

## Saline reclaimed wastewater can be used to produce potted weeping fig (*Ficus benjamina* L.) with minimal effects on plant quality

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### Abstract

The objective of the present study was to investigate the limitations of irrigation with saline reclaimed wastewater (RW) for producing potted weeping fig (*Ficus benjamina* L.). Furthermore, two different levels of leaching were studied to ascertain whether either reduces the negative effects of RW. Three irrigation treatments were applied: a) well water (control), b) RW (5 dS m<sup>-1</sup>) with a constant leaching fraction of 23% (RWL), and c) RW (5 dS m<sup>-1</sup>) with a constant leaching fraction of 15% and 50% flushing every nine irrigation events (RWF). After five months of exposure to the RW, plant size, leaf area, specific leaf area, plant DW, stem diameter and shoot/root ratio were reduced, but both compactness and the appearance of the plants remained high. RWF reduced leaf area, plant dry weight, stem diameter, leaf lightness, leaf chroma and leaf SPAD compared with the RWL. Water consumption per pot was higher in control (50.58 L), followed by RWL (24.29 L) and RWF (19.6 L). Photosynthesis and stomatal conductance were 50% lower in RWL plants than in the control, while the RWF plants had the lowest rates. RWF caused damages in the photochemical apparatus. This study confirms that: a) weeping fig is a good candidate for being grown with saline RW without compromising its aesthetic value; b) RW may be regarded as a good alternative to the retardants used in this plant; and c) the recommended irrigation would be RWL.

**Additional key words:** irrigation; ornamental plant; pot plant; salinity.

### Resumen

**El riego con agua residual depurada salina permite producir ficus (*Ficus benjamina* L.) en maceta con una pérdida de calidad mínima**

El objetivo del estudio fue investigar las limitaciones del riego con agua residual regenerada salina (RW) en la producción de *Ficus benjamina* L. en maceta. Se valoró también la efectividad de dos niveles de drenaje para reducir los efectos negativos del RW. Los tratamientos fueron: a) agua no salina (control), b) RW con 5 dS m<sup>-1</sup> y un 23% de drenaje (RWL), y c) RW con 5 dS m<sup>-1</sup> y un 15% de drenaje, más un 50% de lavado cada 9 riegos (RWF). Después de 5 meses bajo RW el tamaño de la planta, área foliar, área foliar específica, peso seco de la planta, diámetro del tallo y el ratio parte aérea/raíz fueron reducidos, mientras que la compactidad y el aspecto ornamental estuvieron dentro de los patrones comerciales adecuados. RWF produjo una disminución del área foliar, peso seco de la planta, diámetro del tallo, luminosidad y saturación del color de la hoja, y de la clorofila foliar (SPAD) en comparación con RWL. El consumo de agua fue mayor en el control (50,58 L), seguido de RWL (24,29 L) y RWF (19,6 L). La fotosíntesis y la conductancia estomática fueron un 50% menor en las plantas bajo RWL respecto al control, mientras que las plantas bajo RWF presentaron los registros más bajos. RWF produjo daños en el aparato fotoquímico. Este estudio indica que: a) *F. benjamina* puede ser cultivado con RW sin comprometer su valor estético, b) el riego con RW resultó una buena alternativa al uso de retardadores químicos, y c) el riego recomendado es RWL.

**Palabras clave adicionales:** planta de maceta; planta ornamental; riego; salinidad.

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Abbreviations used: DW (dry weight); EC (electric conductivity);  $\Phi$ PSII (light adapted quantum yield of PSII); NPQ (non-photochemical quenching); WUE (water use efficiency);  $F_v/F_m$  (maximum photochemical efficiency of PSII);  $g_s$  (stomatal conductance);  $P_n$  (net photosynthesis rate at midday);  $\Psi_1$  (leaf water potential);  $\Psi_o$  (leaf osmotic potential);  $\Psi_p$  (leaf pressure potential).

## Introduction

A possible supplementary or alternative source of irrigation water for nursery production and landscaping is reclaimed wastewater, especially in arid and semiarid regions of the world. However, a potential problem with reclaimed wastewater is its high salt content, which is detrimental to sensitive plants if not managed properly (Niu & Cabrera, 2010). The high salinity of irrigation water may adversely affect the growth and appearance of ornamental plants, causing leaf damage, such as burning or chlorosis, with a consequent loss of plant quality (Bañón *et al.*, 2011). Salinity may also disrupt physiological functions, reducing growth. However, salinity may also be a tool for controlling plant quality and development.

Selecting crops that can tolerate a degree of salinity stress is fundamental for putting saline water to its best use. Weeping fig (*Ficus benjamina* L.) is an important component in foliage plant production, being used extensively as potted house plant. Many studies on this species have been focused on rooting, *in vitro* culture, indoor acclimatization, environmental effects, nutrition, plant retardants, etc., while the information available concerning saline stress is scarce. Vogelesang (1991) and Black (2003) considered weeping fig as a moderately salt-tolerant plant.

Salt tolerance must also be considered in the light of irrigation management. Oron *et al.* (2002) reported that highly saline water has an agricultural potential in combination with proper leaching and irrigation management. Several studies have indicated that when saline water is used for irrigation, due attention should be given to minimizing root zone salinity (Katerji *et al.*, 2004). The accumulation of salts in the root zone can only be prevented by leaching with extra irrigation water. Different recommendations on leaching fraction have been made considering irrigation water EC and leachate EC, but less is known about combinations of leaching and flushing to reduce salt damage.

The objective of the experiment was twofold: (i) to study the growth, visual quality and physiological responses of weeping fig to saline reclaimed wastewater; and (ii) to assess two strategies of irrigation and drain-off to reduce salinity damage in ficus.

## Material and methods

Three seedlings of one year old weeping fig (cv. Danielle) were transplanted to brown PVC 2.5 L pots

(16 cm upper internal diameter), containing a mixture of black peat, coconut fiber and perlite as substrate (2:2:1 vol.). The transplantation of seedlings to cultivation pots was carried out in the last week of Feb. 2011, and the experiment finished in the last week of Jul. 2011. The study was conducted in a greenhouse at the Agricultural Experimental Station of the Polytechnic University of Cartagena (37° 35' N, 0° 59' W), using nine metal crop tables (3 m long, 1.30 m wide and 0.80 m high). Twenty-four pots were placed on each of the tables. The 24 pots arranged in three rows of 8 pots.

The experiment comprised three irrigation treatments: a) control [water taken from a canal that delivers water (1 dS m<sup>-1</sup>) from the Tagus River to the Segura River for agricultural and municipal use] with a constant leaching fraction of about 15%; b) saline reclaimed wastewater (5 dS m<sup>-1</sup>) with a constant leaching fraction of about 23% (RWL); and c) saline reclaimed wastewater (5 dS m<sup>-1</sup>) with a constant leaching fraction of about 15% and flushing of about 50% every nine irrigation events (RWF).

The irrigation was controlled by a system similar to that described by Nemali & van Iersel (2006) but with a CR1000 data logger, balances (Analytical Sartorius, Model 5201, capacity 5.2 kg and readability of 0.01 g) and an Agrónic 4000 (Sistemas Electrònics PROGRÉS, S. A., Bellpuig, Spain) to control three pumps connected to three 1000 L tanks which contained the different irrigation solutions. Each pot had one emitter (2 L h<sup>-1</sup>) connected to a spaghetti tube. The pressure-compensated drip emitters used were tested for homogeneity before the experiment started (the water flow varied between 1.9 and 2.1 L h<sup>-1</sup>). The CR1000 recorded the weight of the pots every 10 min. Three balances were installed per treatment and, on each balance, a PVC tray slightly inclined to one side and with drainage holes ensured that the leachate could be collected. The datalogger was programmed to record the weight of the pots 30 min after each irrigation event (mean of three pots), and the following irrigation event was triggered when pots had lost 250 g. We programmed the CR1000 to count every irrigation event and flushing automatically. The leaching fraction was adjusted with the irrigation time.

Fertilization was carried out by the irrigation head, and nutrients were provided at constant concentrations in the irrigation water, containing 80 N-40 P<sub>2</sub>O<sub>5</sub>-80 K<sub>2</sub>O (ppm) and a pH 6. This nutrient solution was made by mixing KNO<sub>3</sub>, NH<sub>4</sub>(NO<sub>3</sub>), K(HPO<sub>4</sub>) and nitric acid (HNO<sub>3</sub>). The fertilizers added increased EC by ap-

proximately 0.39 and 0.36 dS m<sup>-1</sup> in control water and RW, respectively. The reclaimed wastewater was refined by a tertiary treatment plant (Los Alcazares, Murcia), and contained the following ion concentrations in mg L<sup>-1</sup>: Na<sup>+</sup> (683.69), K<sup>+</sup> (24.57), Ca<sup>2+</sup> (180), Mg<sup>2+</sup> (137.98), chloride (1083.03), sulfate (969.38), carbonates (< 5), bicarbonate (297.00), nitrates (6.39), ammonia (1.15), phosphate (1.44), boron (1.23), manganese (28.78), iron (52.40), zinc (< 0.04), copper (< 0.04). It had a pH of 7.09 and an EC of 4.99 dS m<sup>-1</sup>.

A datalogger (HOBO H08-004-02, MicroDAQ.com, Ltd., Contoocook, NH, USA) was used to measure air temperature and humidity with a Temperature/RH Smart Sensor S-THB-M008 (MicroDAQ.com, Ltd., Contoocook, NH, USA), and photosynthetic active radiation with a HOBO sensor S-LIA-M003 Smart Sensor (MicroDAQ.com, Ltd., Contoocook, NH, USA). Data were collected at 60 s intervals and averages were recorded every 30 min. Weather conditions were 15.7 ± 4.6°C (minimum) and 33.3 ± 5.3°C (maximum); minimum relative humidity was 38 ± 11% and the maximum 82 ± 4%. The daily light integral (DLI) was calculated by integrating the photosynthetic photon flux measurements throughout the day, giving 13.9 ± 4.6 mol m<sup>-2</sup> s<sup>-1</sup> (mean ± SD) over the 150 days of the experiment.

At the end of the experiment, the dry weight (DW) of roots, stems and leaves was determined in six plants per treatment, gently washing the substrate from the roots with pressurized water using a hose with flat tip. To calculate the DW, leaves, stems and roots were introduced in clearly identified envelopes and placed in a natural convection bacteriological stove (model 2002471, JP Selecta SA, Barcelona, Spain) at 60°C until constant weight was reached. Finally, the DW was determined by weighing with a GRAM ST precision balance (sensitivity of 10 mg and up to 1,200 g, Gram Precision SL, Barcelona, Spain). The leaf area was determined with a LI-3100C (LI-COR Biosciences, Lincoln, NE, USA) in the same plants whose DW was measured. The blade area was calculated by dividing the leaf area by the number of leaves. The growth indices determined were the shoot DW/root DW (S/R) and the specific leaf area (SLA) (leaf area/leaf DW). The plant architecture was determined in six plants per treatment. The compactness index was determined with a photograph taken with an HP CW450 digital camera (Hewlett-Packard Española S.L.) and the formula, compactness index = plant profile area/[ $(\pi/4) \times ((\text{height} + \text{width})/2)\text{exp}2$ ], where the plant profile area is the

area within the plant perimeter. The plant area, and the height and width of the plant, were obtained from the picture using the software UTHSCSA Image Tool (University of Texas, San Antonio, TX, USA). Two indexes were calculated for the side and top image, and the average of both is given as the final index of compactness. The closer the result was to unity, the more compact were the plants. Six plants were used. The base stem diameter was determined with an electronic SYLVAC gauging device (sensitivity of 0.01 mm and maximum 150 mm, TECMICRO SA, Madrid, Spain).

The leachate was collected weekly in plastic containers and measured gravimetrically. Leachate EC was analyzed immediately after collection using an EC meter (Dist<sup>®</sup> 6, Hanna Instruments S.L., Eibar, Spain). We represented an average of all the experiments both leachate and leachate EC. The LF was quantified as the volume of solution leached from the pot divided by the total solution applied. Substrate pore water EC was measured in six replicates per treatment following the pour-through method (Wright, 1986) at the end of the experiment. The water use efficiency (WUE) was calculated as the total DW harvested divided by the water applied (determined by the CR1000).

Leaf color and SPAD measurements were made in six plants of each treatment (four per repetition) at the end of the experiment, selecting representative south-facing, mid-height mature plant leaves. The color was determined with a shot in the middle of the leaf blade with a Minolta CR10 colorimeter (Konica Minolta Sensing, Inc., Osaka, Japan) that calculated the color coordinates (CIELAB): lightness, hue angle and chroma. The SPAD was measured using the same criteria as for color but with a SPAD-502 chlorophyll meter (Konica Minolta Sensing, Inc., Osaka, Japan) which estimates relative chlorophyll content with the light transmitted through the leaf at 650 nm (photosynthetically active wave length) and 940 nm. For each measurement the average of three shots was determined.

Leaf and root dry matter samples were used to determine chlorides and sodium. Dry tissue samples were ground and three sub-samples of 0.2 g were analysed after extraction in 50 mL of distilled water by ion chromatography (ion chromatography system, model 861, Metrohm AG, Herisau, Switzerland) equipped with conductometric detector and an autosampler (Metrohm 838 Advanced Sample Processor), consisting of an anion separator column Metrosep A Supp 5-250 (250 mm × 4.0 mm, 5 µm particle size) with a pre-guard column (Metrosep A Supp 4/5 Guard 5 mm × 4 mm),

and a cation separator column Metrosep C 2-250 (250 mm × 4.0 mm, 7 µm particle size) with a pre-guard column (Metrosep C 2, 5 mm × 4 mm). The suppressors used were MSM II (Metrohm Suppressor Module) for anions. In all cases, mature leaves were taken from the middle of the plant. Six plants per treatment for each species were used for experimental purposes.

Leaf water potential ( $\Psi_l$ ), leaf osmotic potential ( $\Psi_o$ ) and leaf pressure potential ( $\Psi_p$ ) were determined at midday.  $\Psi_l$  was estimated using a Scholander pressure chamber (Soil Moisture Equipment Co, Santa Barbara, CA, USA), for which leaves were enclosed in a plastic bag and sealed in the chamber within 20 s of collection and pressurised at a rate of 0.02 MPa s<sup>-1</sup>. Leaves from the  $\Psi_l$  measurements were frozen in liquid nitrogen (-170°C) and stored at -30°C. After thawing, the sap was extracted from the sample with a small press, and then placed on a filter paper disc in the osmometer chamber and the values of the  $\Psi_o$  were measured using a WESCOR 5520 vapour pressure Osmometer (Wescor Inc., Logan, UT, USA).  $\Psi_p$  was estimated as the difference between  $\Psi_l$  and  $\Psi_o$  for each time. All measurements were taken at midday in six plants per treatment at the end of the experiment.

Stomatal conductance ( $g_s$ ) and the net photosynthesis rate at midday ( $P_n$ ) were measured at midday using a CIRAS-2 Portable Photosynthesis System (PP Systems, Amesbury, MA, USA). The air flow rate through the cuvette was 200 mL min<sup>-1</sup> with a [CO<sub>2</sub>] of 420 µmol mol<sup>-1</sup>, an air temperature of 20°C, a vapour pressure deficit of 1.6 kPa, and a photosynthetic photon flux of 1000 µmol m<sup>-2</sup> s<sup>-1</sup>. The chlorophyll fluorescence was measured using a Pulse Modulated Fluorimeter FMS-2 (Gomensoro Scientific Instrumentation S.A., Madrid). The method and parameters determined were those described by Miralles *et al.* (2011). All measurements were performed in the same leaves at the end of the experiment: six plants and three leaves per plant in each treatment.

The design was a randomized complete block design. There were three blocks of 24 plants per treatment set on a crop table. Treatments were analysed by one-way analysis of variance using Statgraphics Plus for Windows. Treatment means were separated by LSD Test ( $p < 0.05$ ). Regression analyses were also determined between DLI and daily ET, and between days of culture and daily ET. Ratios and percentages were arcsine ( $x$ )<sup>1/2</sup> transformed before statistical analysis to ensure homogeneity of variance.

## Results and discussion

The RW treatments reduced plant height and width by 36% and 13%, respectively, compared with the control, which led to a smaller plant size (Table 1). In calceolaria, Fornes *et al.* (2007) indicated that a strong reduction in size was the main factor protecting plants from saline stress. Niu & Cabrera (2010) suggests that all parts of a plant, including leaves, stems and roots, may be reduced in size under saline conditions. We measured the blade area size, determining that the plants irrigated with RW presented smaller leaves than the control plants as well as the number of leaves (by around 60%). Both foliar reductions led to strong decrease in leaf area in RWL (about 63%) and RWF (about 74%). Specific leaf area (SLA) decreased under RW too, indicating that the leaves become thicker and/or more succulent. Small plant size together with changes in leaf morphology leading to a fall in water extraction from the soil has been proposed as an important mechanism for drought tolerance (Kusaka *et al.*, 2005). Otherwise, a decreased SLA in salinized plants could mean greater amounts of photosynthetic apparatus per leaf area than in non-salinized plants, which could represent an effective water stress resistance mechanism because it would allow photosynthesis to increase (Zwack & Graves, 1998). However, in this experiment this did not occur because the RW irrigated plants were seen to suffer a sharp fall in  $P_n$ .

The treatments with RW reduced plant DW by 53% (RWL) and 67% (RWF) respect to the control, which were accompanied by a reduction in stem diameter. RWF caused a greater reduction in plant DW and stem diameter than RWL (Table 1). Salt tolerance is usually assessed as the percentage of biomass production in saline versus control conditions over a prolonged period of time (Munns *et al.*, 2002). From this point of view we should classify weeping fig as a salinity-sensitive plant. Results for shoot/root ratio showed reduction under the treatments with RW, meaning that the shoot growth was more sensitive to RW than root growth. Usually, growth reduction by salinity in shoots is greater than in roots (Munns *et al.*, 2002), and ficus is not an exception in this respect. A low shoot/root ratio means that roots are abundant with regard to leaf area, and that the plant has a high water stress avoidance potential (Miralles *et al.*, 2009).

As noted above, RWF reduced plant DW, stem diameter and leaf area compared with the RWL, which

suggests that the plants under RWF were subjected to more stressful saline conditions than those under RWL. However, RWF and RWL had similar pore water EC, which does not support these results, suggesting that the final measurement of pore water EC did not provide accurate information on the saline stress level in plants

**Table 1.** Effects of irrigation treatments on variables/parameters studied

Variables/Parameters <sup>1</sup>	Treatments <sup>2</sup>		
	Control	RWL	RWF
Plant height (cm)	76.0 a	48.3 b	43.8 b
Plant width (cm)	66.5 a	57.5 ab	49.5 b
Blade area (cm <sup>2</sup> )	8.8 a	7.5 b	6.8 b
Number of leaves	1,211 a	531 b	412 b
Leaf area (dm <sup>2</sup> )	106.1 a	39.7 b	27.5 c
Specific leaf area (cm <sup>2</sup> g <sup>-1</sup> )	131.0 a	110.0 b	111.2 b
Plant DW (g)	149.5 a	70.6 b	48.8 c
Shoot/Root	5.8 a	4.0 b	3.5 b
Stem diameter (mm)	10.5 a	8.1 b	6.6 c
Final pore water EC (dS m <sup>-1</sup> )	8.9 a	17.0 b	16.7 b
Average leachate EC (dS m <sup>-1</sup> )	5.39 a	9.41 b	9.28 b
Leaf lightness	28.4 b	28.1 b	32.2 a
Leaf chroma	10.6 b	11.7 b	15.9 a
Leaf hue angle	115.6 a	117.1 a	115.3 a
Leaf SPAD	74.1 b	70.2 b	67.0 a
Compactness index	0.75 a	0.67 a	0.69 a
Water consumption (L pot <sup>-1</sup> )	50.6 a	24.3 b	19.6 c
WUE (gDW L <sup>-1</sup> )	3.0 a	2.9 a	2.5 b
Ψ <sub>l</sub> (MPa)	-0.83 a	-0.93 a	-1.01 a
Ψ <sub>p</sub> (MPa)	0.43 a	0.39 a	0.37 a
Ψ <sub>o</sub> (MPa)	-1.26 a	-1.31 b	-1.38 b
Leaf Cl <sup>-</sup> (mg g <sup>-1</sup> DW)	6.62 a	7.12 ab	8.64 b
Leaf Na <sup>+</sup> (mg g <sup>-1</sup> DW)	0.95 a	1.20 a	1.21 a
Root Cl <sup>-</sup> (mg g <sup>-1</sup> DW)	15.45 a	15.15 a	18.10 b
Root Na <sup>+</sup> (mg g <sup>-1</sup> DW)	4.86 a	5.08 a	6.10 b
g <sub>s</sub> (mmol H <sub>2</sub> O m <sup>-2</sup> s <sup>-1</sup> )	103.0 a	56.00 b	3.25 c
P <sub>n</sub> (μmol CO <sub>2</sub> m <sup>-2</sup> s <sup>-1</sup> )	15.8 a	8.98 b	0.58 c
F <sub>v</sub> /F <sub>m</sub>	0.833 a	0.825 a	0.696 b
èPSII	0.232 ns	0.234 ns	0.187 ns
NPQ	2.73 a	2.38 a	1.11 b

<sup>1</sup>DW: dry weight; EC: electric conductivity; WUE: water use efficiency; Ψ<sub>l</sub>: leaf water potential; Ψ<sub>o</sub>: leaf osmotic potential; Ψ<sub>p</sub>: leaf pressure potential; g<sub>s</sub>: stomatal conductance; P<sub>n</sub>: net photosynthesis rate at midday; F<sub>v</sub>/F<sub>m</sub>: maximum photochemical efficiency of PSII; NPQ: non-photochemical quenching; èPSII: light adapted quantum yield of PSII. <sup>2</sup>Control: water taken from a canal that delivers water from the Tagus River to the Segura River for agricultural and municipal use with a constant leaching fraction of about 15%. RWL: saline reclaimed wastewater with a constant leaching fraction of 23%. RWF: saline reclaimed wastewater with a constant leaching fraction of 15% and flushing of 50% every nine irrigation events. Means within each row followed by the same letter do not differ significantly at  $p \leq 0.05$ .

during the cultivation period. Leachate EC was the highest after each flushing event in RWF (data not shown), although the average leachate EC during the experiment was the same for RWL and RWF (Table 1). This can be explained by the tendency of water in drip irrigation to flow downwards rather than to spread horizontally (De Rijck & Schrevels, 1998). In this way, salt accumulated around the wet bulb produced by the dripper and the flushing washed out some of these salts. Therefore, root sphere EC in RWF was less stable than in RWL during the experiment, leading to worse saline conditions in the former.

Ornamental plants, however, are judged by their aesthetic value rather than growth rate or production (Wu & Dodge, 2005). As well as stunted growth, salt stress may cause foliar damage, including leaf necrosis, leaf chlorosis and marginal leaf burn; as salt stress becomes severe, premature leaf drop can occur (Niu & Cabrera, 2010). In our experiment, leaves neither dropped nor presented saline damage symptoms, but maintained a good visual appearance. However, RWF enhanced leaf lightness and chroma compared with the other treatments. None of the treatments produced a statistical difference in hue angle (Table 1).

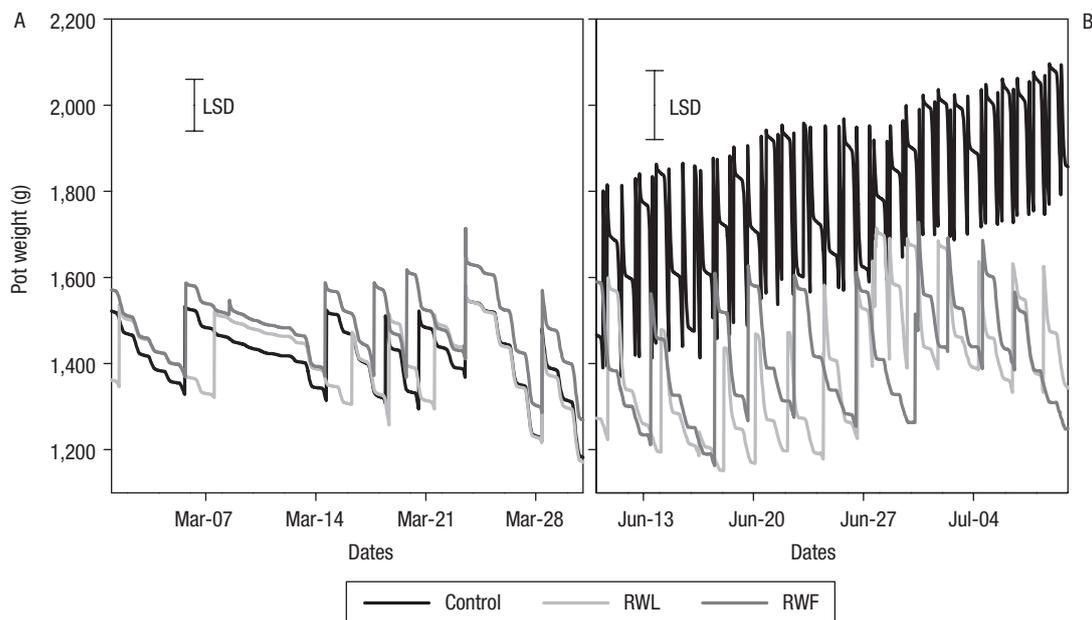
This color change in the RWF-treated plants resulting in lighter leaves with a more saturated green color, is regarded as a typical foliar salt damage symptom (Wu & Dodge, 2005). This change was also reflected by the decline in leaf SPAD readings in RWF. Compactness is another important quality criterion in ornamental plant, especially for potted plants. The compactness index tended to decrease in RW, but there was no statistical difference between treatments, suggesting the same compactness in all plants.

RWL and RWF reduced the amount of water applied to the pots (water consumption) by 52% and 61%, respectively, compared with the control (Table 1), representing an important water saving with RW. While WUE was reduced in RWF, no such effect was observed in RWL since growth reduction in RWF was greater than the reduction in the water applied. Rubio *et al.* (2010) also found lower WUE in salinized pepper plants, whereas Karlberg *et al.* (2006) reported that WUE may remain unchanged or increase at high soil salinities if the plant only responds as if under water stress due to high soil osmotic potential, which leads to stomata closing. All plants had similar Ψ<sub>l</sub> and Ψ<sub>p</sub> values, which pointed to no water stress under RW conditions, because ficus develops different strategies for avoiding water loss (decreased leaf

area, plant size, shoot/root and SLA). The salinized ficus plants also showed an efficient stomatal regulation and probably osmotic adjustment, since they presented lower  $g_s$  and  $\Psi_o$  values than those irrigated with control water (Table 1). Torrecillas *et al.* (2003) also found that gas exchange parameters were reduced in *Cistus* spp. irrigated with saline water, and osmotic adjustment permitted the plants to maintain the  $\Psi_p$ .

On the other hand, leaf  $Cl^-$  concentration increased from 6.62 (control) to 8.64  $mg\ g^{-1}$  DW (RWF), but was unaffected by RWL (Table 1). Neither RWL nor RWF modified leaf  $Na^+$ , although RWF slightly increased root  $Na^+$ . These data indicate that ficus has a mechanism to prevent  $Cl^-$  and  $Na^+$  accumulation in leaves, which partly explains the absence of symptoms due to saline ion toxicity. Karlberg *et al.* (2006) suggested that WUE falls in saline conditions because the plant allocates relatively more photosynthates to counteract the adverse effects of salinity due to ion toxicity. In this way, the salinized ficus plants reduced their growth to avoid saline ions toxicity. The scarce differences in leaf  $Na^+$  and  $Cl^-$  contents in both leaf and root among treatments suggests that the ficus root has the ability to limit  $Na^+$  and  $Cl^-$  uptake.

The differences in water consumption among treatments are mainly related to the differences in the number of irrigation events (Fig. 1). The evolution of pot weight in the first month of the experiment points to no difference in water consumption among treatments (Fig. 1A), whereas in the last month the control plants were irrigated much more frequently than those under RWL; and the plants under RWF were irrigated less frequently than the plants under RWL (Fig. 1B). An increasing pot weight during the cultivation period indicates that the plants are growing, which was true in the control pots and partially true in RW irrigated plants because the pot weight decreased at the end of June (Fig. 1B). The reason why pot weight diminished might be due to the possibility that: a) salinity negatively affected substrate ability to retain water, and b) lower irrigation frequency under RW caused worse rehydration for the top part of the substrate. Mostafazadeh-Fard *et al.* (2007) reported that salinity decreases soil structure stability, and Tilt *et al.* (1987) found that plant growth was significantly correlated with the water retention properties of substrates. This behavior observed under RW points to an important limitation of using balances to control irrigation when there are problems in substrate rehydration or/and water retention.

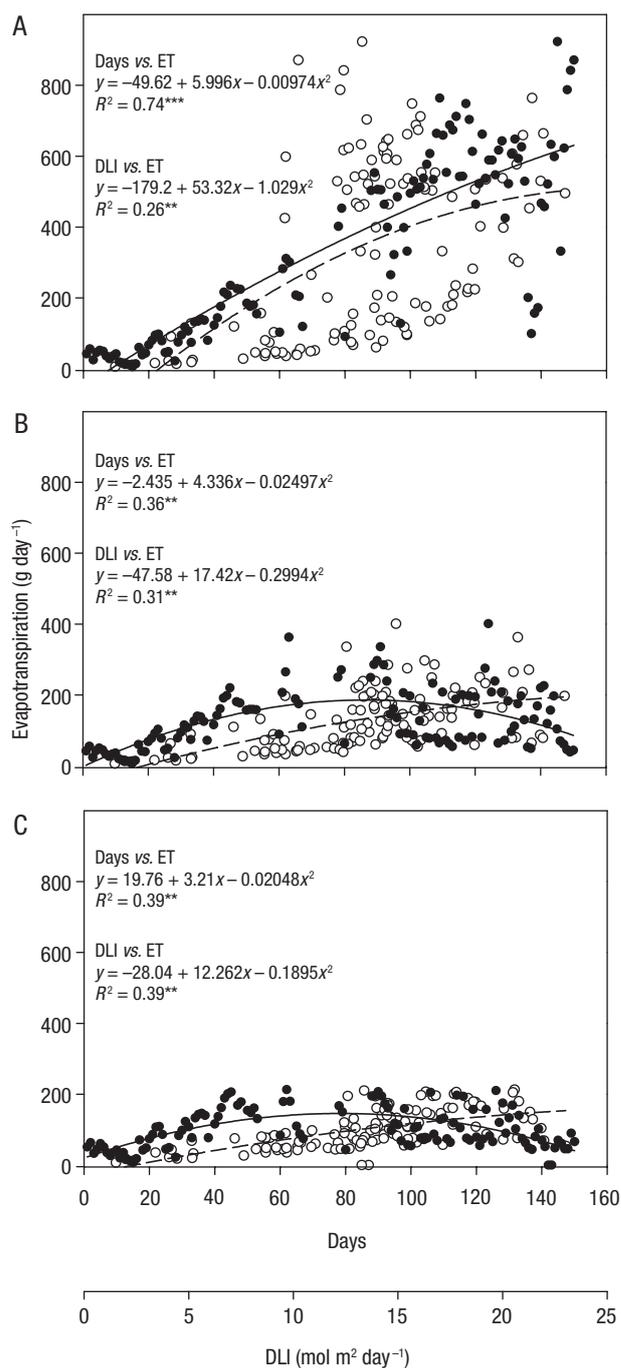


**Figure 1.** Thirty representative days of pot weight evolution at the beginning of experiment (from 1-March to 30-March) (A), and at the end of the experiment (from 10-June to 10-July) (B). Saline reclaimed wastewater with a constant leaching fraction of 23% (RWL); saline reclaimed wastewater with a constant leaching fraction of 15% and flushing of 50% every nine irrigation events (RWF); water taken from a canal that delivers water from the Tagus River to the Segura River for agricultural and municipal use with a constant leaching fraction of about 15% (control). Vertical bars indicate SE ( $n = 3$ ).

Water consumption is generally related to evapotranspiration (ET). In this experiment, the control plants received more water than those irrigated with RW (Table 1), which can be attributed to a relatively high ET under good-quality water compared with saline water (Katerji *et al.*, 2000). In fact, the control had a higher ET rate than the two RW treatments (Fig. 2). The ET was lower in RWF than RWL, presumably as a result of decreasing evaporation (lower water retained in bulk) and transpiration (lower leaf area and  $g_s$ ) (Figs. 2B and C). A regression study showed a significant quadratic relationship between ET and days of culture, and between ET and daily light integral (DLI) in all the treatments studied (Fig. 2). In the control, ET was strongly related with the days of culture but weakly related with DLI (Fig. 2A). Under RW, the  $R^2$  increased for ET vs. DLI and decreased for ET vs. days of culture; so, the state of development had less influence over ET when plants were irrigated with RW. The form of the curves for ET vs. days of culture changed in RW treatments compared with the control (Fig. 2). Figure 2A shows that ET increased as the days of culture increased in control conditions. However, in saline conditions, ET increased as the days of culture increase up to about 80 days, after which the slope changed and the relationship became negative, so that the ET rate fell to similar levels as in the first weeks of culture (Figs. 2B and C).

At the end of the experiment, the root zone presented a higher substrate osmotic potential due to the increasing salt content, and the salinized plants decreased their levels of  $g_s$  to maintain leaf turgor (Munns, 1988). In our experiment, a substantial fall in the  $g_s$  was observed in RWL [from 103 (control) to 56  $\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$ ], while the plants under RWF closed their stomata (3.25  $\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$  of  $g_s$ ). This led to a substantial fall in  $P_n$  (43% in RWL), while RWF showed minimal photosynthetic activity (Table 1). Ali-Dinar *et al.* (1999) found that salinity reduced the  $P_n$  in guava (*Psidium guajava* L.), which they attributed to stomatal closure. A fall in  $P_n$  under salinity may be due to lower  $g_s$ , the depression of specific metabolic processes in carbon uptake, inhibition of photochemical capacity, or a combination of these (Seemann & Critchley, 1985). In our experiment, the decrease in  $P_n$  seems to be mainly related to stomatal factors because the decrease in  $g_s$  was followed by proportional decrease in  $P_n$ .

To know whether the plants irrigated with RW were photochemically damaged, a chlorophyll fluorescence study was made. The results did not show statistical



**Figure 2.** Daily evapotranspiration (ET) as a function of days of culture (days) and day light integral (DLI) for control (A), saline reclaimed wastewater (5  $\text{dS m}^{-1}$ ) with a constant leaching fraction of 23% (RWL) (B), and saline reclaimed wastewater with a constant leaching fraction of 15% and flushing of 50% every nine irrigation events (RWF) (C). The continuous regression curves (open circle) indicate days vs. ET and discontinuous regression curves (filled circle) indicate DLI vs. ET. \*\*, \*\*\*: significant at  $p \leq 0.01$  and at  $p \leq 0.001$ , respectively.

differences in maximum photochemical efficiency of PSII ( $F_v/F_m$ ) or non-photochemical quenching (NPQ) when control and RWL were compared, whereas RWF decreased both parameters with respect to RWL (Table 1). No statistical difference in light adapted quantum yield of PSII ( $\Phi_{PSII}$ ) was observed among all treatments. The  $F_v/F_m$  in RWF was 0.696, which is below the 0.83 regarded as the optimal value for most plant species (Johnson *et al.*, 1993), indicating that the photosynthetic apparatus was injured. The stable  $\Phi_{PSII}$  and decreased  $P_n$  in RWF suggested that photochemical production was hardly used for photosynthesis, but was dissipated in photorespiration and other photochemical processes (Foyer *et al.*, 1994). NPQ reflects the efficiency of heat dissipation, which is an essential mechanism in protecting the leaf from light-induced damage (Horton *et al.*, 1996). In our study, NPQ remained lower in RWF than in RWL, signifying that the heat dissipation mechanism was damaged. Miralles *et al.* (2011) found that the decrease in NPQ was closely related to freezing injury in oleander (*Nerium oleander* L.). Thus, we believe that the diminution in NPQ with respect to the control confirms that the plants were photochemically damaged.

In conclusion, saline reclaimed wastewater can be used to produce potted weeping Fig with minimal effects on plant quality, while saving water. Plant size was clearly reduced as a result of the sensitivity of ficus to salinity, which suggests that RW may be a good alternative to the chemical retardants employed in ficus production. The salinized ficus plants showed attractive foliage because tolerance mechanisms prevented plants from suffering damage during saline stress (higher ability to limit  $Na^+$  and  $Cl^-$  uptake, stomata closing, osmotic adjustment, and reduction in leaf size, plant size, shoot/root and SLA). With regard to the two variations in LF, RWF saved more water than RWL. However, the RWF irrigated plants had a lower dry biomass and WUE than those under RWL, showed leaf discoloration and their PSII photochemistry was harmed. These findings indicate that RWL was the better irrigation-drainage combination when RW is used.

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