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Pot-in-pot Reduces Salinity, Chloride Uptake, and Maintains Aesthetic Value in *Euonymus japonicus* Thunb. Under Saline Irrigation

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***Abstract*. The appropriate management of crop conditions can reduce the salt damage suffered by ornamental species and produce high-quality plants even when saline irrigation water is used. The aim of this study was to determine whether the pot-in-pot (PIP) cultivation system can improve the saline irrigation tolerance of *Euonymus japonicus* compared with aboveground potting (AGP) in terms of growth and development, aesthetic quality, ion accumulation, and leaf potentials. A 5-month experiment started on 6 Mar., and the interaction between the cultivation system (PIP or AGP) and water quality (fresh water and saline water, with 1.76 and 9.04 dS·m^{-1}, respectively) was assessed. The substrate used was a mixture of white peat, coconut fiber, and perlite (40/40/20, v/v/v). A soil moisture sensor-controlled system was used to irrigate all the treatments when the AGP treatment irrigated with fresh water reached a volume water content (θ) of 0.33–0.35 m^3·m^{-3}. An interaction effect reduced the salinity effects in PIP and saline irrigation (PIP-s) compared with AGP and saline irrigation (AGP-s) in terms of damaged leaf area, plant dry weight (DW), and the compactness index. The PIP-s plants showed a survival rate of 93% compared with 57% in AGP-s. The substrate temperatures were milder in PIP regardless of the irrigation water, and the pore water electrical conductivity (EC) was 36% lower in PIP-s than in AGP-s. PIP reduced the Cl$^-$ accumulated in leaves but did not influence Na$^+$, Ca^{2+}, Mg^{2+}, or the K$^+$/Na$^+$ ratio. The lower amount of Cl$^-$ accumulated increased leaf water potential (Ψ_o) in PIP. Saline irrigation produced a general accumulation of Cl$^-$ and Na$^+$ in leaves and decreased Ca^{2+}, Mg^{2+}, the K$^+$/Na$^+$ ratio, Ψ_o, the shoot to root ratio, and height. In general, PIP reduced the salinity damage to *Euonymus japonicus*, the main effect being the lower Cl$^-$ ion uptake, which improved its aesthetic value (less damage and greater compactness and growth).**

The supply of high-quality water has become increasingly limited in many areas of the world, especially in arid and semiarid regions such as the Mediterranean area. However, this area has a great potential for growing crops as a result of its high solar radiation, and many ornamental growers try to make the most of these climatic conditions although only low-quality water is available. However, the use of low-quality water for irrigation affects plants in different ways, depending on the degree of salt tolerance of the species (Alarcón et al., 1993), the level of water salinity, and the characteristics of the water itself. Typical plant responses to salinity include reduced shoot and root growth and reduced whole plant size (Munns, 2002). As salinity stress becomes more severe, foliar damage such as leaf burn, scorch, necrosis, and premature defoliation may occur (Niu et al., 2010a), negatively affecting plant quality, which is of serious concern because the visual quality of ornamental plants is more important than maximum growth (Niu et al., 2008). Growth reduction is related with the osmotic effect of salinity, which limits a plant's ability to extract water from the soil (Rodriguez et al., 1997). Leaf damage is related to the Na$^+$ and Cl$^-$ accumulated in leaves which are not compartmentalized in vacuoles, making them metabolically toxic (Shannon and Grieve, 1999).

Plant salt tolerance is the ability to withstand the effects of high salt concentrations in the root zone without a significant adverse effect (Shannon and Grieve, 1999). This tolerance may be determined by: 1) the ability to limit uptake and/or transport of Na$^+$ and Cl$^-$ to aerial parts, because these ions are retained in the root (Murillo-Amador et al., 2006); 2) the capacity to maintain nutrient uptake (Chaparzadeh et al., 2003); 3) the capacity of plants to maintain a high K$^+$/Na$^+$ ratio in their tissues (Maathuis and Amtmann, 1999); and 4) the capacity to maintain a positive water balance through osmotic adjustment, which involves an active increase in tissue solute concentration (Torrecillas et al., 2003).

As well as environmental conditions, the management of factors that affect humidity and temperature in the substrate can help produce good-quality plants depending on the level of exposure to salinity and the salt tolerance of the species in question. Niu et al. (2010b) reported that salt accumulation in the root zone is affected by the substrate properties, plant size, and environmental conditions because these factors influence the substrate moisture content and cation exchange capacity in the root zone. The factors that can reduce salt accumulation in the substrate may reduce the negative effect of salinity. In this respect, the PIP system compared with AGP reduces root zone temperature stress (Young and Bachman, 1996), which improves roots development (Mathers, 2003), and enhances efficient water use by decreasing container evapotranspiration (Martin et al., 1999), which will reduce salt accumulation as a result of lower substrate water evaporation (Miralles et al., 2009).

The PIP crop system was introduced ≈1990 (Parkerson, 1990) in the United States and combines some of the benefits of both field and container production. In a PIP system, a holder or socket pot is permanently placed in the ground with the top rim remaining above. The container-grown plant is then placed within the holder pot for the production cycle (Ruter, 1998b).

The *Euonymus japonicus* (Japanese Spindle) is a commercial woody perennial shrub with good aesthetic qualities, which is frequently planted in public areas such as streets, recreation areas, and car parks. Previous studies with this species before this experiment with different levels of salinity showed that it was quite tolerant to saline irrigation (≈6 dS·m^{-1}).

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The objectives of this study were to evaluate the potential benefits of the PIP system for reducing the salt stress effect on *Euonymus japonicus*. To this end, plants of this species were grown in PIP and AGP and irrigated with fresh and saline water. The following points were studied: 1) substrate temperature and leachate EC and pH; 2) growth and development of the plant and any salt damage; and 3) pore water EC, leaf potentials, and ion concentrations.

Material and Methods

Plant material. One-year-old seedlings of commercial *Euonymus japonicus* var. Marieke were transplanted from 96-plug trays (each plug 56 cm^3) into 2.5-L black plastic pots (16 cm Ø × 15 height) in Jan. 2010. The substrate was a mixture of white peat, coconut fiber, and perlite (40/40/20, v/v/v) and was amended with 3 $g \cdot L^{-1}$ of a slow-release fertilizer (Osmocote plus 14-14-14; 14N–6.1P–11.6K; release time 2–3 months at 21 °C; The Scotts Company LLC, Marysville, OH). The plants were 5–7 cm in height at the beginning of the experiment. The fertilization was applied on 1 Mar. and 1 June with 7.5 g per pot of slow-release fertilizer (described previously).

The experiment was conducted in an open-air plot of 70 m^2 at the "Tomás Ferro" Experimental Agro-Food Station of the Polytechnic University of Cartagena (lat. 37°35' N, long. 0°59' W) starting on 6 Mar. 2010 and ending the last week of July 2010. Weather conditions were taken from a meteorological station sited 100 m from the experimental plot. The mean, maximum, and minimum daily values of environmental temperature and daily precipitation were registered.

Experimental design. The PIP system consisted of placing cultivation pots in pots already buried in the ground. The buried pots were made of black polyvinyl chloride with a grilled bottom to ensure drainage (5.5 L, 17 cm upper exterior diameter and 30 cm height). An air chamber of 15 cm separated the bases of both pots. Once the pots were buried in the ground, the plot was covered with a plastic permeable mulch (Horsol® 140 $g \cdot m^{-2}$; PROJAR S.A., Valencia, Spain), which was covered with a 4-cm layer of gravel (≈2 cm Ø). The cultivation pots were placed in 10 irrigation rows, each one 60 cm from the other. Five irrigation rows provided saline water (9.04 $dS \cdot m^{-1}$) and the other five fresh water (1.76 $dS \cdot m^{-1}$) in a random arrangement. A drip irrigation system was installed with one autocompensated 2 $L \cdot h^{-1}$ dripper (Netafim USA, Fresno, CA) per plant connected to one spaghetti tube. Each row had 22 cultivation pots [buried pots (PIP) in odd position alternating with aboveground pots (AGP) in even positions], which were placed 55 cm apart. This resulted in a total of four treatments: AGP with fresh water (AGP-c), PIP with fresh water (PIP-c), AGP with saline water (AGP-s), and PIP with saline water (PIP-s).

A data logger, CR1000 (Campbell Scientific, Ltd., Logan, UT) was installed in the center of the plot. This registered data of substrate temperature with two temperature probes (Termistor 107; Campbell Scientific, Ltd.) per treatment and volume water content (θ) with five EC-5 soil moisture sensors (Decagon Devices, Ltd., Pullman, WA) per treatment. The EC-5 probes were totally introduced in the pot at an angle of 45° at the side of the dripper. Soil moisture sensors were connected to the CR1000 through a multiplexer. The CR1000 was programmed to collect data every 10 min and to calculate average θ and its SE per treatment. The θ was obtained from the voltage readings of the soil moisture sensor using our own substrate-specific calibration ($\theta = 3.765 \times \text{voltage } 0.451$, $R^2 = 0.93$) determined using the procedure of Nemali et al. (2007). The CR1000 determined the mean, maximum, and minimum daily temperatures of the substrate.

The treatments were watered all at once for the same time (≈15 min and 550 mL) when the average θ of the AGP-c treatment, measured by the EC-5 probes, reached the threshold of 0.33–0.35 $m^3 \cdot m^{-3}$, which was ≈–2.5 kPa estimated according to the retention curve of the substrate. The AGP-c treatment had an average leaching of 25%, which was 40% more than that recommended for irrigation with fresh water in the area to avoid salt accumulation in the fresh water treatments. This irrigation criterion was established because previous studies with the PIP system concluded that it saves water (Miralles et al., 2009), whereas Navarro et al. (2007) observed less water consumption in the salinity irrigation treatments. The automated irrigation control was based on that described by Nemali and van Iersel (2006) but using a CR1000 instead of a CR10X with the 12 VDC switch connected to a relay, which controlled the fresh water and saline water pumps through an Agronic 3000 irrigation controller (Sistemes Electronics Progres, S.A., Lerida, Spain). The saline solution was obtained by adding ≈4 $g \cdot L^{-1}$ of sodium chloride to the irrigation water in a 1000-L water tank.

Water quality and substrate electrical conductivity. The pH and EC of irrigation water and leachate was registered once a week with a pH meter (pHep® 4; Hanna Instruments S.L., Eibar, Spain) and a conductivity meter (Dist® 6; Hanna Instruments S.L., Eibar, Spain). The samples were collected from May to July. There were a total of five replicates for each treatment.

Substrate pore water EC was measured in five replicates per treatment in the last week of July following the pour-through method (Wright, 1986).

Measurements of bulk EC (EC of the substrate mass including, air, solids, salts, and water) were taken weekly with a WET sensor (Delta-T Devices Ltd., Cambridge, U.K.). A total of 10 measurements per replicate was taken. Each sample was taken near the dripper because the EC-5 sensors were placed in that position. This measurement were taken simply to control that bulk EC did not exceed 8 $dS \cdot m^{-1}$, the maximum level of salinity that does not interfere with the θ estimates of the EC-5 sensor.

Measurements of plant morphology and salt damage. On 30 July 2010, the plant morphology (height and profile area) was determined with a flexometer and a side view picture was taken with a digital camera HP CW450 (Hewlett-Packard Española S.L., Madrid, Spain) of five plants per treatment. The compactness index was determined as follows: compactness index = profile area/$\{[(\pi/4)*[(\text{height} + \text{width})/2]^2\}$, where the profile area, and the height and width of the plant profile, were obtained from the picture and the software UTHSCSA Image Tool (University of Texas, San Antonio, TX). The closer the result was to unit, the more compact the plants.

Leaf area and the DW of roots, stem, and leaves were also determined in the same plants. The leaf area of both healthy and damaged leaves was determined with a LI-3100C (LI-COR Biosciences, Lincoln, NE). Individual leaf area was calculated as plant leaf area/total number of leaves. To calculate the DW, leaves (healthy, damaged, and fallen), stems, and roots were washed with distilled water and introduced in clearly identified envelopes and placed in a natural convection bacteriological stove at 60 °C until constant weight was reached. Finally, the DW was determined by weighing with a GRAM ST series precision balance.

The growth indices determined were the shoot DW/root DW (S:R), the specific leaf area (SLA) (leaf area/leaf DW) and leaf DW/total DW.

The DW of fallen leaves, damaged leaves, and the damaged leaf area were determined. The percentage of damaged leaf area was calculated with the image analysis software for plant disease quantification ASSESS 2.0 (University of Manitoba, Winnipeg, Canada).

Leaf potentials. Midday leaf water potential (Ψ_{hmd}), midday leaf ψ_S (Ψ_{omd}), and midday leaf turgor potential (Ψ_{pmd}) were measured in the last week of July 2010. A total of five repetitions per treatment were used. The leaf water potential was estimated according to the method described by Scholander et al. (1965) using a pressure chamber (Soil Moisture Equipment Co, Santa Barbara, CA) following the recommendations of Turner (1988). Leaves from the Ψ_{omd} measurements were frozen in liquid nitrogen. The values of the ψ_S (Ψ_{omd}) was measured using a Wescor 5520 vapor pressure osmometer (Wescor Inc., Logan, UT) according to Gucci et al. (1991). Estimates of Ψ_{pmd} were based on the difference between Ψ_{hmd} and Ψ_{omd} for each time.

Determination of inorganic ions. The inorganic ion content (Cl^-, Na^+, Ca^{2+}, Mg^{2+}, and K^+) were determined in roots and healthy leaves of all treatments and in damaged leaves of AGP-s. A total of five samples per treatment were collected, each sample consisting of 40 g fresh weight. The samples were processed to be analyzed in the SAIT laboratory of the laboratory by ion chromatography, as described by Bañón et al. (2011).

Statistical analysis. A split plot design was used: salinity as the main plot with five blocks and crop system as the subplot. Data were subjected to a split plot analysis of variance with a $P \leq 0.05$ using the software Statgraphics Plus 5.1 software (StatPoint Technologies Inc., Warrenton, VA).

Results

Substrate temperature. Substrate temperature was unaffected by the irrigation water (Fig. 1). Maximum daily temperatures were higher in AGP [average maximum of 33.17 ± 7.31 °C in AGP and 27.14 ± 7.05 °C in PIP during the experimentation period (mean ± SD)]. Minimum daily temperatures were greater in PIP (average minimum of 16.74 ± 5.28 °C in AGP and 20.39 ± 5.86 °C in PIP). Mean average temperatures were similar in both treatments (average mean of 24.02 ± 6.01 °C in AGP and 23.58 ± 6.28 in PIP). Environmental mean and maximum temperatures were lower than those recorded in the substrate, especially in summer (June, July, and August), but minimum environmental temperatures were similar to substrate temperatures in AGP (Fig. 1).

pH and electrical conductivity. The pH in the different leachate was 7.50 ± 0.33 (Fig. 2A). The irrigation water pH was 8.06 ± 0.58 without significant differences between the saline and control water. Leachate EC differed between irrigation water treatments. However, the crop production systems made little difference (Fig. 2B). In the case of saline irrigation (which was maintained at 9.04 ± 0.26 $dS \cdot m^{-1}$; Fig. 2C), the leachate EC was 12.31 ± 1.34 $dS \cdot m^{-1}$ and in the control irrigation (which was maintained at 1.76 ± 0.21 $dS \cdot m^{-1}$; Fig. 2C), it was 2.49 ± 0.73 $dS \cdot m^{-1}$. Several days before 20 June, a sharp increase in leachate EC in all the treatments (especially in saline treatments) was registered as a result of a strong precipitation event (Figs. 1C and 2B).

The pore water EC presented an interactive effect (Tables 1 and 2). In this case, the pore EC was similar in both control irrigation systems but was ≈34% lower in PIP-s than in AGP-s, although it was still 62% higher than in the control irrigated treatments.

The bulk EC was related with the leachate EC (Fig. 3). In general terms, the greater the bulk EC, the greater the leachate EC, except at the beginning of the experiment, when bulk EC was similar for all treatments.

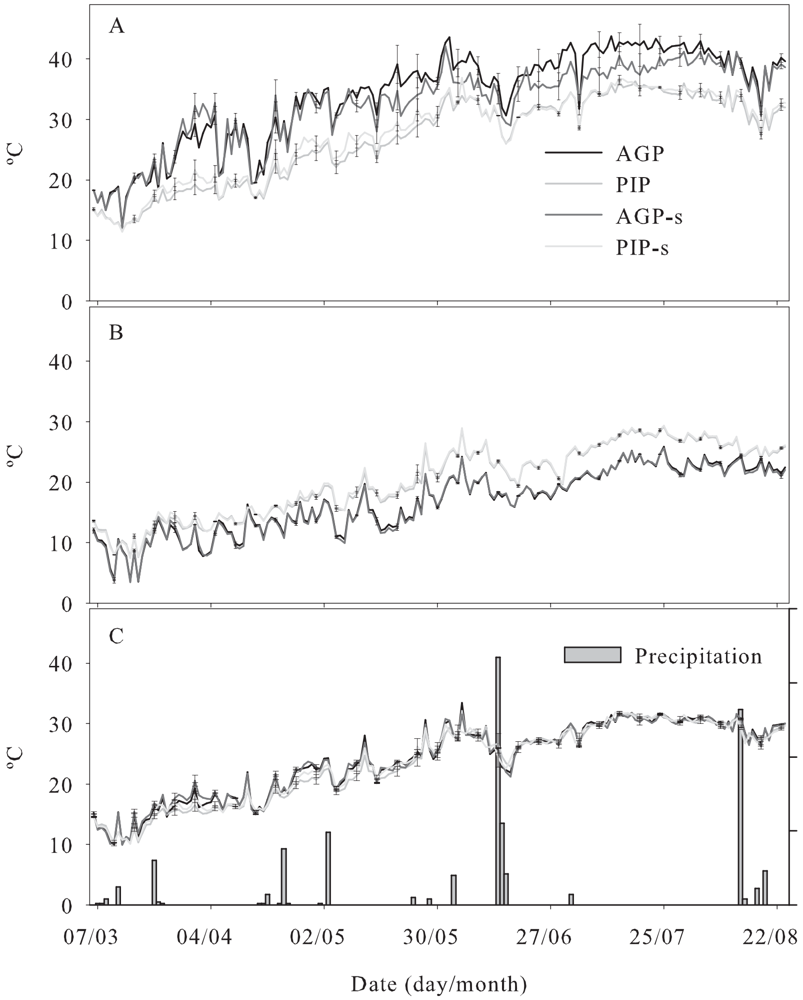

Fig. 1. Evolution of daily precipitation and substrate temperatures. Maximum temperatures (**A**), minimum temperatures (**B**), and average temperatures and precipitation (**C**). Error bars are SES (n = 2).

Growth and development. Root and stem DW were unaffected significantly by either salinity or crop system (Table 1). Leaf DW and plant DW had an interactive effect: the negative effects of saline irrigation (AGP-s reduced leaf DW by more than 69% and halved plant DW) were avoided by the PIP system (Tables 1 and 2). Similar results were obtained for the leaf area, the number of leaves, the compactness index and the fallen leaves, damaged leaves, and damaged leaf area. However, the individual leaf area was similar in all the treatments. The S:R ratio showed an interaction effect; it was reduced by salinity and under fresh water irrigation in PIP. The SLA only increased in AGP-s. Leaf DW/plant DW was reduced by salinity. The height was reduced by salt irrigation and in the AGP system. The percentage of plants with visual damage was 0% for the control irrigation and 85% and 29% in AGP-s and PIP-s, respectively. The survival rates of *Euonymus japonicus* in AGP-c, PIP-c, AGP-s, and PIP-s were 97%, 100%, 57%, and 93%, respectively.

Leaf potentials and ion concentrations. The Ψ_{hmd} was slightly lower in PIP. The Ψ_{omd} decreased with saline irrigation and AGP, whereas Ψ_{pmd} behaved in the opposite way (Table 3).

The leaf concentration of Cl^- increased with saline irrigation and AGP but was unaffected in roots. The Na^+ concentration increased with saline irrigation in both leaf and roots, contrary to Ca^{2+} and Mg^{2+} (Table 4). K^+ had an interactive effect in leaf, whereas in roots, it decreased with saline irrigation and AGP. The leaf K^+ concentration decreased in AGP-s (Table 5). However, PIP-c decreased leaf K^+ (contrary behavior to saline irrigation). The K^+/Na^+ ratio had an interactive and similar effect in both plant parts. The K^+/Na^+ ratio was significantly greater in PIP-c. Saline irrigation reduced this ratio compared with control irrigation treatments (Table 5).

AGP-s healthy leaves had lower ion concentrations than damaged leaves, especially Cl^- and Na^+ (≈39% and 43% less, respectively). The K^+/Na^+ ratio was unaffected.

Discussion

Substrate temperature is a crucial factor in root development and function. For example, above or below the range 15–27 °C, root growth decreases, water and nutrient uptake is affected, and root morphology may be altered (Adam et al., 2003). In the present experiment, the roots in PIP suffered less stressful temperatures than AGP (PIP had lower maximum and higher minimum substrate temperatures). Neal (2010) reported root zone temperatures in AGP and PIP of 44.4 and 37.4 °C, respectively, when the air temperature was 38.8 °C, which is similar to our findings. This author reported that visual observation of the root systems confirmed areas of root death in the southwest quadrants of plastic containers in AGP. These results would help explain the better root functioning under saline conditions in PIP, which

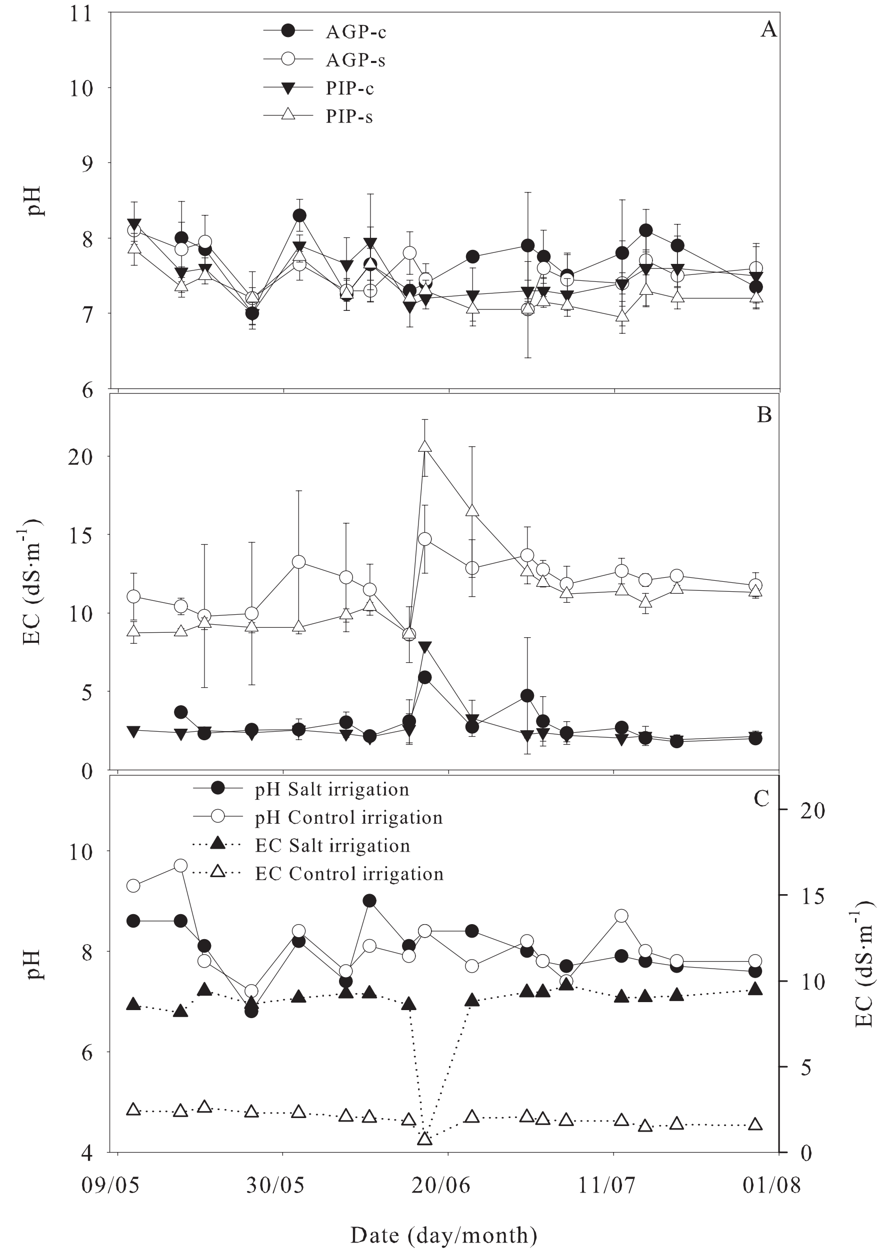

Fig. 2. Evolution of the leachate pH (**A**) and leachate electrical conductivity (EC) (**B**). Evolution of the irrigation water pH and EC (**C**). Error bars are SES (n = 5 for leachate, n = 3 for irrigation).

encouraged greater biomass production than AGP, but not in fresh water-irrigated plants, as reported by Miralles et al. (2009) in *Myrtus communis* (Table 2). However, many studies have found greater shoot and root growth in PIP; for example, Ruter (1998a) in Heritage river birch reported a 20% and 31% higher shoot DW and root DW, respectively, than in AGP. Miralles et al. (2009) reported an increase in the S:R ratio in AGP, like in this experiment. Neal (2010) reported that lilac grown in PIP had the lowest S:R ratios of all the cultivation systems analyzed (AGP, bag-in-pot, field-grown, and plastic container).

Saline irrigation also produces lower plant DW and affects the S:R ratio. Niu et al. (2010b) found that shoot DW of ornamental pepper 'Black Pearl' and vinca (*Catharanthus roseus*) 'Rose' decreased linearly as salinity increased from 0.8 to 7.4 dS·m^{-1}. Munns (2002) indicated that plants typically respond to salinity stress by reducing shoot and root growth, shoot growth reduction occurring earlier. In *Euonymus japonicus,* S:R was also reduced by salinity, mainly as a result of lower leaf DW (Tables 1 and 2).

The AGP-s treatment increased the SLA. Torrecillas et al. (2003) found that SLA increased in *Cistus albidus* and *C. monspeliensis* under saline irrigation. This fact could be the result of greater quantities of solutes in the leaves (accumulation of Na^+ and Cl^-; Table 4), which would have increased the density of leaves and reduced their Ψ_{omd} in AGP-s (Table 3). Torrecillas et al. (2003) also found that leaf DW/plant DW decreased with salinity, like in our results, as a result of leaf abscission in *Euonymus japonicus*. Leaf abscission is considered a tolerance mechanism of this species, allowing the elimination of leaves that have accumulated Na^+ and Cl^- ions (Tables 1, 2, and 4). Kchaou et al. (2010) reported the same behavior in olive trees subjected to salinity, especially in old leaves.

The AGP-s plants showed less growth in general, but their aesthetic value was seriously affected by the burned leaves, because, for ornamental landscape plants, visual quality is more important than maximum growth (Niu et al., 2008). The height was reduced under saline irrigation conditions, as reported by Navarro et al. (2007) in *Arbutus unedo* seedlings. The presence of visible leaf salt damage as the salinity of irrigation water increases has already been reported in ornamental bedding plants (Niu et al., 2010b); in herbaceous perennials (Niu and Rodriguez, 2006a, 2006b); and in woody shrubs (Wu et al., 2001). However, the PIP system presented the plants from the negative effects related with the compactness index and leaf damage and increased height. This increase in plant height (by ≈80%) was also reported in *Acacia smallii* by Martin et al. (1999). However, this effect depends on the species, because the same author found no such effect on *Cercidium floridum*, and Miralles et al. (2009) reported a decrease in height growth in *Myrtus communis* grown in PIP compared with AGP.

The positive effects of the PIP system in preventing salinity damage can be explained by both the milder substrate temperature (Fig. 1) and the greater substrate moisture content (data not shown), which reduced pore EC in PIP-s by 34% compared with AGP-s. This decrease in pore EC could be explained by the 11% increase in leaching in PIP-s (data not shown), because a smaller leaching fraction would lead to salt accumulation (Niu et al., 2008). However, there were no differences in leachate EC at the end of the experiment within the same irrigation water treatments (Fig. 2). This absence of differences, despite different leaching fractions, may be related with drip irrigation, in which the water has a greater tendency to flow downward than to spread horizontally, as reported by De Rijck and Schrevens (1998), so the salts accumulated around the wet bulb produced by the dripper. This, in turn, can be explained by two factors: 1) the bulk EC increased as the experiment progressed in the saline treatments (Fig. 3); and 2) the substantial increase in leachate EC as a result of heavy precipitation ≈20 June, which would have irrigated the whole substrate of the pot, flushing out the accumulated salts. This last aspect would also explain the high pore EC registered by the pour-through method at the end of the experiment.

The Ψ_{hmd} was unaffected by salinity, unlike the findings of Navarro et al. (2007) in *Arbutus unedo* and Torrecillas et al. (2003) in *Cistus albidus* and *C. monspeliensis,* who found that Ψ_{hmd} decreased with salinity. The PIP system produced slightly more negative values, which could have been the result of

Table 1. Effect of salinity (S) and crop system (CS) on *Euonymus japonicus* biomass, leaf plant parameters, shoot:root ratio (S:R), leaf dry weight (DW)/total DW, height, compactness index, and pore water electrical conductivity (EC) at the end of the experiment and level of significance from the analysis of variance split plot conducted to determine the effects of S, CS, and interaction (S × CS).

	Salinity (S)		Crop system (CS)		Significance level		
Parameters	Salt	Control	AGP	PIP	S	CS	S × CS
Root DW (g)	3.21	3.04	2.67	3.58	NS	NS	NS
Stem DW (g)	1.34	1.46	1.25	1.55	NS	NS	NS
Leaf DW (g)	3.34	5.62	4.17	4.78	*	NS	*
Plant DW (g)	7.89	10.11	8.09	9.91	*	NS	*
Leaf area (cm^2)	461	584	489	556	*	NS	*
Number of leaves (no.)	104	133	107	129	NS	NS	*
Individual leaf area (cm^2)	4.60	4.49	4.66	4.43	NS	NS	NS
S:R	1.52	2.35	2.04	1.83	*	NS	*
SLA (g·cm^{-2})	162	110	152	120	*	NS	*
Leaf DW/total DW	0.42	0.55	0.48	0.49	**	NS	NS
Height (cm)	16.9	19.1	17.4	18.6	**	*	NS
Compactness index	0.85	0.93	0.85	0.93	NS	NS	*
Fallen leaves (no.)	8.00	0.13	7.63	0.50	**	*	*
Damaged leaves (no.)	7.13	0.13	6.25	1.00	****	***	***
Damaged leaf area (cm^2)	5.56	0.00	4.97	0.59	****	***	***
Pore EC (dS·m^{-1})	36.1	11.9	28.6	19.4	****	***	**

Asterisks indicate differences between means. *Significant at $P \leq 0.05$, **significant at $P \leq 0.005$, ***significant at $P \leq 0.0005$, ****significant at $P \leq 0.00005$, and nonsignificant (NS).

Table 2. Interactive effects of salinity and crop system on *Euonymus japonicus* biomass, plant leaf parameters, shoot:root ratio (S:R) compactness index, and pore water electrical conductivity (EC) at the end of the experiment.

	Treatment			
Parameter	AGP-c	PIP-c	AGP-s	PIP-s
Leaf DW (g)	6.35 a^z	4.89 a	1.98 b	4.69 a
Plant DW (g)	10.8 a	9.43 a	5.39 b	10.4 a
Leaf area (cm^2)	613 a	555 a	365 b	557 a
Number of leaves (no.)	137 a	129 a	77 b	130 a
S:R	2.67 a	2.03 b	1.41 c	1.63 c
SLA (g·cm^{-2})	103 c	117 b	201 a	123 b
Compactness index	0.96 a	0.92 a	0.75 b	0.94 a
Fallen leaves (no.)	0.00 a	0.25 a	15.3 b	0.75 a
Damage leaves (no.)	0.00 a	0.25 a	12.50 b	1.75 a
Damage leaf area (no.)	0.00 a	0.00 a	9.94 b	1.17 a
Pore EC (dS·m^{-1})	13.7 c	10.1 c	43.5 a	28.7 b

^{z}Different letters in the same row indicate significant differences between means at a $P \leq 0.05$ according to the least significant difference test.

AGP-c = aboveground potting with fresh water; PIP-c = pot in pot with fresh water; AGP-s = aboveground potting with saline irrigation; PIP-s = pot in pot with saline irrigation; DW = dry weight; SLA = specific leaf area.

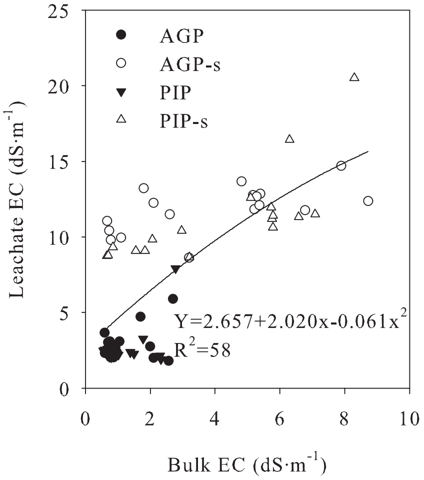

Fig. 3. Leachate electrical conductivity (EC) as a function of bulk EC measured with the WET sensor. The data were collected once a week and averaged for each treatment (PIP, PIP-s, AGP, AGP-s). PIP = pot in pot; PIP-s = PIP with saline irrigation; AGP = aboveground potting; AGP-s = AGP with saline irrigation.

the lower transpiration in AGP in summer. In *Myrtus communis,* Miralles et al. (2009) reported that the lower soil matric potential in AGP could have activated the messages sent by roots, perhaps of a chemical kind, which might have lowered stomatal conductance rates, thus maintaining greater Ψ_{hmd} in AGP. The Ψ_{omd} was reduced by both salinity and the AGP system. A decrease in Ψ_{omd} as the salinity of the irrigation water increased was reported by Niu et al. (2010b) and Navarro et al. (2007) in bedding plants and *Arbutus unedo*, respectively. Niu et al. (2008) reported that this effect was the result of the accumulation of Na^+ and Cl^- in leaves, which is associated with osmotic adaptation (Heuer and Nadler, 1998; Pardossi et al., 1999). Navarro et al. (2007) reported that *Arbutus unedo* underwent osmotic adjustment as a result of an imposed salt stress, which would have led to Ψ_{pmd} increasing, like in our results. The accumulation of Cl^- and Na^+ was responsible for this leaf potential because saline irrigation increased their concentration, and AGP increased Cl^- as well. The opposite behavior of Ψ_{omd} and Ψ_{pmd} in the PIP system is explained by lower Cl^- accumulation in leaves (Tables 3 and 4). The greater pore EC in the saline irrigation and AGP system would explain these results (Tables 1 and 2).

Ion accumulation could also be responsible for leaf damage and decreased growth, especially in the case of Cl^- with its increased concentration, so its reduction by PIP would justify the reduction in any damage. The strong toxicity of Cl^- was reported by Martinez-Barroso and Alvarez (1997) in strawberry, where the leaf scorch observed was basically the result of the increase in leaf Cl^- concentrations. Navarro et al. (2007) also found that the harmful consequences of NaCl salinity on net photosynthesis were associated with high leaf Cl^- in *Arbutus unedo*. The nonsignificant effects of the crop system on other nutrients (Ca^{2+} and Mg^{2+}) and on the K^+/Na^+ ratio would confirm this hypothesis, because salt tolerance is also expressed by the capacity to maintain nutrient uptake (Chaparzadeh et al., 2003) and a high K^+/Na^+ ratio (Cassaniti et al., 2009). The greater Na^+ concentration in roots under saline irrigation may be related with salt tolerance, because, as reported by Colmer et al. (2005), controlling the salt concentration of the aerial parts by restricting entry through the roots and limiting transport to the shoots is an important mechanism that allows plants to survive and grow in saline conditions. However, Cl^- was not retained in roots, which confirms that the lower concentration in leaves was the result of a lower concentration in pore water, which is supported by the lower pore water EC in fresh water treatments and PIP-s compared with AGP-s (Tables 2 and 4).

Conclusion

Euonymus japonicus grown in PIP with fresh water grew to a greater height and had a lower S:R ratio compared with AGP-grown plants. Even when saline water was used, PIP avoided many of the negative effects of AGP, which reduced growth (less biomass production), the aesthetic value (damaged leaf and less compactness), and plant survival (57% vs. 93% in PIP). These benefits are explained by the less extreme substrate temperatures, leading to both a greater leaching fraction and higher water content in the substrate, which produced a lower pore EC (34% lower than AGP-s). These conditions allowed better root development and reduced the Cl^- accumulation in leaves because this species did not accumulate Cl^- in the roots, unlike Na^+ (ion accumulation in roots prevents its transportation to leaves). The absence of differences in Na^+, other nutrients including Ca^{2+} and Mg^{2+}, and the K^+/Na^+ ratio with respect to AGP suggested that Cl^- ion was mainly responsible for leaf damage. On the other hand, saline irrigation had other effects compared with the control irrigation: a lower decrease in the S:R ratio, shorter plants, decreased Ψ_{omd}, increased Ψ_{pmd}, lower Ca^{2+}, Mg^{2+}, and K^+/Na^+ ratio, and the accumulation of Cl^- and Na^+ in leaves.

Table 3. Effects of salinity (S) and crop system (CS) on *Euonymus japonicus* leaf potentials at the end of experiment and level of significance from the analysis of variance split plot conducted to determine the effects of S, CS, and interaction (S × CS).

	Salinity (S)		Crop system (CS)		Significance level		
Parameters	Salt	Control	AGP	PIP	S	CS	S × CS
Ψ_{hmd}	–1.40	–1.40	–1.19	–1.62	NS	****	NS
Ψ_{omd}	–2.30	–1.94	–2.25	–2.00	**	*	NS
Ψ_{pmd}	0.90	0.54	1.06	0.39	**	****	NS

Asterisks indicate differences between means. *Significant at $P \leq 0.05$, **significant at $P \leq 0.005$, ***significant at $P \leq 0.0005$, ****significant at $P \leq 0.00005$, and nonsignificant (NS).

AGP = aboveground potting; PIP = pot in pot; Ψ_{hmd} = midday leaf water potential; Ψ_{omd} = midday leaf ψ_S; Ψ_{pmd} = midday leaf turgor potential.

Table 4. Effects of salinity (S) and crop system (CS) on the leaf and roots ions (mg·g^{-1} of DW) of *Euonymus japonicus* at the end of experiment and level of significance from the analysis of variance split plot conducted to determine the effects of S, crop system CS, and interaction (S × CS).

		Salinity (S)		Crop system (CS)		Significance level		
Plant part	Ions	Saline	Control	AGP	PIP	S	CS	S × CS
Leaf	Cl^-	38.9	10.9	28.6	21.1	*	*	NS
	Na^+	15.8	1.6	9.9	7.5	*	NS	NS
	Ca^{2+}	1.5	1.9	1.8	1.6	*	NS	NS
	Mg^{2+}	1.4	2.0	1.7	1.7	*	NS	NS
	K^+	13.5	14.8	14.4	13.9	NS	NS	*
	K^+/Na^+	0.85	9.17	1.45	1.85	*	*	*
Root	Cl^-	12.21	9.17	10.00	11.37	NS	NS	NS
	Na^+	11.64	5.17	8.37	8.44	**	NS	NS
	Ca^{2+}	1.56	2.57	2.04	2.08	***	NS	NS
	Mg^{2+}	1.28	2.40	1.77	1.92	***	NS	NS
	K^+	2.00	5.41	2.75	4.67	****	***	NS
	K^+/Na^+	0.18	1.04	0.47	0.74	****	****	***

Asterisks indicate differences between means. *Significant at $P \leq 0.05$, **significant at $P \leq 0.005$, ***significant at $P \leq 0.0005$, ****significant at $P \leq 0.00005$, and nonsignificant (NS).

DW = dry weight; AGP = aboveground potting; PIP = pot in pot.

Table 5. Interactive effects of salinity and crop system on the leaf and roots ions (mg·g^{-1} of DW) of *Euonymus japonicus* at the end of the experiment.

		Treatment			
Plant part	Parameter	AGP-c	PIP-c	AGP-s	PIP-s
Leaf	K^+	16.6 a^z	13.0 bc	12.3 c	14.8 ab
	K^+/Na^+	6.5 b	19.5 a	0.7 c	1.0 c
Root	K^+/Na^+	0.82 b	1.26 a	0.12 c	0.23 c

^{z}Different letters in the same row indicate significant differences between means at a $P \leq 0.05$ according to the least significant difference (test.

DW = dry weight; AGP-c = aboveground potting with fresh water; PIP-c = pot in pot with fresh water; AGP-s = aboveground potting with saline irrigation; PIP-s = pot in pot with saline irrigation.

The PIP system can be recommended for the cultivation of this species using saline water, because salinity damage will be avoided for at least 4 months.

Literature Cited

Adam, M.L., J.M. Kelly, W.R. Graves, and P.M. Dixon. 2003. Net nitrate uptake by red maple is a function of root-zone temperature. J. Plant Nutr. 26:203–222.

Alarcón, J.J., M.C. Bolarín, M.J. Sánchez-Blanco, and A. Torrecillas. 1993. Water relations and osmotic adjustment in *Lycopersicon esculentum* and L. Pennellii during short-term salt exposure and recovery. Physiol. Plant. 89:441–447.

Bañón, S., J. Miralles, J. Ochoa, J.A. Franco, and M.J. Sánchez-Blanco. 2011. Effects of diluted and undiluted treated wastewater on the growth, physiological aspects and visual quality of potted lantana and polygala plants. Sci. Hort. 129:869–876.

Cassaniti, C., C. Leonardi, and T.J. Flowers. 2009. The effects of sodium chloride on ornamental shrubs. Sci. Hort. 122:586–593.

Chaparzadeh, N., R.A. Khavari-Nejad, F. Navari-Izzo, and R. Izzo. 2003. Water relations and ionic balance in *Calendula officinalis* L. under saline conditions. Agrochimica XLVII:69–79.

Colmer, T.D., R. Munns, and T.J. Flowers. 2005. Improving salt tolerance of wheat and barley: Future prospects. Aust. J. Exp. Agr. 45:1425–1443.

De Rijck, G. and E. Schrevens. 1998. Distribution of nutrients and water in rockwool slabs. Sci. Hort. 72:277–285.

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

Heuer, B. and A. Nadler. 1998. Physiological responses of potato plants to soil salinity and water deficit. Plant Sci. 137:43–51.

Kchaou, H., A. Larbi, K. Gargouri, M. Chaieb, F. Morales, and M. Msallem. 2010. Assessment of tolerance to NaCl salinity of five olive cultivars, based on growth characteristics and Na^+ and Cl^- exclusion mechanisms. Sci. Hort. 124:306–315.

Maathuis, F.J.M. and A. Amtmann. 1999. K^+ nutrition and Na^+ toxicity: The basis of cellular K^+/Na^+ ratios. Ann. Bot. (Lond.) 84:123–133.

Martin, C.A., L.B. McDowell, and B. Shiela. 1999. Below-ground pot-in-pot effects on growth of two southwest landscape trees was related to root membrane thermostability. J. Environ. Hort. 17:63–68.

Martinez-Barroso, M.C. and C.E. Alvarez. 1997. Toxicity symptoms and tolerance of strawberry to salinity in the irrigation water. Sci. Hort. 71:177–188.

Mathers, H.M. 2003. Summary of temperature stress issues in nursery containers and current methods of protection. HortTechnology 13: 617–624.

Miralles, J., P.A. Nortes, M.J. Sánchez-Blanco, J.J. Martínez-Sánchez, and S. Bañón. 2009. Above-ground and pot-in-pot production systems for *Myrtus communis* L. Trans. ASABE 52:93–101.

Munns, R. 2002. Comparative physiology of salt and water stress. Plant Cell Environ. 25:239–250.

Murillo-Amador, B., E. Troyo-Diéguez, J.L. García-Hernández, R. López-Aguilar, N.Y. Ávila-Serrano, S. Zamora-Salgado, E.O. Rueda-Puente, and C. Kaya. 2006. Effect of NaCl salinity in the genotypic variation of cowpea (*Vigna unguiculata*) during early vegetative growth. Sci. Hort. 108:423–441.

Navarro, A., S. Bañon, E. Olmos, and M.J. Sánchez-Blanco. 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.

Neal, C.A. 2010. Crabapple and lilac growth and root-zone temperatures in Northern nursery production systems. HortScience 45:30–35.

Nemali, K.S., F. Montesano, S.K. Dove, and M.W. van Iersel. 2007. Calibration and performance of moisture sensors in soilless substrates: ECH2O and Theta probes. Sci. Hort. 112:227–234.

Nemali, K.S. and M.W. van Iersel. 2006. An automated system for controlling drought stress and irrigation in potted plants. Sci. Hort. 110: 292–297.

Niu, G. and D.S. Rodriguez. 2006a. Relative salt tolerance of five herbaceous perennials. HortScience 41:1493–1497.

Niu, G. and D.S. Rodriguez. 2006b. Relative salt tolerance of selected herbaceous perennials and groundcovers. Sci. Hort. 110:352–358.

Niu, G., D.S. Rodriguez, and L. Aguiniga. 2008. Effect of saline water irrigation on growth and physiological responses of three rose rootstocks. HortScience 43:1479–1484.

Niu, G., D.S. Rodriguez, and M. Gu. 2010a. Salinity tolerance of *Sophora secundiflora* and *Cercis canadensis* var. mexicana. HortScience 45:424–427.

Niu, G., D.S. Rodriguez, and T. Starman. 2010b. Response of bedding plants to saline water irrigation. HortScience 45:628–636.

Pardossi, A., F. Malorgio, and F. Tognomi. 1999. Salt tolerance and mineral relations for celery. J. Plant Nutr. 22:151–161.

Parkerson, C.H. 1990. P & P: A new field-type nursery operation. Proc. Inter. Plant Prop. Soc. 40:417–419.

Rodriguez, P., J. Dell'Amico, D. Morales, M.J. Sánchez-Blanco, and J.J. Alarcón. 1997. Effects of salinity on growth, shoot water relations and root hydraulic conductivity in tomato plants. J. Agr. Sci. 128:439–444.

Ruter, J.M. 1998a. Fertilizer rate and pot-in-pot production increase growth of Heritage river birch. J. Environ. Hort. 16:135–138.

Ruter, J.M. 1998b. Pot-in-Pot production and cyclic irrigation influence growth and irrigation efficiency of 'Okame' cherries. J. Environ. Hort. 16:159–162.

Scholander, P.F., H.T. Hammel, E.D. Bradstreet, and E.A. Hemmingsen. 1965. Sap pressure in vascular plants. Science 148:339–346.

Shannon, M.C. and C.M. Grieve. 1999. Tolerance of vegetable crops to salinity. Sci. Hort. 78:5–38.

Torrecillas, A., P. Rodríguez, and M.J. Sánchez-Blanco. 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.

Turner, N.C. 1988. Measurement of plant water status by the pressure chamber technique. Irrig. Sci. 9:289–308.

Wright, R.D. 1986. The pour-through nutrient extraction procedure. HortScience 21:227–229.

Wu, L., X. Guo, and A. Harivandi. 2001. Salt tolerance and salt accumulation of landscape plants irrigated by sprinkler and drip irrigation systems. J. Plant Nutr. 24:1473–1490.

Young, R.E. and G.R. Bachman. 1996. Temperature distribution in large, pot-in-pot nursery containers. J. Environ. Hort. 14:170–176.