Torrecillas, A., Alarcón, J.J. 1998. Changes in the growth, leaf water relations and cell ultrastructure in *Argyranthemum coronopifolium* plants under saline conditions. *J. Plant Physiol.* 153:174-180.
- Morales, M.A., Olmos, E., Torrecillas, A., Sánchez-Blanco, M.J., Alarcón, J.J. 2001. Differences in water relations, leaf ion accumulation and excretion rates between cultivated and wild species of *Limonium* sp. grown in conditions of saline stress. *Flora* 196:345-352.
- Moreno, F., Fernandez, J.E., Clothier, B.E., Green, S.R. 1996. Transpiration and root water uptake by olive trees. *Plant Soil* 184:85-96.
- Mounzer, O., Pedrero-Salcedo, F., Nortes, P.A., Bayona, J.M., Nicolás-Nicolás, E., Alarcón J.J. 2013. Transient soil salinity under the combined effect of reclaimed water and regulated deficit drip irrigation of Mandarin trees. *Agric. Water Manage.* 120:23–29.
- Möller, M., Alchanatis, V., Cohen, Y., Meron, M., Tsipris, J., Naor, A., Ostrovsky, V., Sprintsin, M., Cohen, S. 2007. Use of thermal and visible imagery for estimating crop water status of irrigated grapevine. *J. Exp. Bot.* 58:827–838.
- Mujeriego, R. 1990. Riego con agua residual municipal regenerada. Manual práctico. Universidad Politécnica de Cataluña, 48 1 pp.
- Munns, R. 2002. Comparative physiology of salt and water stress. *Plant Cell Environ.* 25: 239-250.
- Munns, R., Tester, M. 2008. Mechanisms of salinity tolerance. *Ann. Rev. Plant Biol.* 59:651- 681.
- Muralev, E., Nazarenko, P.I., Poplavskij, V.M., Kuznetsov, I.A. 1997. Seawater desalination. In *Nuclear Desalinization of Seawater. Proceedings of a*

- Symposium in Taejon, Republic of Korea International Atomic Energy Agency, Austria, Vienna. p. 355-366.
- Naor, A., Cohen, S. 2003. Sensitivity and variability of maximum trunk shrinkage, midday stem water potential, and transpiration rate in response to withholding irrigation from field grown apple trees. *HortScience* 38:547–551.
- Naor, A. 2006. Irrigation scheduling and evaluation of tree water status in deciduous orchards. *Hortic. Rev.* 32:111–166.
- Navarro, A., Bañón, S., Olmos, E., Sánchez-Blanco, M.J., 2007. Effects of sodium chloride on water potential components, hydraulic conductivity, gas exchange and leaf ultrastructure of *Arbutus unedo* plants. *Plant Sci.* 172:473-480.
- Navarro, A., Bañón, S., Conejero, W., Sánchez-Blanco, M.J. 2008. Ornamental characters, ion accumulation and water status in *Arbutus unedo* seedlings irrigated with saline water and subsequent relief and transplanting. *Environ. Exp. Bot.* 62:364-370.
- Navarro, A., Bañón, S., Morte, A., Sánchez-Blanco, M.J. 2011. Effects of nursery preconditioning through mycorrhizal inoculation and drought in *Arbutus unedo* L. plants. *Mycorrhiza* 21:53–64.
- Navarro, A., Elia, A., Conversa, G., Campi, P., Mastroilli, M. 2012. Potted mycorrhizal Carnation plants and saline stress: Growth, quality and nutritional plant responses. *Sci. Hortic.* 140:131-139.
- Navarro-Ródenas, A., Bárzana, G., Nicolás, E., Carra, A., Schubert, A., Morte, A. 2013. Expression analysis of aquaporins from desert truffle mycorrhizal symbiosis reveals a fine-tuned regulation under drought. *Mol. Plant Microbe. Interact.* 26:1068–1078.
- Neumann, P.M. 1997. Salinity resistance and plant growth revisited. *Plant Cell. Environ.* 20:1193–1198.
- Nicolás, E., Maestre-Valero, J.F. Alarcón, J.J., Pedrero, F., Vicente-Sánchez, J., Bernabé, A., Gómez-Montiel, J., Hernández, A., Fernández, F. 2014. Effectiveness and persistence of arbuscular mycorrhizal fungi on the physiology, nutrient uptake and yield of Crimson seedless grapevine. *The J. Agr. Sci. Camb.* In press.

- Nidchaporn, N. 2005. Effects of long-term fertilization on diversity of arbuscular mycorrhizal fungi under a maize cropping system in Thailand. Ph.D. Thesis, Kasetsart University, Thailand.
- Niu, X., Bressan, R.A., Hasegawa, P.M., Pardo, J.M. 1995. Ion homeostasis in NaCl stress environments. *Plant Physiol.* 109:735-742.
- Niu, G., Rodríguez, D.S. 2006a. Relative salt tolerance selected herbaceous perennials and groundcovers. *Sci. Hortic.* 110:352- 358.
- Niu, G., Rodríguez, D.S. 2006b. Relative salt tolerance of five herbaceous perennials. *HortScience* 41:1493–1497.
- Niu, G., Rodríguez, D.S., Aguiniga, L., Mackay, W. 2007. Salinity tolerance of *Lupinus havardii* and *Lupinus texensis*. *HortScience* 42:526-528.
- Niu, G., Cabrera, R.I. 2010a. Growth and physiological responses of landscape plants to saline water irrigation - a review. *HortScience* 45:605-1609.
- Niu, G., Rodríguez, D.S., Call, E., Bosland, P., Ulery, A., Acosta, E. 2010b. Responses of eight Chile peppers to saline water irrigation. *Sci. Hortic.* 126:215–222.
- Norton-Brandao, D., Scherrenberg, S.M., van Lier J.B. 2013. Reclamation of used urban waters for irrigation purposes – A review of treatment technologies. *J. Environ. Manage.* 122:85–98.
- Ortuño, M.F., Conejero, W., Moreno, F., Moriana, A., Intrigliolo, D.S., Biel, C., Mellisho, C.D., Pérez-Pastor, A., Domingo, R., Ruiz-Sánchez, M.C., Casadesu, J., Bonany, J., Torrecillas, A. 2010. Could trunk diameter sensors be used in woody crops for irrigation scheduling? A review of current knowledge and future perspectives. *Agric. Water Manage.* 97:1–11.
- Ouzounidou, G., Giamporova, M., Moustakas, M., Karataglis, S. 1995. Response of maize (*Zea mays* L.) plants to copper stress. I. Growth, mineral content and ultrastructure of roots. *Environ Exp Bot* 35:167-176.
- Padhi, J., Misraa, R.K., Payerob, J.O. 2012. Estimation of soil water deficit in an irrigated cotton field with infrared thermography. *Field Crop Res.* 126:45–55.
- Palaniswami, C., Sree Ramulu, U.S. 1994. Effects of continuous irrigation with paper factory effluent on soil properties. *J. Indian Soc. Soil Sci.* 42:139-140.

- Paranychianakis, N.V., Aggelides, S., Angelakis, A.N. 2004. Influence of rootstock, irrigation level and recycled water on growth and yield of Soultamina grapevines. *Agric. Water Manage.* 69:13–27.
- Parida, A.K., Das, A.B. 2005. Salt tolerance and salinity effects on plants. *Ecotoxicol. Environ.* 60:324–349.
- Parnell, J.R. 1998. Project Greenleaf Final Report. City of St. Petersburg, Public Utilities Department. pp 500.
- Parsons, L.R., Wheaton, T.A., Castle, W.S. 2001. High application rates of reclaimed water benefits citrus tree growth and fruit production. *HortScience* 36:1273-1277.
- Passioura, J.B., Munns, R. 2000. Rapid environmental changes that affect leaf water status induce transient surges or pauses in leaf expansion rate. *Aust. J. Plant Physiol.* 27:941-948.
- Pedrero, F., Alarcón, J.J., 2009. Effects of treated wastewater irrigation on lemon trees. *Desalination* 246:631-639.
- Pedrero, F., Kalavrouziotis, I., Alarcón, J.J., Koukoulakis, P., Asano T., 2010a. Use of treated municipal wastewater in irrigated agriculture-Review of some practices in Spain and Greece. *Agr. Water Manage.* 97:1233-1241.
- Pedrero, F. 2010b. Manejo sostenible del riego con aguas regeneradas. PhD thesis. Dept Irrigation. CEBAS-CSIC. Murcia.
- Pedrero, F., Allende, A., Gil, M.I., Alarcón, J.J., 2012. Soil chemical properties, leaf mineral status and crop production in a lemon tree orchard irrigated with two types of wastewater. *Agr. Water Manage.* 109, 54-60.
- Pedrero, F, Mounzer, O., Alarcon, J.J, Bayona, J.M., Nicolás, E. 2013. The viability of irrigating mandarin trees with saline reclaimed water in a semi-arid Mediterranean region: a preliminary assessment. *Irrig. Sci.* 31:759–768.
- Percival, G.C. 2005. Identification of foliar salt tolerance of woody perennials using chlorophyll fluorescence. *HortScience* 40:1892–1897.
- Perner, H., Schwarz, D., Bruns, C., Mader, P., George, E. 2007. Effect of arbuscular mycorrhizal colonization and two levels of compost supply on nutrient uptake and flowering of pelargonium plants. *Mycorrhiza* 17:469–474.

- Petkova, V., Denev, I.D., Cholakov, D., Porjazov, I. 2007. Field screening for heat tolerant common bean cultivars (*Phaseolus vulgaris* L.) by measuring chlorophyll fluorescence induction parameters. *Sci Hortic.* 111:101–106.
- Phillips, J.M., Hayman, D.S. 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *T. Brit. Mycol. Soc.* 55:157-160.
- Pitman, M.G. 1975. Ion transport in whole plants. In: Baker, D.A., Hall, J.L. (Eds.), *Ion transport in plant cells and tissues*. Amsterdam: North-Holland Publishing Co, pp. 267-308.
- Porras-Soriano, A., Soriano-Martin, M.L., Porras-Piedra, A., Azcon, R. 2009. Arbuscular mycorrhizal fungi increased growth, nutrient uptake and tolerance to salinity in olive trees under nursery conditions. *J. Plant Physiol.* 166:1350–1419.
- Pou, A., Diago, M.P., Medrano, H., Baluja, J., Tardaguila, J. 2014. Validation of thermal indices for water status identification in grapevine. *Agric. Water Manage.* 134:60–72.
- Qadir, M., Wichelns, D., Raschid-Sally, L., Minhas, P.S., Drechsel, P., Bahri, A., McCornick, P. 2007. Agricultural use of marginal-quality water—opportunities and challenges. In: Molden D (Ed) *Water for food, water for life: A comprehensive assessment of water management in agriculture*. Earthscan, London, UK.
- Rabie, G.H., Almadini, A.M. 2005. Role of bioinoculants in development of salt-tolerance of *Vicia faba* plants under salinity stress. *Afr. J. Biotec.* 4:210–222.
- Rashid, A. 1986. Mechanism of salt tolerance in wheat (*Triticum aestivum* L.). PhD thesis. Dept Soil Sci Univ of Agri. Pakistan.
- Rattan, R.K., Datta, S.P., Chhokar, P.K., Suribabu, K., Singh, A.K. 2005. Long-term impact of irrigation with sewage effluents on heavy metal content in soils, crops and groundwater, a case study. *Agric. Ecosyst. Environ.* 109:310- 322.
- Reboll, V., Cerezo, M., Roig, A., Flors, V., Lapeña, L., García-Agustín, P. 2000. Influence of wastewater vs. groundwater on young Citrus trees. *J. Sci. Food Agric.* 80:1441–1446.

- Reich, P.B., Walters, M.B., Kloeppel, B.D., Ellsworth, D.S. 1995. Different photosynthesis-nitrogen relations in deciduous hardwood and evergreen coniferous tree species. *Oecologia* 112:492-501.
- Rengel, Z. 1992. The role of calcium in salt toxicity. *Plant Cell Environ.* 15:625-632.
- Reynolds, M.P., Rajaram, S., Sayre, K.D. 1999. Physiological and genetic changes of irrigated wheat in the post-green revolution period and approaches for meeting projected global demand. *Crop Sci.* 39:1611–1621.
- Rillig, M.C., Wright, S.F., Kimball, B.A., Pinter, P.J., Wall, G.W., Ottman, M.J., Leavitt, S.W. 2001. Elevated carbon dioxide and irrigation effects on water stable aggregates in a Sorghum field: a possible role for arbuscular mycorrhizal fungi. *Glob. Change Biol.* 7:333–337.
- Rillig, M.C. 2004. Arbuscular mycorrhizae, glomalin, and soil aggregation. *Can J Soil Sci* 84:355–363.
- Rillig, M.C., Mummey, D.L. 2006. Mycorrhizas and soil structure. *New Phytol.* 171:41-53.
- Rodríguez, P., Torrecillas, A., Morales, M.A., Ortuño, M.F., Sánchez-Blanco, M.J. 2005. Effects of NaCl salinity and water stress on growth and leaf water relations of *Asteriscus maritimus* plants. *Environ. Exp. Bot.* 53:113-123.
- Ruiz-Lozano, J.M., Azcón, R., Gómez, M. 1996. Alleviation of salt stress by arbuscular-mycorrhizal *Glomus* species in *Lactuca sativa* plants. *Physiol. Plant* 98:767–772.
- Ruiz-Sánchez, M.C., Domingo, R., Save, R., Biel, C., Torrecillas, A. 1997. Effects of water stress and rewatering on leaf water relations of lemon plants. *Biol. Plantarum* 39:623-631.
- Ruiz-Sánchez, M.C., Domingo-Miguel, R., Castel-Sánchez, J.R. 2010. Deficit irrigation in fruit trees and vines in Spain. *Span. J. Agric. Res.* 8:5-20.
- Rush, D., Epstein, E. 1981. Comparative studies on the sodium, potassium and chloride relations of a wild halophytic and domestic salt sensitive tomato species. *Plant Physiol.* 68:1308-1313.
- Sánchez-Blanco, M.J., Rodríguez, P., Morales, M.A., Ortuño, M.F., Torrecillas, A. 2002. Comparative growth and water relations of *Cistus albidus* and *Cistus*

- monspeliensis* plants during water deficit conditions and recovery. *Plant Sci.* 162:107–113.
- Sánchez-Blanco, M.J., Rodríguez, P., Morales, M.A., Torrecillas, A. 2003. Contrasting physiological responses of dwarf sea-lavender and marguerite to simulated sea aerosol deposition. *J. Environ. Qual.* 32:2238-2244.
- Sánchez-Blanco, M.J., Ferrández, T., Morales, M.A., Morte, A., Alarcón, J.J. 2004. Variations in water status, gas exchange, and growth in *Rosmarinus officinalis* plants infected with *Glomus deserticola* under drought conditions. *J. Plant Physiol.* 161:675-682.
- Sánchez-Blanco MJ, Álvarez S, Ortuño MF, Ruiz-Sánchez MC. 2014. Root System Response to Drought and Salinity: Root Distribution and Water Transport. In: Morte, A., Varma, A. (Eds) *Soil Biology*, Springer-Verlag, Berlin Heidelberg, pp 325-352.
- Santos, C.V. 2004. Regulation of chlorophyll biosynthesis and degradation by salt stress in sunflower leaves. *Sci. Hortic.* 103:93–99.
- Scholander, P.F., Hammel, H.T., Bradstreet, E.D., Hemingsen, E.A. 1965. Sap pressure in vascular plants. *Science* 148:339-346.
- Schuch, U. 2005. Effect of reclaimed water and drought on salt-sensitive perennials. *HortScience* 40:1095 (Abstract).
- Sepulcre-Cantó, G., Zarco-Tejada, P.J., Sobrino, J.A., Berni, J.A., Jiménez-Muñoz, J.C., Gastellu-Etchegorry, J.P. 2009. Discriminating irrigated and rainfed olive orchard with thermal ASTER imagery and DART 3D simulation. *Agric. For Meteorol.* 149:962–975.
- Shannon, M.C., Grieve, C.M. 1999. Tolerance of vegetable crops to salinity. *Scient. Hort.* 78:5–38.
- Sharifi. M., Ghorbanli, M., Ebrahimzadeh, H. 2007. Improved growth of salinity-stressed soybean after inoculation with salt pre-treated mycorrhizal fungi. *J. Plant Physiol.* 164: 1144-1151.
- Sheng, M., Tang, M., Chen, H., Yang, B., Zhang, F., Huang, Y. 2008. Influence of arbuscular mycorrhizae on photosynthesis and water status of maize plants under salt stress. *Mycorrhiza* 18:287-296.

- Shillo, R., Ding, M., Pasternak, D., Zaccai, M. 2002. Cultivation of cut flower and bulb species with saline water. *Sci. Hortic.* 92:41–54.
- Siddiqui, M.H., Khan, M.N., Mohammad, F., Khan, M.M.A. 2008. Role of Nitrogen and Gibberellin (GA₃) in the Regulation of Enzyme Activities and in Osmoprotectant Accumulation in *Brassica juncea* L. under Salt Stress. *J. Agron. Crop Sci.* 194:214-224.
- Sirault, X.R.R., James, R.A., Furbank, R.T. 2009. A new screening method for osmotic component of salinity tolerance in cereals using infrared thermography. *Funct. Plant Biol.* 36:970–977.
- Sixto, H., Aranda, I., Grau, J.M. 2006. Assessment of salt tolerance in *Populus alba* clones using chlorophyll fluorescence. *Photosynthetica* 44:169–173.
- Smith, S.E., Gianinazzi-Pearson, V. 1990. Phosphate uptake and vesicular arbuscular activity in mycorrhizal *Allium cepa* L.: Effect of photon irradiance and phosphate nutrition. *Aust. J. Plant Physiol.* 17:177-188.
- Smith, S.E., Read, D.J. 2008. *Mycorrhizal Symbiosis*, Ed 3. Academic Press, New York.
- Strullu, D.G., Harley, J.L., Gourret, J.P., Garrec, J.P. 1983. A note on the relative phosphorous and calcium contents of metachromatic granules in *Fagus mycorrhizas*. *New Phytol.* 94:89-94.
- Sun, Y., Niu, G., Osuna, P., Ganjegunte, G., Auld, D., Zhao, L., Peralta-Videa, J.R., Gardea-Torresdey, J.L. 2013. Seedling emergence, growth, and leaf mineral nutrition of *Ricinus communis* L. cultivars irrigated with saline solution. *Ind. Crop Prod.* 49:75–80.
- Talaat, N.B., Shawky, B.T. 2014. Protective effects of arbuscular mycorrhizal fungi on wheat (*Triticum aestivum* L.) plants exposed to salinity. *Env. Exp. Bot.* 98:20-31.
- Tanji, K.K. 1990. *Agricultural Salinity Assessment and Management*. American Society of Civil Engineers. New York.
- Tanji, K.K. 2002. Salinity in the soil environment. In: Läuchli, A., Lüttge, U. (eds.). *Salinity: Environment–plants–molecules*. Kluwer Academic Publishers, Dordrecht, The Netherlands. p. 21–51.

- Tester, M., Davenport, R. 2003. Na⁺ tolerance and Na⁺ transport in higher plants. *Ann. Bot.* 91:503–527.
- Tian, C.Y., Feng, G., Li, X.L., Zhang, F.S. 2004. Different effects of arbuscular mycorrhizal fungal isolates from saline or non-saline soil on salinity tolerance of plants. *Appl. Soil Ecol.* 26:143–148.
- Tisserant, B., Gianinazzi-Pearson, V., Gianinazzi, S., Gollotte, A. 1993. In planta histochemical staining of fungal alkaline phosphatase activity for analysis of efficient arbuscular mycorrhizal infections. *Mycol. Res.* 97:245–250.
- Torrecillas, A., Rodriguez, P., Sánchez-Blanco, M.J. 2003. Comparison of growth, leaf water relations and gas exchange of *Cistus albidus* and *C. monspeliensis* plants irrigated with water of different NaCl salinity levels. *Sci. Hort.* 97:353–368.
- Treseder, K.K., Turner, K.M. 2007. Glomalin in ecosystems. *Soil Sci. Soc. Am. J.* 71:1257–1266.
- Turner N.C. 1988. Measurements of plant water status by the pressure chamber technique. *Irrig. Sci.* 9:289–308.
- Unesco Water Portal. 2007. <http://www.unesco.org/water>. Accessed 25. October 2007.
- Valdés, R., Miralles, J., Ochoa, J., Sánchez-Blanco, M.J., Bañón, S. 2012. Saline reclaimed wastewater can be used to produce potted weeping fig (*Ficus benjamina* L.) with minimal effects on plant quality. *Span. J. Agric. Res.* 10:1167–1175.
- Valdés, R., Miralles, J., Ochoa, J., Sánchez-Blanco, M.J., Bañón, S., 2014a. The number of emitters alters salt distribution and root growth in potted gerbera. *HortScience* 49, 160–165.
- Valdés, R., Miralles, J., Franco, J.A. Sánchez-Blanco, M.J., Bañón, S. 2014b. Using soil bulk electrical conductivity to manage saline irrigation in the production of potted poinsettia. *Sci. Hort.* 170:1–7.
- Valdez-Aguilar, L.A., Grieve, C.M., Poss, J. 2009. Salinity and alkaline pH in irrigation water affect marigold plants: I. Growth and shoot dry weight partitioning. *HortScience* 44:1719–1725.

- Van den Driessche, R. 1991. Effects of nutrients on stock performance in the forest. In: den Driessche R (ed) Mineral Nutrition of Conifer Seedlings. CRC Press, Boca Raton, USA, pp 229–260.
- Veatch-Blohm, M.E., Morningstar, L. 2011. Calla lily growth and development under saline irrigation. *HortScience* 46:222–227.
- Veatch-Blohm, M.E., Malinowski, M., Keefer, D. 2012. Leaf water status, osmotic adjustment and carbon assimilation in colored calla lilies in response to saline irrigation. *Sci. Hortic.* 144:65–73.
- Vicente-Sánchez, J., Nicolás, E., Pedrero, F., Alarcón, J.J., Maestre-Valero, J.F., Fernández F. 2013. Arbuscular mycorrhizal symbiosis alleviates detrimental effects of saline reclaimed water in lettuce plants. *Mycorrhiza* 24:339–348.
- Vinocur, B., Altman, A. 2005. Recent advances in engineering plant tolerance to abiotic stress: achievements and limitations. *Curr. Opin. Biotech.* 16:123-132.
- Watanabe, F.S., Olsen, S.R. 1965. Test of an ascorbic acid method for determining phosphorus in water and NaHCO_3 , extracts from soils. *Soil Sci. Soc. Am. Pro.* 29:677-678.
- Weber, B., Avnimelech, Y., Juanico, M. 1996. Salt enrichment of municipal sewage: new prevention approaches in Israel. *Environ. Manage.* 20:487–495.
- Wei, W., Bilsborrow, P.E., Hooley, P., Fincham, D.A., Lombi, E., Forster, B.P. 2003. Salinity induced differences in growth, ion distribution and partitioning in barley between the cultivar Maythorpe and its derived mutant Golden Promise. *Plant Soil* 250:183-191.
- West, C.P., Oosterhuis, D.M., Wulschleger, S.D., 1990. Osmotic adjustment in tissues of tall fescue in response to water deficit. *Environ. Exp. Bot.* 30:149-156.
- Wright, S.F., Franke-Snyder, M., Morton, J.B., Upadhyaya, A. 1996. Time-course study and partial characterization of a protein on hyphae of arbuscular mycorrhizal fungi during active colonization of roots. *Plant Soil* 181:193–203.
- Wu, L., Skaggs, T.H., Shouse, P.J., Ayars, J.E. 2001a. State space analysis of soil water and salinity regimes in a loam soil underlain by shallow groundwater. *Soil Sci. Soc. Am. J.* 65:1065-1074.

- Wu, L., Guo, X., Harivandi, A. 2001b. Salt tolerance and salt accumulation of landscape plants irrigated by sprinkler and drip irrigation systems. *J. Plant Nutr.* 24:473–490.
- Wu, L., Dodge, L. 2005. A special report for the Elvenia J. Slosson Endowment Fund: Landscape salt tolerance selection guide for recycled water irrigation. University of California, Davis, CA.
- Wyn Jones, R.G., Gorham, J. 2002. Intra- and inter-cellular compartmentation of ions. In: Läuchli A, Lüttge U, eds. *Salinity: Environment – Plants – Molecules*. Dordrecht, the Netherlands: Kluwer, p. 159–180.
- Yadav, R.K., Goyal, B., Sharma, R.K., Dubey, S.K., Minhas, P.S. 2002. Post-irrigation impact of domestic sewage effluent on composition of soils, crops and ground water-a case study. *Environ. Int.* 28:481-486.
- Yermiyahu, U., Ben-Gal, A., Keren, R., Reid, R.J., 2008. Combined effect of salinity and excess boron on plant growth and yield. *Plant Soil* 304:73-87.
- Zandonadi, R.S., Pinto, F.A.C., Sena, D.G., Queiroz, D.M., Viana, P.A., Mantovani, E.C. 2005. Identification of lesser cornstalk borer–attacked maize plants using infrared images. *Biosyst. Eng.* 91:433–439.
- Zhu, J.K. 2001. Plant salt tolerance. *Trends Plant Sci.* 6:66-71.
- Zhu, Y.G., Miller, R.M. 2003. Carbon cycling by arbuscular mycorrhizal fungi in soil-plant systems. *Trends Plant Sci.* 8:407–409.
- Zollinger, N., Koeing, R., Cerny-Koeing, T., Kjelgren, R. 2007. Relative salinity tolerance of intermountain Western United States native herbaceous perennials. *HortScience* 42:529-534.
- Zuccarini, P., Okurowska, P. 2008. Effects of mycorrhizal colonization and fertilization on growth and photosynthesis of sweet basil under salt stress. *J. Plant Nutr.* 31:497–513.

Abbreviations

ALP	Alkaline phosphatase
AMF	Arbuscular mycorrhizal fungi
ANOVA	Analysis of variance
C	Control
C	Chroma
CEBAS	Centro de Edafología y Biología Aplicada del Segura
Chl a	Chlorophyll a
Chl b	Chlorophyll b
Chl t	Chlorophyll total
cm	Centimetre
CO ₂	Carbon dioxide
C _t	Total carbon
C _{org}	Organic carbon
CWSI	Crop water stress index
d	Day
dS	Decisiemen
DP	Percentage of dry plants
DW	Dry weight
E	Transpiration
EC	Electrical conductivity
EEG	Easily Extractable glomalin of soil
ET	Evapotranspiration
F _v /F _m	Maximal photochemical efficiency of photosystem II
F' _v /F' _m	Energy conversion efficiency in photosystem II

g	Gram
g _s	Stomatal conductance
h	Hour
HUE	Angle Hue
I _G	Stomatal conductance index
IR	Infrared
IRTs	Infrared thermometers
IW	Irrigator's water
J	Absorption rate of ions by roots
Kg	Kilogram
KPa	Kilopascal
L	Liter/ Luminosity
m	Metre
mg	Miligram
min	Minute
mL	Milliliter
mm	Milimoles
Mm	Milimolar
MPa	Megapascal
NBT	Nitro blue tetrazodium
N _t	Total nitrogen
NTU	Nephelometrical Turbidity Units
ns	No significance

OM	Organic matter content
P	Significant level
PAC	Percentage of plants in acceptable conditions
PAR	Photosynthetically active radiation
PDB	Percentage of plants in with dry branches
PIC	Percentage of plants in ideal conditions
P _n	Net photosynthetic rate
ppm	Parts per million
PS II	Photosystem II
RH	Relative humidity
RW	Reclaimed water
s	Second
SDH	Succinate dehydrogenase
T ^a	Temperature
T _a	Air temperature
T _c	Canopy temperature
T _{c,m}	Daily mean canopy temperature
T _{c,max}	Daily maximum canopy temperature
T _{c,midday}	Daily midday canopy temperature
T _{c,min}	Daily minimum canopy temperature
TDR	Time Domain Reflectometry
T _{dry}	Dry reference temperature
TDS	Total dissolved solids
T _{md}	Temperature at midday
T _{wet}	Wet reference temperature

V	Volume
VDP	Vapour pressure deficit
WW	Wastewater

Symbols

Ψ_o	Osmotic potential
Ψ_{stem}	Stem water potential
Ψ_t	Turgor potential
Ψ_{100s}	Osmotic potential at full turgor
$\emptyset$	Diameter
θ_v	Volumetric water content
ΔT	Differences between $T_{c,\text{min}}$ and $T_{c,\text{max}}$;
$\Delta(T_c - T_a)$	Differences between $(T_c - T_a)_{\text{max}}$ and $(T_c - T_a)_{\text{min}}$
μmol	Micromoles
%	Percentage
$^{\circ}\text{C}$	Degree Celsius