UNIVERSIDAD POLITÉCNICA DE CARTAGENA
DEPARTAMENTO DE PRODUCCIÓN VEGETAL

Adecuación de técnicas viverísticas en la producción de arbustos
ornamentales

Julián Miralles Crespo

2011

UNIVERSIDAD POLITÉCNICA DE CARTAGENA
DEPARTAMENTO DE PRODUCCIÓN VEGETAL

Adecuación de técnicas viverísticas en la producción de arbustos
ornamentales

Julián Miralles Crespo

2011

Directores:

Sebastián del Pilar Bañón Arias

Juan José Martínez Sánchez

UNIVERSIDAD POLITÉCNICA DE CARTAGENA
DEPARTAMENTO DE PRODUCCIÓN VEGETAL

Management of nursery techniques in the production of ornamental
shrubs

Julián Miralles Crespo

2011

Supervisors:

Sebastián del Pilar Bañón Arias

Juan José Martínez Sánchez

UNIVERSIDAD POLITÉCNICA DE CARTAGENA

AUTORIZACIÓN DE LA PRESENTACIÓN DE LA TESIS DOCTORAL
POR LOS DIRECTORES

D. Sebastián del Pilar Bañón Arias, Catedrático de Universidad del Área de Producción Vegetal en el Departamento de Producción Vegetal de la Universidad Politécnica de Cartagena y D. Juan José Martínez-Sánchez, Catedrático de Universidad del Área de Producción Vegetal en el mismo Departamento.

A U T O R I Z A N:

La presentación de la Tesis Doctoral titulada “Adecuación de técnicas viverísticas en la producción de arbustos ornamentales”, realizada por D. Julián Miralles Crespo, bajo nuestra dirección y supervisión, en el Departamento de Producción Vegetal, y que presenta para la obtención del grado de Doctor por la Universidad Politécnica de Cartagena.

En Cartagena, a _____ de _____ de _____

LOS DIRECTORES DE LA TESIS

Fdo.: Sebastián del Pilar Bañón Árias __ Juan José Martínez Sánchez

UNIVERSIDAD POLITÉCNICA DE CARTAGENA

Comisión de Doctorado

**AUTORIZACIÓN DE LA PRESENTACIÓN DE LA TESIS DOCTORAL
POR LA COMISIÓN ACADÉMICA RESPONSABLE DEL PROGRAMA DE
DOCTORADO**

D. Marcos Egea Cortines, Coordinador de la Comisión Académica del Programa Técnicas Avanzadas en Investigación y Desarrollo Agrario y Alimentario

INFORMA:

Que la Tesis Doctoral titulada “Adecuación de técnicas viverísticas en la producción de arbustos ornamentales”, ha sido realizada por D. Julián Miralles Crespo, bajo la dirección y supervisión de D. Sebastián del Pilar Bañón Árias y D. Juan José Martínez Sánchez y que la Comisión Académica ha dado su conformidad para que sea presentada ante la Comisión de Doctorado.

La rama de conocimiento por la que esta tesis ha sido desarrollada es:

- ☐ Ciencias Básicas
- ☐ Ciencias Sociales y Jurídicas
- ☒ Ingeniería y Arquitectura

En Cartagena, a _____ de _____ de _____

EL COORDINADOR DE LA COMISIÓN ACADÉMICA DEL PROGRAMA

Fdo.: D. Marcos Egea Cortines

Comisión de Doctorado

RECONOCIMIENTOS

El Ingeniero de Montes Julián Miralles Crespo, ha contado durante el periodo de realización de esta tesis, con una beca de Formación de Profesorado Universitario del Ministerio de Educación.

Este trabajo se encuadra dentro de las actividades de los proyectos de investigación de la Fundación Séneca de la Región de Murcia 02991/PI/05, 05660/PI/07, 08669/PI/08; de los proyectos CICYT AGL 2005-05588-C02-1 y 2, AGL2008-05258-CO2-1 y 2; del proyecto de la Consejería de Agricultura y Agua de la Región de Murcia programa UPCT-CEBAS-IMIDA 2008 y del proyecto Fred C. Gloeckner Foundation and USDA-NIFA-SCRI Award no. 2009-51181-05768.

Los resultados de la presente tesis doctoral han sido publicados o aceptados en:

Revistas científicas:

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.

Miralles, J., M.J. Sánchez-Blanco, A. Navarro, J.J. Martínez-Sánchez, J.A. Franco, and S. Bañón. 2010. Comparison of stem diameter variations in three small ornamental shrubs under water stress. HortScience, 45:1681-1689.

Miralles, J., J.J. Martínez-Sánchez, J.A. Franco, and S. Bañón. 2011. *Rhamnus alaternus* growth under four simulated shade environments: morphological, anatomical and physiological responses. Scientia Horticulturae, 127:562-570.

Miralles, J. and M.W. van Iersel. 2011. A calibrated TDT soil moisture sensor can be used for precise automated irrigation of container-grown Plants. Hortscience. Accepted for publication.

Congresos:

Miralles, J., P. Nortes, M.J. Sánchez-Blanco, J.J. Martínez-Sánchez, and S. Bañón. Producción viverística de *Myrtus communis* en macetas dentro de macetas enterradas. Presentación oral en las IV Jornadas Ibéricas de Horticultura Ornamental, Innovación y futuro en la jardinería titulada. Pontevedra, del 14 al 18 de Octubre de 2008. Actas de Horticultura 52: 113-117.

Miralles, J., R. Valdes, J.J. Martínez-Sánchez, A. Navarro, M.J. Sánchez-Blanco, and S. Bañón. Respuesta de las contracciones diarias del tronco y la evapotranspiración en tres arbustos ornamentales sometidas a estrés hídrico severo. Presentación oral en el VI

Congreso Ibérico, XII Nacional de Ciencias Hortícolas. Logroño, del 25 al 29 de mayo de 2009. Actas de Horticultura 54: 307-308.

Miralles, J., R. Loira, S. Bañon, and J.J. Martínez-Sánchez. Response of *Rhamnus alaternus* to five shading levels. Oral presentation at the 28th International Horticultural Congress, Lisboa (Portugal), 22-27 de agosto de 2010. Abstracts Volume II. pp. 187.

Proyectos fin de carrera:

Efectos del sombreado sobre el crecimiento, desarrollo, relaciones hídricas y estado fotoquímico de *Rhamnus alaternus*. (2009). Raúl Espín Loira. Universidad Politécnica de Cartagena. Escuela Técnica Superior de Ingeniería Agronómica.

AGRADECIMIENTOS

Una vez hechos los trabajos de investigación que forman esta tesis, llega el momento de recopilarlos y escribirla. Esta ardua tarea, será más fácil si empiezo por los agradecimientos, porque recordar toda la gente que me ha ayudado, me va a dar fuerzas para culminar esta etapa de la mejor manera posible. En vez de ir nombrando gente como si de una lista se tratara, he decidido hacerlo de otra forma. Voy a contar en tres páginas mi experiencia vivida durante los cuatro años de duración de mi beca de formación de profesorado universitario (FPU) del Ministerio de Educación. En esta “aventura” he tenido la suerte de encontrarme con personas y situaciones que me han hecho “crecer”, tanto a nivel investigador como personal y que forman parte de la historia que les voy a contar. Por lo tanto, agradezco a todos los que aquí aparecéis el “abono” que me habéis aportado y que ha contribuido en mi crecimiento. Espero no olvidarme de nadie, porque sois muchos, pero en tal caso, le presento mis disculpas de antemano. Pues bien, sin más preámbulos relataré esta historia, que todavía me queda mucho por redactar y tres páginas no dan para enrollarse mucho.

Mi llegada a la Universidad Politécnica de Cartagena (UPCT) se debió a una conversación entre los Doctores Juan José Martínez Sánchez (desconocido por mí) y Eduardo Orozco Bayo (antiguo director de proyecto de la Ingeniería Técnica Forestal). En ese momento yo me encontraba realizando el proyecto fin de carrera para optar a Ingeniero de Montes cuando el Dr. Martínez-Sánchez me ofreció una beca para montar un vivero en la finca “Tomás Ferro”. Desde el momento en que la acepté, todo fueron facilidades por su parte para terminar el proyecto y calentar motores para pedir la beca FPU (cosa que él veía más clara que yo).

Mi “aterrizaje” en la Estación Experimental Agroalimentaria “Tomás Ferro” se centró ese primer año en producir planta forestal en bandeja para la repoblación de la ampliación del puerto de Cartagena. En dicha tarea recibí ayuda de D. Francisco Javier Solano, D. Jose Anastasio Egea, D. Pedro Mateo y D. Vicente Aparicio. Con ellos aprendí a trasplantar tomillos con ligereza (Vicente) a programar riegos y abonos (Francisco), y a instalar sistemas de riego (José Anastasio y Pedro). Además, hemos tenido tiempo de pasar buenos ratos tanto fuera como dentro del trabajo.

Terminado el primer año, llegó la beca FPU, cuyos directores fueron los doctores Sebastian del Pilar Bañón Arias y Juan José Martínez Sánchez. En ese momento, me puse a trabajar especialmente con el Dr. Bañón. Él me enseñó con generosidad y sabiduría, siempre escuchándome con paciencia y tratándome con cercanía. Ha sido clave en mi

formación y me ha demostrado su confianza al dejarme libertad de actuación en los ensayos finales. No obstante, el mejor regalo que he recibido ha sido la amistad que se ha forjado entre nosotros y el acogimiento por parte de sus hijos, Dani y Sebas, y su mujer, Maribel.

Ahora bien, la tesis no se hace con un par de directores y un doctorando. Hace falta gente que te ayude a medir diversos parámetros a pleno sol en verano. Ahí ha estado especialmente mi gran amiga Raquel, aunque también he contado con Victor, Marina, Paula, Eli, el personal de la finca y el Dr. Jesús Ochoa. También necesitas personas que te enseñen cómo se usa tal o cual cosa, donde encontré a Pedro Nortes, Gregorio Egea, Alejandra Navarro y Manuel García. Y, no podemos olvidar a los que te hacen comentarios críticos cuando tienes los resultados y los quieres publicar. En este punto he tenido la supervisión especial de la Dra. María Jesús Sánchez-Blanco, aunque también he recibido comentarios constructivos de los doctores D. Arturo Torrecillas Melendreras, Doña María Fernández Ortuño y D. José Antonio Franco Leemhuis. “Lastly, I should like to thank Philip Thomas for his help with the English version and for teaching me how to drink tea when the temperature is 35°C in the shade”.

Otro punto de crucial importancia fue la estancia durante cuatro meses en el Departamento de Horticultura de la Universidad de Georgia en Athens (USA). Aquí estuve bajo la dirección del Dr. Marc W. van Iersel, quien me enseñó a programar dataloggers de Campbell Scientific para el riego automatizado, y para encender LEDs. Marc, también me aportó, junto con su mujer la Dra. Lynne Seymour, un hogar durante 15 días, todas las facilidades para mi adaptación y muy buenos ratos (con cerveza Terrapin incluida), lo que acabó forjando una buena amistad. Este viaje, mejoró mis recetas culinarias, pues como agradecimiento a la técnica del laboratorio Sue Dove, a mi buen amigo Yong Yum Kim (“El Koreano”) y a algunos más, les invitaba a cenar tortilla de patatas o trucha arcoíris al horno. Además, descubrí el “Catholic Center”, que dirigían los padres franciscanos Tom y David. Lo vivido aquí fue algo extraordinario, desde el punto de vista humano (aparte de la mejora de mi vida social, principalmente por Alex Brown) me incorporé de tutor para ayudar a niños mejicanos con sus deberes en el Oasis Tutor bajo la dirección de la hermana Margarita (española por cierto), que me preparó como futuro padre para educar con cariño. Desde el espiritual, trabajé la parte de la libertad responsable del cristiano con el libro “Crossing the Dessert” y las reuniones de “Thursday Night Theology (TNT)”. La semana de mi despedida, Alex, Drew, Michael, Devin, Robin y muchos más me invitaron a una bolera y a beber cerveza Terrapin en la propia fábrica. El día antes de volver (que fue

domingo), el padre Tom me hizo decir unas palabras de despedida a la comunidad cristiana en el atril, al final de la misa.

La vuelta a casa, fue muy especial, porque a los 13 días de llegar nació mi hija Ana. Ni que decir tiene que tenía muchas ganas de volver a ver a mi mujer Ana y mis padres Julián y M^a Isabel. Los cuales pudieron descansar de mí cuatro meses, porque reconozco que les ha tocado aguantar mis preocupaciones y agobios por la “dichosa tesis”. Ellos han contribuido también a su manera, escuchándome y ayudándome de la mejor manera (entreteniéndome lo menos posible y diciéndome “descansa”). Respecto a los descansos, me ha ayuda mucho Sierra Espuña, lugar donde he encontrado un espacio ideal para hacer oración ignaciana que me han enseñado los Jesuitas en el centro Loyola. Oración donde, en general, he encontrado paz interior al discernir sobre mis inquietudes personales y también sobre los ensayos de investigación.

La vuelta al trabajo, fue muy revolucionaria, pues Sebas apostó por las cosas que le conté que había aprendido y confió en mí para que las pusiera en marcha. Fruto de esto, son los capítulos V y VI de esta tesis. Además siempre ha contado conmigo para los congresos, donde hemos tenido momentos de ciencia y de ocio (los más). Y en estos congresos recuerdo grandes momentos, especialmente el de Sardinia y el taxi para “chincue” con Mayra Aguado, José Antonio Franco, Sebastián y María José Vicente.

Por último sólo me queda comentar tres cosas: a) los “almuerzos” con los profesores, donde me tenía que esforzar para pagar alguna vez (siempre me querían invitar). Todos ellos me han tratado con cariño y quiero mencionar a aquellos a los que aún no lo he hecho: Encarna Conesa, Josefina Contreras, Pablo Bielza, Juan Antonio Fernández, Alejandro Pérez, Rafael Domingo, Juan Antonio Martínez. b) Las comidas compartidas, pero especialmente las de la finca y la fiesta de San Isidro Labrador donde el personal de la finca, los becarios y las becarias hemos reforzado nuestra amistad. Aquí mencionaré a personas que no han trabajado directamente conmigo pero que forman parte de este apartado: Diana, Adriana, Almudena, Conchi y Carolina (amiga de autobús). c) Dedicar un abrazo a mi hija Isabel, que nació el día 10 de mayo, justo después de realizar la solicitud de depósito para la Tesis.

Abstract/Resumen

ABSTRACT

The availability of irrigation water is increasingly limited compared to a nursery sector that crops many varieties of ornamental plants with different water requirements. In this situation, the crop of ornamental shrubs has to reduce water consumption without losing quality. The aim of this thesis is to study the application of four nursery techniques that can reduce water consumption, and the crop optimization of *Rhamnus alaternus* (native Mediterranean shrub with little water requirements) with different levels of shading. The tests conducted were: 1) The study of the "pot-in-pot (PIP), which is a holder or socket pot permanently placed in the ground with the top rim remaining above and a pot-grown plant that is placed within the holder pot. The container-grown plant is then placed within the holder pot for the production cycle. We measured substrate matric potentials, substrate temperature, water relations, and the development of *Myrtus communis* grown in PIP and "above-ground-pot" (AGP). 2) The use of dendrometers in shrubs of small diameter. Dendrometers were set up in three shrubs with ornamental interest and were subjected to water stress treatments. The dendrometer measures linear changes in the stem diameter, which were related to leaf water potential and evapotranspiration. 3) The effect of five levels of shading on *R. alaternus* and its changes on growth and plant development, aesthetic, water relations and consumption. 4) The possibility of using the system Acclima (automated irrigation system based on a soil moisture sensor TDT) to produce potted plant (soilless crop) and its suitability for researching projects. *Begonias semperflorens* were studied at six levels of substrate volumetric water content (θ) controlled by the system. 5) The irrigation with saline water on *Euonymus japonicus* and the possibility of the system PIP to increase its salinity tolerance. We evaluated growth, water consumption, aesthetic, water relations and ions accumulation.

The results showed the following: 1) The PIP system reduces the consumption of *M. communis* and improved its tolerance to water stress due to the lower extreme temperatures in the root, which reduced the ratio shoot dry weight/root dry weight (S/R). 2) The use of dendrometer allows the detection of stress in the shrubs of small diameter, but was less sensitive compared to the leaf water potential, which limits their use to situations where moderate stress is not a problem (hardening or growth control). 3) *Rhamnus alaternus* is a shrub with plasticity to different light levels, but its aesthetic value is compromised with levels above 84%, so that 25% and 50% of shading is recommended in spring and summer, respectively. 4) The Acclima system allows precise control of θ , which allowed us to assess that *B. semperflores* growth increases linearly up to a moisture level of $0,348 \text{ m}^3 \cdot \text{m}^{-3}$, above

which a greater consumption did not increase its growth. 5) Irrigation with saline water damages the aesthetic and growth of *Euonymus japonicus*, however, the PIP reduces these damages because produces a lower consumption which increased the leachate and reduced the pore water electrical conductivity. In addition, less extreme temperatures in the root favored better root development, which reduced its S/R.

In conclusion, the use of the techniques studied, would allow a reduction in water consumption and the optimization of *R. alaternus* production by light management. In addition, the dendrometer allows estimating the plant water status and the PIP system increases the salinity tolerance.

RESUMEN

La disponibilidad de agua para riego es cada vez más limitada frente a un sector viverístico que produce numerosas variedades de plantas ornamentales con requerimientos hídricos diferentes. Ante esta situación, la producción de arbustos ornamentales tiene que reducir el consumo de agua sin perder la calidad del producto. El objetivo de la tesis, es estudiar la aplicación de cuatro técnicas viverísticas susceptibles de reducir el consumo hídrico, y la optimización de la producción de *Rhamnus alaternus* (arbusto mediterráneo autóctono con pocos requerimientos hídricos) mediante distintos niveles de sombreado. Los ensayos realizados fueron los siguientes: 1) El estudio del sistema “pot-in-pot” (PIP), que consiste en introducir la maceta de cultivo en otra enterrada en el suelo donde encaja. Se evaluaron los potenciales mátricos y la temperatura del sustrato, las relaciones hídricas, y el desarrollo de *Myrtus communis* producido en PIP y en maceta sobre suelo, sistema “above-ground-pot” (AGP). 2) El uso de dendrómetros en arbustos de diámetro pequeño. Se instalaron dendrómetros en tres arbustos con interés ornamental y se sometieron a tratamientos de estrés. El dendrómetro mide los cambios diametrales y éstos se relacionaron con el potencial hídrico y la evapotranspiración de los arbustos. 3) El efecto de cinco niveles de sombreado sobre *R. alaternus* y sus efectos en el crecimiento y desarrollo de la planta, estética, relaciones hídricas y consumo. 4) La posibilidad de usar el sistema Acclima (sistema de riego automatizado basado en un sensor de humedad del suelo TDT) para producir planta en maceta (cultivo sin suelo) y para realizar investigación. Se estudiaron *Begonias semperflorens* en seis niveles de humedad del suelo (θ) controlados por el sistema. 5) El riego con aguas salinas sobre *Euonymus japonicus* y la posibilidad del sistema PIP de aumentar su tolerancia a la salinidad. Se evaluaron crecimiento, consumo, estética, relaciones hídricas y acumulación de iones.

Los resultados mostraron lo siguiente: 1) el sistema PIP reduce el consumo de *M. communis* y mejora su tolerancia al estrés hídrico por las menores temperaturas extremas sufridas en la raíz, que redujeron el ratio peso seco aéreo/peso seco radical (ratio PSA/PSR). 2) El uso del dendrómetro permite la detección del estrés en los arbustos de diámetro pequeño, pero es menos sensible que el potencial hídrico, lo que limita su uso a situaciones donde un estrés moderado no sea un problema (situaciones de endurecimiento o de reducción del crecimiento). 3) *Rhamnus alaternus* es un arbusto con plasticidad a distintos niveles de luz, pero su estética se deteriora con niveles superiores al 84%, mientras que se mejora con sombreados del 25% y el 50% en primavera y verano respectivamente. 4) El sistema Acclima permite un control preciso del θ , lo que nos permitió verificar que *B. semperflores* aumenta su

crecimiento de forma linear hasta un nivel de humedad de $0.348 \text{ m}^3 \cdot \text{m}^{-3}$, por encima del cual un mayor consumo no lo incrementa. 5) El riego con aguas salinas, daña la estética y el crecimiento de *Euonymus japonicus*, sin embargo, el PIP reduce estos daños porque produjo un menor consumo que incrementó el drenaje y redujo la conductividad eléctrica de los poros del sustrato. Además, las temperaturas menos extremas en la raíz favorecieron el mejor desarrollo radical, lo que redujo su ratio PSA/PSR.

En conclusión, el uso de las técnicas estudiadas, permitirían una reducción del consumo de agua y la optimización de la producción de *R. alaternus* mediante el manejo de la luz. Además, el dendrómetro permite una estimación del estado hídrico de la planta y el sistema PIP aumenta la tolerancia a la salinidad.

INDEX

INDEX	17
TABLE INDEX	22
FIGURE INDEX	24
PICUTRE INDEX	27
 BACKGROUND AND OBJECTIVES.....	 29
1. Pot-in-Pot	31
2. Sensors	32
2.1. Dendrometers	32
2.2. Soil moisture sensors	33
3. Light management	35
4. Salinity	36
References	38
 Chapter 1: Above-ground and pot-in-pot production systems for <i>Myrtus communis</i> L.	 45
Abstract	46
1. Introduction.....	46
2. Materail and methods.....	48
2.1. Plant material and growing conditions.....	48
2.2. Experimental design and statistical analysis.....	49
2.3. Growth and development.....	51
2.4. Soil matric potential (SMP) and temperature.....	51
2.5. Leaf water potential and gas exchange.....	51
3. Results and Discussion.....	52
3.1. SMP and plant water relations.....	52
3.2. Growth and development.....	64
4. Conclusion.....	69
Acknowledgements.....	66
References.....	70

Chapter 2: Comparison of stem diameter variations in three small ornamental shrubs under water stress.....75

Abstract.....	76
1. Introduction.....	76
2. Materials and methods.....	78
2.1. Plant material and growing conditions.....	78
2.2. Plant water relations.....	80
2.3. Determination of stem diameter variations.....	81
2.4. Evapotranspiration determination.....	82
2.5. Treatments, design and statistical analysis.....	82
3. Results.....	82
3.1. Study of the indices derived by the stem diameter variations (SDV).....	82
3.2. Evapotranspiration.....	86
3.3. Plant water relations.....	88
3.4. Regression analysis between daily plant ET and stem diameter indices.....	88
4. Discussion.....	92
5. Conclusion.....	95
Acknowledgements.....	95
References.....	96

Chapter 3: *Rhamnus alaternus* growth under four simulated shade environments; Morphological, anatomical and physiological responses.....101

Abstract.....	102
1. Introduction.....	102
2. Material and methods.....	104
2.1. Plant material and crop conditions.....	104
2.2. Measurements of DW, leaf area and growth indexes.....	108
2.3. Measurements of water evapotranspired, leaching and water use efficiency.....	108
2.4. Measurements of leaf color and relative chlorophyll content (RCC).....	108
2.5. Measurement of gas exchange and leaf water potential.....	109
2.6. Measurement of plant architecture and compactness index.....	109
2.7. Measurement of leaf cell layers in cross section.....	110
2.8. Measurement and extraction of soluble sugar contents.....	110
2.9. Stomatal density and pore size.....	111

2.10. Measurement of chlorophyll fluorescence.....	112
2.11. Statistical analysis.....	112
3. Results.....	112
3.1. Shoot evolution.....	112
3.2. Plant growth and leaf color.....	113
3.3. Leaf soluble sugars concentration.....	114
3.4. Leaf blade thickness and stomatal characteristics.....	115
3.5. Irrigation and leaching.....	117
3.6. Photosynthesis, stomatal conductance and leaf water potential.....	118
3.7. Photochemical efficiency and relative chlorophyll content (RCC).....	119
4. Discussion.....	120
5. Conclusion.....	124
Acknowledgements.....	124
References.....	124

Chapter 4: A calibrated TDT soil moisture sensor can be used for precise automated irrigation of container-grown Plants.....131

Abstract.....	132
1. Introduction.....	133
2. Material and methods.....	135
2.1. Sensor calibration.....	135
2.2. Plant material and crop conditions.....	135
2.3. Independent measurements of θ and EC.....	137
2.4. Measurements at the end of the experiment.....	138
2.5. Experimental Design and Statistical analysis.....	138
3. Results and discussion.....	138
3.1. Calibration of sensors.....	138
3.2. Substrate Water Content Control.....	140
3.3. Theta and Sigma probe correlations.....	141
3.4. Evapotranspiration and plant growth.....	143
3.5. Photosynthetic activity.....	145
4. Conclusion.....	147
Acknowledgements.....	147
References.....	148

Chapter 5: Pot-in-pot increases salt tolerance in *Euonymus japonicus*; Maintains plant growth and reduces water consumption.....151

Abstract.....	152
1. Introduction.....	152
2. Material and methods.....	154
2.1. Plant material and crop conditions.....	154
2.2. Experimental design.....	155
2.3. Irrigation water evapotranspired and water use efficiency.....	158
2.4. Gas exchange and chlorophyll fluorescence.....	158
2.5. Study of correlations.....	159
2.6. Statistical analysis.....	160
3. Results and discussion.....	160
3.1. Substrate water content and stomatal conductance.....	160
3.2. Water use.....	164
3.3. Dendrometry and photochemical study.....	168
4. Conclusions.....	170
Acknowledgements.....	170
References.....	171

Chapter 6: Pot-in-pot increases salt tolerance in *Euonymus japonicus*; Maintains aesthetic value and reduces chloride uptake.....175

Abstract.....	176
1. Introduction.....	176
2. Material and methods.....	178
2.1. Plant material and crop conditions.....	178
2.2. Experimental design.....	179
2.3. Water quality and substrate electrical conductivity.....	180
2.4. Measurements of architecture, anatomy and salt damage.....	180
2.5. Leaf water and osmotic potentials.....	181
2.6. Determination of inorganic ions.....	182
2.7. Statistical analysis.....	182
3. Results.....	182
3.1. Substrate temperature.....	182
3.2. Water quality, leaching and substrate.....	184

3.3. Growth, development and survival rate.....	186
3.4. Leaf potentials and ion concentrations.....	188
4. Discussion.....	190
5. Conclusion.....	193
Acknowledgements.....	194
References.....	194
General conclusions.....	197

TABLE INDEX

Chapter 1: Above-ground and pot-in-pot production systems for *Myrtus communis* L., 45

Table 1. Dry weight production after 12 months.....	68
--	----

Chapter 2: Comparison of stem diameter variations in three small ornamental shrubs under water stress.....75

Table 1. Regression analysis between daily ET and stem diameter indices during water stress and recovery periods.....	90
--	----

Table 2. Regression analysis between daily ET and stem diameter indices during irrigation periods.....	91
---	----

Chapter 3: *Rhamnus alaternus* growth under four simulated shade environments; Morphological, anatomical and physiological responses.....101

Table 1. Effect of shading on growth, development and leaf color.....	114
--	-----

Table 2. Effect of shading on leaf soluble sugar concentrations ($\text{mg}\cdot\text{g}^{-1}$ DW).....	115
---	-----

Table 3. Effect of shading on transversal leaf section dimensions (μm) and stomatal characteristics.....	116
--	-----

Table 4. Photochemical efficiency and relative chlorophyll content (RCC) study.....	120
--	-----

Chapter 4: A calibrated TDT soil moisture sensor can be used for precise automated irrigation of container-grown Plants.....131

Table 1. Effect of different volumetric water content (θ) threshold values for irrigation scheduling on stomatal conductance (gs), net photosynthesis (Pn), instantaneous water use efficiency (WUEi), area of the fully expanded leaves, and shoot dry weight (DW) of <i>Begonia semperflorens</i> after two months of irrigation using an Acclima CS3500 irrigation controller.....	145
---	-----

Chapter 5: Pot-in-pot increases salt tolerance in *Euonymus japonicus*; Maintains plant growth and reduces water consumption.....151

Table 1. Regression analysis between irrigated water evapotranspired (IWET) and both DLI and days after first irrigation (DAFI).....	165
---	-----

Chapter 6: Pot-in-pot increases salt tolerance in <i>Euonymus japonicus</i>; Maintains aesthetic value and reduces chloride uptake	175
Table 1. Level of significance from the two-way anova conducted to determine the effects of salinity (S), crop system (CS) and interaction (S x P) on the studied parameters	184
Table 2. Interactive effects of salinity and crop system on parameters studied	187
Table 3. Level of significance from the two-way anova conducted to determine the effects of salinity (S), crop system (CS) and interaction (S x CS) on the leaf potentials parameters	189
Table 4. Level of significance from the two-way anova conducted to determine the effects of salinity (S), crop system (CS) and interaction (S x P) on the ions concentration ($\text{mg}\cdot\text{g}^{-1}$ of DW)	189
Table 5. Interactive effects of salinity and crop system on ions concentration ($\text{mg}\cdot\text{g}^{-1}$ of DW)	190
Table 6. Ions concentration ($\text{mg}\cdot\text{g}^{-1}$ of DW) in AGP-s of healthy leaves and damaged leaves	190

FIGURE INDEX

Chapter 1: Above-ground and pot-in-pot production systems for *Myrtus communis* L., 45

Fig. 1. Pot-in-Pot general design.....	50
Fig. 2. Mean monthly temperature and SMP evolution in substrate of PIP and AGP treatments, and mean monthly environmental temperature and solar radiation.....	52
Fig. 3. Mean monthly maximum temperature and SMP evolution in substrate of PIP and AGP treatments, and mean monthly maximum environmental temperature and solar radiation.....	53
Fig. 4. Mean monthly minimum temperature and SMP evolution in substrate of PIP and AGP treatments, and mean monthly minimum environmental temperature and solar radiation.....	53
Fig. 5. Representative 31-day period for summer, showing daily maximum and minimum substrate temperature in PIP and AGP systems and daily minimum substrate SMP registers in PIP and AGP systems.....	55
Fig. 6. Representative 31-day period for autumn, showing daily maximum and minimum substrate temperature in PIP and AGP systems and daily minimum substrate SMP registers in PIP and AGP systems.....	56
Fig. 7. Representative 31-day period for winter, showing daily maximum and minimum substrate temperature in PIP and AGP systems and daily minimum substrate SMP registers in PIP and AGP systems.....	57
Fig. 8. Representative 31-day period for spring, showing daily maximum and minimum substrate temperature in PIP and AGP systems and daily minimum substrate SMP registers in PIP and AGP systems.....	58
Fig. 9. Mean stomatal conductance values in PIP and AGP.....	59
Fig. 10. Mean leaf water potential values in PIP and AGP.....	59
Fig. 11. Mean photosynthesis values in PIP and AGP.....	60
Fig. 12. Hourly environmental temperature, VPD, substrate temperature, and SMP evolution in PIP and AGP systems during a 10-day period in summer.....	62
Fig. 13. Hourly environmental temperature, VPD, substrate temperature, and SMP evolution in PIP and AGP systems during a 10-day period in winter.....	63
Fig. 14. Number of shoots evolution in PIP and AGP systems.....	65
Fig. 15. Crown area evolution in PIP and AGP systems.....	65
Fig. 16. Plant height evolution in PIP and AGP systems.....	66

Chapter 2: Comparison of stem diameter variations in three small ornamental shrubs under water stress	75
Fig. 1. Daily evolution during 25 days of MXDS, MNDS and MDS for winter, spring and summer for drought and recovery period.....	84
Fig. 2. Daily evolution during 31 days of SGR and MDS for drought to death in summer.....	85
Fig. 3. Figures A, B and C show daily plant ET evolution during 25 days of <i>R. alaternus</i> , <i>C. citrinus</i> and <i>P. tobira</i> for winter, spring and summer with drought and recovery period.....	86
Fig. 4. Figure A shows daily plant ET evolution during 31 days of <i>R. alaternus</i> , <i>C. citrinus</i> and <i>P. tobira</i> for drought to death in summer.....	87
Fig. 5. Leaf water potential evolution for drought and recovery periods winter (A), spring (B) and summer (C) and drought to death period (D).....	89
Fig. 6. Analysis of regression between daily ET-SGR (A), daily ET- MDS (B), Ψ_{hmd} -SGR (C) and Ψ_{hmd} -MDS (D) during drought to death period.....	92
 Chapter 3: <i>Rhamnus alaternus</i> growth under four simulated shade environments; Morphological, anatomical and physiological responses	101
Fig. 1. <i>R. alaternus</i> after pinching at 20 cm of height in 0% shading treatment.....	105
Fig. 2. Mean maximum daily photosynthetic photon flux density (PPFD) in the different shading treatments (from 1 August 2007 to 31 October 2008).....	106
Fig. 3. Seasonal evolution of average shoot length (those born from the main stem) (A), main stem diameter (B), number of shoots per plant (C) and plant height (D).....	113
Fig. 4. Average seasonal irrigation and leaching in the different shading treatments.....	118
Fig. 5. Seasonal evolution of (A) photosynthesis (P_n), (B) stomatal conductance (g_s) and (C) leaf water potential (Ψ_{hmd}) in the different shading treatments.....	119
 Chapter 4: A calibrated TDT soil moisture sensor can be used for precise automated irrigation of container-grown Plants	131
Fig. 1. The relationship between the substrate water content (θ) of a bark-based soilless substrate, as measured by TDT sensors using the factory calibration for mineral soil, and the θ determined based on wet and dry weight and volume of the substrate (volumetric θ).....	139
Fig. 2. Average volumetric water content (θ) of the substrate as measured by TDT sensors over a 66 d period, during which begonias were grown in 3.65 L pots.....	141

Fig. 3. The relationship between substrate volumetric water content (θ) measurements by time domain transmissometry (TDT) sensors (using our substrate-specific calibration) and a Theta probe.....	142
Fig. 4. The relationship between bulk electrical conductivity (EC) measurements collected with time domain transmissometry (TDT) sensors and the pore water EC (top) and bulk EC (bottom) as measured using a SigmaProbe.....	143
Fig. 5. Cumulative evapotranspiration from treatment initiation until the termination of the experiment (66 d), as a function of the substrate volumetric water content (θ) threshold at which begonias were irrigated.....	144
Fig. 6. Shoot dry weight of begonias as a function of total evapotranspiration since the start of the irrigation treatments.....	146
 Chapter 5: Pot-in-pot increases salt tolerance in <i>Euonymus japonicus</i>; Maintains plant growth and reduces water consumption	151
Fig. 1. Average volume water content (θ) of each treatment.....	161
Fig. 2. Daily light integral (DLI) and daily mean vapour pressure deficit (VPD) from 6 March 2010 to the 24 august 2010.....	162
Fig. 3. Photosystem II efficiency (ϵ PSII) (A), Stomatal conductance (gs) (B), and net photosynthesis (Pn) (C).....	163
Fig. 4. Volume of accumulated irrigation water (control and saline), accumulated irrigation water evapotranspired (IWET), and daily precipitation.....	164
Fig. 5. Accumulated stem growth diameter (A), Stem growth rate (SGR) (B) and maximum daily shrinkage (MDS) (C).....	167
Fig. 6. Correlation between maximum daily shrinkage (MDS) and volume water content (θ) during the period 12 July to 2 August.....	169
 Chapter 6: Pot-in-pot increases salt tolerance in <i>Euonymus japonicus</i>; Maintains aesthetic value and reduces chloride uptake	175
Fig. 1. Maximum daily (A), minimum daily (B) and average daily (C) temperatures in substrate of PIP and AGP treatments, and maximum daily (A), minimum daily (B) and mean daily (C) environmental temperature.....	183
Fig. 2. Weakly leachate pH (A) and leachate electrical conductivity (EC) (B) of saline irrigation (AGP-s and PIP-s) and control irrigation (AGP-c and PIP-c) treatments. C) Irrigation water pH and EC.....	185

PICTURE INDEX

Chapter 1: Above-ground and pot-in-pot production systems for *Myrtus communis* L., 45

Picture 1. Experimental plot, pot-in-pot (PIP) and above ground pot (AGP) and datalogger in the center of the plot.....50

Picture 2. Above-ground-pot (AGP) and pot-in-pot (PIP) myrtle plants after one year of experimentation67

Picture 3. Experimental plot after two weeks without irrigation at the end of the experiment69

Chapter 2: Comparison of stem diameter variations in three small ornamental shrubs under water stress.....75

Picture 1. Detail of dendrometer and balance installed at *C. citrinus*.....81

Chapter 3: *Rhamnus alaternus* growth under four simulated shade environments; Morphological, anatomical and physiological responses.....101

Picture 1. Experimental table design, 0% of shading (front) and 32% of shading (behind).....107

Picture 2. Representative plants after nearly one year of experimentation. Left *R. alaternus* represents 0% of shading, right *R. alaternus* with 93% of shading.....115

Picture 3. Images obtained with optical microscopy. Cell layers thickness of treatments 0% of shading (left) and 93% of shading (right).....116

Picture 4. Images obtained with optical microscopy. Cuticle thickness of treatments 0% of shading (left) and 93% of shading (right).....117

Picture 5. Images obtained with electron microscopy. Stomatal density of treatments with 0% shading (left) and 93% of shading (right).....117

Chapter 4: A calibrated TDT soil moisture sensor can be used for precise automated irrigation of container-grown Plants.....131

Picture 1. Acclima CS3500 irrigation controller (left) and TDT-sensor (right).....135

Picture 2. Experimental plot. The Acclima CS3500 irrigation controller, Begonias, solenoid valves, flow meters and TDT-sensor can be observed.....137

Chapter 5: Pot-in-pot increases salt tolerance in <i>Euonymus japonicus</i>; Maintains plant growth and reduces water consumption	151
Picture 1. Experimental plot at the end of the experiment	155
Picture 2. Detail of plant with dendrometer and soil moisture sensor (only the wire is visible)	157
Picture 3. Detail of bags for leaching collection. AGP (left) with the mesh structure to support the plant, PIP (right) with the bag inside the space between pots	159
 Chapter 6: Pot-in-pot increases salt tolerance in <i>Euonymus japonicus</i>; Maintains aesthetic value and reduces chloride uptake	 175
Picture 1. From left to right, above ground pot with saline irrigation (AGP-s), pot-in-pot with saline irrigation (PIP-s), above ground pot with control irrigation (AGP-c), pot-in-pot with control irrigation (PIP-c)	187
Picture 2. Differences between AGP (left) and PIP (right) with saline irrigation	188
Picture 3. Leaf damage by salt in above ground pot and pot-in-pot under saline irrigation (AGP-s up and PIP-s down)	188

Background and Objectives

This doctoral thesis was written within the context of the nursery production of ornamental plants produced in pots. The aim of this work was to provide the tools and knowledge to face some of the threats faced by the industry and to correct some of its weaknesses. To carry out this objective, we investigated the adaptability of some techniques not fully exploited yet and attempted to enhance some of the strengths the sector already has.

At present, the main threat that the ornamental nursery is facing is the scarcity of good quality water. Population growth and increased urbanization have increased intensive agriculture and competition for water (Jury and Vaux, 2005). Furthermore, the ornamentals industry will also need to comply with existing and new laws, regulating the environmental impact of production (Fernández et al., 2009). This threat should be added to the greatest weakness of the sector, which is the unknown real water needs of many ornamental crops by growers, who usually make irrigation decisions based on visual appearance of the substrate or plants or by simply using timers (Nemali and van Iersel, 2006). The result is often excessive irrigation, resulting in leaching and runoff of water and fertilizer, which may cause eutrophication and algal blooms (Majsztrik et al., 2011) as well as CO₂ emissions through excessive energy use in water pumping. On the other hand, the sector has strengths, which have been developed over the years, such as the efforts to improve irrigation-fertilization systems (drip irrigation and sprinkler) using modern programmers and the production of high quality ornamental plants. Besides, there are the favorable growing conditions of the Mediterranean coastal region, with high solar radiation (Rubio et al., 2010). Amidst all this, there are new opportunities for the sector to be exploited, such as the new technologies (soil moisture sensors, dendrometers, PAR sensors, etc.), which allow plant water needs to be evaluated, thus correcting one weakness of the sector. As well as this, there is increasing interest in agricultural sustainability on the part of both consumers and governments, which may well result in market opportunities for ornamental crops (Dennis et al., 2010) with the “sustainable” label.

This thesis focuses on: a) system of pot-in-pot (PIP) culture, b) the use of soil moisture sensors and dendrometers, c) solar light management, and d) irrigation with saline water and its interaction with the PIP system. The potential use of these systems and technologies in reducing plant water consumption and improving plant quality was assessed. A general description of previous studies of these points follows.

1.-Pot-in-Pot

Pot-in-pot (PIP) production, introduced around 1990 in the USA (Parkerson, 1990), is a nursery production method that 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). Previous research into PIP compared with above ground potting (AGP) determined that PIP improves biomass production (Ruter, 1998a), reduces root zone temperature stress (Young and Bachman, 1996) and enhances efficient water use by decreasing container evapotranspiration (Martin et al., 1999). An additional advantage over AGP is the elimination of extensive staking and blowover and a more easy mechanization. Recent studies by Neal (2010) reported that crabapple and lilac root or shoot mass were greater in PIP compared with another four production systems: field-grown, plastic container, bag-in-pot and above ground system.

The disadvantages of the PIP system include high initial cost of pots and installation, potential drainage problems of socket pots in poorly drained soils, and the possibility of root elongation into the socket pot and surrounding soil. In PIP production, containers may stick together; the bottom of the insert pot may sag, causing an uneven base; and there is little or no spacing flexibility once the socket pots are established (Adrian et al., 1998). So, careful planning of the layout should be undertaken because of the large initial outlay for production and the cost of changing the system (Tilt et al., 1994). The economic analysis reported by Adrian et al. (1998) concluded that the PIP system had the highest total capital outlay and fixed cost compared with AGP, which related primarily to higher costs associated with purchasing and installing socket pots. The PIP system, however, was least costly on a per harvested plant basis due to less intensive, labor-saving cultural practices and the ability to grow larger plants quickly.

In chapter I the benefits of this system in the development of ornamental shrubs grown in little pots (about 2.5 L) was assessed. The differences in substrate temperature and soil matric potential (estimated with watermark sensors) were investigated in native *Myrtus communis* L. cultivated in PIP and AGP. The way each system affected growth and development, water status and gas exchange parameters was also studied.

2. Sensors

The use of sensors is crucial fact for assessing the plant water status, so that irrigation decisions can be made in order to give the plant the water it really needs. In this case, we have mainly worked with dendrometers and soil moisture sensors.

2.1. Dendrometers

Several studies have suggested that one of the most sensitive water indicators is plant growth and, more specifically, trunk diameter growth. One of the consequences of this has been the proposed use of the dendrometer, an electronic sensor which measures the stem diameter variations (SDV) originated by water changes in the trunk tissues (Molz and Klepper, 1973). The high frequency measurements obtained with this sensor usually point to a maximum diameter value just before sunrise (MXDS) and a minimum diameter value at sometime during the afternoon (MNDS), the difference between them being the maximum daily shrinkage (MDS). The stem growth rate (SGR) is calculated as the difference between two consecutive MXDS.

The dendrometer is generally a linear variable differential transducer (LVDT) which provides precise, accurate and stable measurements under field conditions. The LVDT sensors are attached to the trunk by a special bracket made of invar (FeNi36), an alloy with a thermal expansion coefficient close to zero, and aluminum (Zweifel et al., 2000). The linear variable differential transducer is a sensor that converts a linear displacement of position from a mechanical reference (or zero) into a proportional electrical signal containing phase (for direction) and amplitude (for distance) information. Brackets can be made to measure SDV of very different trunk sizes, from small stems typical of herbaceous crops (Gallardo et al., 2006a,b) up to very large trunk sizes as in mature olive trees (Moreno et al., 2006). In addition SDV measurements can also be performed with a strain gauge type of sensor (Morandi et al., 2007), a device based on a linear potentiometer, which is cheaper than the LVDT.

It has been suggested that the MDS and the SGR are key indicators of the plant water status depending on the species, and numerous works have studied the relationship between dendrometric indices and plant water status indicators (the leaf and stem water potential, gas exchange, etc.) and environmental variables (vapor pressure deficit, PAR, temperature, evapotranspiration, etc.) as reported by Ortuño et al. (2010) and Fernández and Cuevas (2010)

in their revisions. However, this research line has focused on fruit trees and some horticultural crops (pepper and tomato). So, its potential use in low diameter ornamental crops has not been studied yet. However, it is a promising tool for evaluating plant water status for growers. Furthermore, it is quite easy to use compared with other instruments (the pressure chamber, gas exchange systems, etc.) but more appropriate for research than for nursery production.

The objective was to assess whether the dendrometric indices could be used as plant water status indicator in well irrigated and water stress conditions in three ornamental shrubs grown in pots. The species used were Mediterranean shrubs of ornamental and landscaping interest, one of them native (*Rhamnus alaternus* L.), one representative of Australian flora (*Callistemon citrinus* [Curtis]. Skeels.cv. Splendens) and the other a very popular commercial shrub from China and Japan (*Pittosporum tobira* [Thunb.] Ait. cv. Nana). The present research is developed in chapter II.

2.2. Soil moisture sensors

The measurement of the soil water content (θ) by means of *in situ* dielectric methods is increasingly used because they are non-destructive, provide almost instantaneous measurements, require little or no maintenance, can remain in the soil during the winter time and can provide continuous readings; they are non-radioactive, and accurate measurements may be made near the soil surface, while their cost has decreased substantially in recent years (Cardenas-Lailhacar and Dukes, 2010). Some of the techniques based on dielectric methods have been classified as time domain reflectometry (TDR), time domain transmissometry (TDT), and frequency domain reflectometry (FDR). Most of these instruments operate by sending an electromagnetic signal to a probe buried in the soil. When the signal is reflected back (TDR), reaches the end of the loop-probe (TDT), or changes its frequency (FDR). The signal is then detected and analyzed. The time taken for the signal (TDT and TDR) or the frequency of the reflected wave (FDR) varies with the soil dielectric properties, which are mainly governed by the water content of the soil surrounding the probes (Topp, 2003; Blonquist et al., 2005a). More in depth estimations of the water content using these electromagnetic sensors are based on the ability of sensors to measure the ‘real’ part of the dielectric permittivity (ϵ), or property of an electromagnetic signal directly relating to ϵ , which directly relates to θ owing to the ϵ contrast of soil constituents; $\epsilon_a \sim 1$, $\epsilon_s \sim 2-9$ and $\epsilon_w \sim 80$; where the subscripts a, s and w represent air, solids and water, respectively (Blonquist et al., 2006). Other substrate-related factors like electrical conductivity, temperature and bulk

density can cause dielectric loss and affect the permittivity of a substrate (called ‘imaginary permittivity’). The effects of imaginary permittivity on ‘real’ permittivity measurement accuracy is to a larger extent dependent on measurement frequency of the sensor, with higher frequency broadband sensing systems generally yielding better measurements (Blonquist et al., 2005b).

Granular matrix sensors (GMSs) such as watermark, have been used for many years to estimate θ . The GMS is a device that measures soil electrical resistance, which is converted to calibrated readings of soil water potential (Ψ_{soil}). The GMS has two electrodes embedded in a granular matrix material, which is generally surrounded by a protective synthetic membrane and held in a stainless case. This device is buried in intimate contact with soil, and reaches equilibrium with the soil water content. Since the development of the GMS, many researchers have used it in irrigation scheduling. However, in coarse textured soils (e.g. sand) reduced soil/sensor contact may lead to incorrect estimation of the soil water tension (Irmak and Haman, 2001). In addition, GMSs exhibit hysteretic behavior (Thompson et al., 2006) and a high variability of readings (Taber et al., 2002; Intrigliolo and Castel, 2004), so individual sensors should be calibrated for accurate readings (Leib et al., 2003). However, they can be appropriate when a relative indication of soil wetness is sufficient, as indicated by reports of their successful use for monitoring θ in urban tree-landscapes (Connellan et al., 2000), and for irrigation scheduling in onion (Shock et al., 1998a), potato (Shock et al., 1998b), tomato and walnut trees (Hanson et al., 2000).

At present, modern commercially available soil moisture sensors systems include not only a sensor to be buried in the soil but also a controller to interface with the irrigation timer. The controller is a milestone in the development of the soil moisture sensor industry because it sends a signal to the buried sensor, interprets the signal behavior, and converts it to a “sensed” soil water content. At the same time, the controller acts as a switch that allows the operator to choose a desired soil water content threshold (θ_{Th}), above which scheduled irrigation events would not be allowed. Typically, the adjustable θ_{Th} can be set between relatively dry to relatively wet soil moisture conditions; depending on the plant material, soil type, installation depth of the sensor, etc.

Chapter IV of this thesis focuses on assessing the accuracy of the Acclima CS3500 irrigation controller, a commercially available TDT soil moisture sensor with irrigation controller. This system was tested with *Begonia semperflorens* L. grown in pots, and its data was compared with a well-known automated irrigation system developed by Nemali and van Iersel (2006) which can maintain substrate θ at specific thresholds. This irrigation system

determines θ with capacitance sensors (FDR) connected to a datalogger (basic knowledge of programming and wiring dataloggers is required, which may be a limitation for growers).

3. Light management

The growth and productivity of a species are affected by changes in canopy light interception as a result of differences in carbon assimilation by the whole canopy (Cannell et al., 1987). The plant ability to acclimate to different light environments is determined by the genetic adaptation of a given species to the light environment of its native habitat (Boardman, 1977; Pearcy, 1998). This adaptation ability include alterations both at leaf level, associated with morphological, anatomical and physiological characteristics and at the whole-plant level, mainly related to shoot architecture and biomass allocation patterns (Givnish, 1988; Muraoka et al., 1997; Niinemets, 1997a; Valladares and Pearcy, 1998). Comparative studies on leaf characteristics of plants grown in high and low light can provide insights into leaf acclimation to sun and shade environments, through adjustments that enable the greatest possible efficiency of radiant energy use (Givnish, 1988). While plants in shade usually present an increased assimilate investment in leaf area to improve light interception (Pearcy and Sims, 1994; Niinemets et al., 1998) and an increase in leaf longevity (Nilsen et al., 1987; Reich et al., 1992), sun leaves are comparatively thicker, with a high leaf dry mass per area, reflected in a more efficient quantum utilization and so a high photosynthetic capacity per unit leaf area (Wayne and Bazzaz, 1993; Niinemets and Tenhunen, 1997). Adaptation to irradiance also involves changes in foliar chemistry. The content of foliar non-structural carbohydrates tends to relate positively with irradiance (Niinemets 1995, 1997b; Johnson et al., 1997), reflecting increased daily photosynthetic production, due in part to the high stomatal conductance that enables greater carbon acquisition. However, in a comparison of sun and shade leaves of evergreen sclerophylls, Meletiyou-Christou et al. (1994) did not find any significant differences in the soluble sugars concentration and even observed in some species a higher starch content in the leaves at low light.

Rhizopoulou et al. (1991), studying leaves from the same canopy in evergreen sclerophylls, observed that sun and shade leaves developed different mechanisms to withstand microclimatic conditions. Sun leaves, subject to great evaporation, usually follow an avoidance strategy by reducing water loss through a decrease in leaf size and stomatal conductance and an increase in leaf thickness. In contrast, shade leaves develop low solute

potentials to maintain turgor (Rhizopoulou et al., 1991). Also, Niinemets et al. (1999) reported that stomata may exert greater control over water use with increasing irradiance in the canopy. There is also evidence of these adjustments in the foliage chemical and morphological structure in response to water stress (Niinemets and Kull, 1998). These authors observed a higher ability for osmotic adjustment in the upper canopy leaves of *Populus tremula* L. and *Tilia cordata* Mill., provided by the increase of carbohydrates.

It is therefore necessary to know the shade plasticity of any species for both nursery production and reforestation success because, as reported by Villar-Salvador (2004), low quality plants might exhibit morphological and physiological characteristics that impair their performance under stressful conditions. Appropriate conditioning techniques used in the nursery during seedling production are crucial for the establishment, survival and subsequent growth of plants after transplanting (Franco et al., 2006).

Chapter III of this thesis focuses on the light plasticity of *Rhamnus alaternus* L., an important understory species of the Mediterranean area with a high ornamental value because of its bright green leathery foliage, which, with its small red fruits, creates a very pleasing effect. Its potential use in gardening and the best light levels for its nursery production was assessed.

4. Salinity

Of nearly 160 million hectares of cultivated land under irrigation worldwide, about one-third is already affected by salt (Singh and Chatrath, 2001). In most coastal or semiarid areas this problem is accentuated by competition for high quality water for urban use. Thus marginal quality water, somewhat saline, will become important in these areas (Chartzoulakis et al., 2002), and it would be desirable to irrigate plants with saline water or to blend saline with high quality water, as well as to grow selected salt-tolerant crops (Flowers et al., 2005) and/or use systems which improve salinity tolerance of species.

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), on the level of water salinity, and on the characteristics of the water itself. In general, under salinity, plants have smaller and fewer leaves, shorter stature and, generally, show a reduction in the length and mass of roots (Shannon and Grieve, 1999). These phenomena occur due to the disturbance that high salt concentrations cause to, among other things, membrane permeability, water

channel activity, stomatal conductance, photosynthesis and ion balance (Sonneveldt, 1988; Carvajal et al., 1999; Navarro et al., 2003; Cabañero et al., 2004).

Depending upon the composition of the saline solution, ion toxicities or nutritional deficiencies may arise because of competition between cations or anions (Shannon and Grieve, 1999). When Na^+ and Cl^- are present in the rhizosphere, they can disrupt the uptake of nutrients directly by interfering with transporters, such as those for K^+ and NO_3^- in the root plasma membrane (Tester and Davenport, 2003) or, if accumulated and not compartmentalised in vacuoles, can be metabolically toxic, resulting in “scorched” or “burned” leaves (Shannon and Grieve, 1999). Chloride toxicity manifests itself as slight bronzing and leaf-tip yellowing followed by tip death and general necrosis, whereas Na^+ toxicity starts as a marginal yellowing followed by a progressive necrosis (Ferguson and Grattan, 2005). In addition high external salt concentrations can inhibit root growth by osmotic effects (Wild, 1988).

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 depends on species, climatic conditions, type of substrate or soil, and irrigation method. Although salinity levels of irrigation water were kept constant, root zone salinity increased with time, especially when peat-based substrate or clay soil was used (Miyamoto et al., 2005; Niu and Rodriguez, 2006a,b). Salt accumulation in the root zone is affected by the physical and chemical properties of the substrate, plant size, and environmental conditions because these factors influence the substrate moisture content and cation exchange capacity in the root zone (Niu et al., 2010). Plants were more susceptible to salinity stress under sprinkler irrigation than drip irrigation as a result of direct contact with salts (Wu et al., 2001). Foliar salt injuries of a number of landscape plants irrigated with saline water were most affected during the hottest and driest periods of summer (Fox et al., 2005; Niu et al., 2007).

According to species tolerance, halophytes are able to complete their life cycle in 200mM NaCl or more (Flowers and Colmer, 2008), while sensitive glycophytes are injured by one tenth these salt concentrations. In many glycophytes, tolerance is correlated with an ability to limit uptake and/or transport of Na^+ and Cl^- to aerial parts, these ions being retained in the root and lower stem (Maathuis and Amtmann, 1999; Murillo-Amador et al., 2006). For woody perennials (species like citrus and grapevines), Cl^- is more problematic than Na^+ which usually tends to be sequestered in roots and woody tissue (Ferguson and Grattan, 2005); for other plants, like grasses, Na^+ is the primary cause of ion specific damage (Tester and Davenport, 2003).

Another key determinant of salt tolerance is likely to be the capacity of plants to maintain a high K^+/Na^+ ratio in their tissues (Maathuis and Amtmann, 1999; Munns and James, 2003), which is liable to be reduced because of the competitive effect of Na^+ concentration in the rhizosphere on K^+ uptake (Carvajal et al., 1999; Del-Amor et al., 2001; Aktas et al., 2006). Munns and Tester (2008) highlighted the fact that species differ in their ability to retain Na^+ in lignified roots and stems, while Cl^- is not retained and continues to be transported to the leaves. Ornamental shrubs might be expected to contain more woody tissues than annual species and so tolerance to external NaCl might be more closely related to leaf Cl^- concentrations than to those of Na^+ .

Chapters V and VI of this thesis focus on the effects of salinity on a commercial ornamental plant *Euonymus japonicus* Thunb. cv. Marieke, and its possible interaction with the PIP system (different environmental conditions in the rhizosphere) compared with AGP. For this study, a similar automated irrigation system described by Nemali and van Iersel (2006) was installed. The possible increase in salinity tolerance with this system was assessed.

References

- Adrian, J.L., C.C. Montgomery, B.K. Behe, P.A. Duffy, and K.M. Tilt. 1998. Cost comparisons of infield, above ground container and pot-in-pot production systems. *J. Environ. Hort.* 16:65-68.
- Aktas, H., K. Abak, and I. Cakmak. 2006. Genotypic variation in the response of pepper to salinity. *Sci. Hort.* 110:260-266.
- 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.
- Blonquist J.M., S.B. Jones, and D.A. Robinson. 2005a. A time domain transmission sensor with TDR performance characteristics. *J. Hydrol.* 314:235-245.
- Blonquist J.M. S.B. Jones, and D.A. Robinson. 2005b. Standardizing characterization of electromagnetic water content sensors: Part 2 Evaluation of seven sensing systems. *Vadose Zone J.* 4:1059-1069.
- Blonquist Jr, J.M., S.B. Jones, and D.A. Robinson. 2006. Precise irrigation scheduling for turfgrass using a subsurface electromagnetic soil moisture sensor. *Agr. Water Mgt.* 84:153-165.

- Boardman, N.K. 1977. Comparative photosynthesis of sun and shade plants. *Annu. Rev. Plant Physiol.* 28:355-377.
- Cabañero, F.J., V. Martínez, and M. Carvajal. 2004. Does calcium determine water uptake under saline conditions in pepper plants, or is it water flux which determines calcium uptake? *Plant Sci.* 166:443-450.
- Cannell, M.G.R., R. Milne, L.J. Sheppard, and M.H. Unsworth. 1987. Radiation interception and productivity of willow. *J. Appl. Ecol.* 24:261-278.
- Cardenas-Lailhacar, B., and M.D. Dukes. 2010. Precision soil moisture sensor irrigation controllers under field conditions. *Agr. Water Mgt.* 97:666-672.
- Carvajal, M., V. Martínez, and A. Cerdá. 1999. Influence of magnesium and salinity on tomato plants grown in hydroponic culture. *J. Plant Nutr.* 22:177-190.
- Chartzoulakis, K., M. Loupassaki, M. Bertaki, and I. Androulakis. 2002. Effects of NaCl salinity on growth, ion content and CO₂ assimilation rate of six olive cultivars. *Sci. Hort.* 96:235-247.
- Connellan, G.C., P.B. May, and K.R. James. 2000. Developing irrigation management strategies for urban trees using soil moisture sensing technology. In: American Society of Agricultural Engineers (Eds.), National Irrigation Symposium, Proceedings of the 4th Decennial Conference. ISBN 1-892769r-r3-1, pp. 456-461.
- Del-Amor, F.M., V. Martínez, and A. Cerdá. 2001. Salt tolerance of tomato plants as affected by stages of plant development. *HortScience* 36:1260-1263.
- Dennis, J. L., R.G. Lopez, B.K. Behe, C.R. Hall, C. Yue, and B.L. Campbell. 2010. Sustainable production practices adopted by greenhouse and nursery plant growers. *HortScience* 45:1232-1237.
- Ferguson, L. and S.R. Grattan. 2005. How salinity damages citrus: osmotic effects and specific ion toxicities. *HortTechnology* 15:95-99.
- Fernández, J.E. and M.V. Cuevas. 2010. Irrigation scheduling from stem diameter variations: A review. *Agric. Forest Meteorol.* 150:135-151.
- Fernandez, R.T., J. Lea-Cox, G. Zinati, C. Hong, R. Cabrera, D. Merhaut, J. Albano, M. van Iersel, T. Yeager and D. Buhler. 2009. NCDC216: A new multistate group for water management and quality for ornamental crop production and health. *Proc, SNA Res. Conf.* 54:35-38.
- Flowers, T.J. and T.D. Colmer. 2008. Salinity tolerance in halophytes. *New Phytol.* 179:945-963.

- Flowers, T.J., R. Ragab, N. Malash, G. Abdel-Gawad, J. Cuartero, and A. Arslan. 2005. Sustainable strategies for irrigation in salt-prone Mediterranean: SALTMED. *Agric. Water Mgt.* 78:3-14.
- Fox, L.J., J.N. Grose, B.L. Appleton, and S.J. Donohue. 2005. Evaluation of treated effluent as an irrigation source for landscape plants. *J. Environ. Hort.* 23:174-178.
- Franco, J. A., J.J. Martínez-Sánchez, J.A. Fernández, and S. Bañón. 2006. Selection and nursery production of ornamental plants for landscaping and xerogardening in semi-arid environments. *J. Hort. Sci. Biotech.* 81:3-17.
- Gallardo, M., R.B. Thompson, L.C. Valdez, and M.D. Fernández. 2006a. Response of stem diameter variations to water stress in greenhouse grown vegetable crops. *J. Hort. Sci. Biotech.* 81:483-495.
- Gallardo, M., R.B. Thompson, L.C. Valdez, and M.D. Fernández. 2006b. Use of stem diameter to detect plant water stress in tomato. *Irrig. Sci.* 24:241-255.
- Givnish, T.J. 1988. Adaptation to sun and shade: a wholeplant perspective. *Aust. J. Plant Physiol.* 15:63-92.
- Hanson, R.B., S. Orloff, and D. Peters. 2000. Monitoring soil moisture helps refine irrigation management. *Calif. Agric.* 54:38-42.
- Intrigliolo, D.S. and J.R. Castel. 2004. Continuous measurement of plant and soil water status for irrigation scheduling in plum. *Irrig. Sci.* 23:93-102.
- Irmak, S. and D.Z. Haman. 2001. Performance of the Watermark granular matrix sensor in sandy soils. *Appl. Eng. Agric.* 17:787-795.
- Johnson, J.D., R. Tognetti, M. Michelozzi, S. Pinzauti, G. Minotta, and M. Borghetti. 1997. Ecophysiological responses of *Fagus sylvatica* seedlings to changing light conditions. II. The interaction of light environment and soil fertility on seedling physiology. *Physiol. Plant.* 101:124-134.
- Jury, W.A. and H. Vaux. 2005. The role of science in solving the world's emerging water problems. *Proc. Nat. Acad. Sci.* 102:15715-15720.
- Leib, B.G., J.D. Jabro, and G.R. Matthews. 2003. Field evaluation and performance comparison of soil moisture sensors. *Soil Sci.* 168:396-408.
- Maathuis, F.J.M. and A. Amtmann. 1999. K^+ nutrition and Na^+ toxicity: the basis of cellular K^+/Na^+ ratios. *Ann. Bot.* 84:123-133.
- Majsztrik, J., A.G. Ristvey and J.D. Lea-Cox. 2011. Water and nutrient management in the production of container-grown ornamentals. *Hort. Rev.* 38:253-296.

- 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.
- Meletiyou-Christou, M.S., S. Rhizopoulou, and S. Diamantoglou. 1994. Seasonal changes of carbohydrates, lipids and nitrogen content in sun and shade leaves from four Mediterranean evergreen sclerophylls. *Environ. Exp. Bot.* 34:129-140.
- Miyamoto, S., A. Chacon, M. Hossain, and I. Martinez. 2005. Soil salinity of urban turf areas irrigated with saline water. I. Spatial variability. *Landsc. Urban Plan.* 71:233-241.
- Molz, F. J. and B. Klepper. 1973. On the mechanism of water-stress-induced stem deformation. *Agron. J.* 65:304-306.
- Morandi, B., L. Manfrini, M. Zibordi, M. Noferini, G. Fiori, and L.C. Grappadelli. 2007. A low-cost device for accurate and continuous measurement of fruit diameter. *Hortscience* 42:1380-1382.
- Moreno, F., W. Conejero, M.J. Martín-Palomo, I.F. Girón, and A. Torrecillas. 2006. Maximum daily trunk shrinkage reference values for irrigation scheduling in olive trees. *Agric. Water Mgt.* 84:290-294.
- Munns, R. and R.A. James. 2003. Screening methods for salinity tolerance: a case study with tetraploid wheat. *Plant Soil* 253:201-218.
- Munns, R., and M. Tester. 2008. Mechanisms of salinity tolerance. *Ann. Rev. Plant Physiol.* 59:651-681.
- Muraoka, H., Y. Tang, H. Kolzumi, and I. Washitani. 1997. Combined effects of light and water availability on photosynthesis and growth of *Arisaema heterophyllum* in the forest understory and an open site. *Oecologia* 112:26-34.
- 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, J.M., C. Garrido, V. Martínez, and M. Carvajal. 2003. Water relations and xylem transport of nutrients in pepper plants grown under two different salts stress regimes. *Plant Growth Regul.* 41:237-245.
- Neal, C.A. 2010. Crabapple and lilac growth and root-zone temperatures in northern nursery production systems. *Hortscience* 45:30-35.
- 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.

- Niinemets, Ü. 1995. Distribution of foliar carbon and nitrogen across the canopy of *Fagus sylvatica*: adaptation to a vertical light gradient. *Acta Oecol.* 16:525-541.
- Niinemets, Ü. 1997a. Acclimation to low irradiance in *Picea abies*: influences of past and present light climate on foliage structure and function. *Tree Physiol.* 17:723-732.
- Niinemets, Ü. 1997b. Role of foliar nitrogen in light harvesting and shade-tolerance of four temperate deciduous woody species. *Func. Ecol.* 11:518-531.
- Niinemets, Ü., O. Kull, and J.D. Tenhunen. 1998. An analysis of light effects on foliar morphology, physiology and light interception in temperate deciduous woody species of contrasting shade tolerance. *Tree Physiol.* 18:681-696.
- Niinemets, Ü. and O. Kull. 1998. Stoichiometry of foliar carbon constituents varies along light gradients in temperate woody canopies: implications for foliage morphological plasticity. *Tree Physiol.* 18:467-479.
- Niinemets, Ü and J.D. Tenhunen. 1997. A model separating leaf structural and physiological effects on carbon gain along light gradients for the shade-tolerant species *Acer saccharum*. *Plant Cell Environ.* 20:845-866.
- Niinemets, Ü., A. Söber, O. Kull, W. Hartung, and J.D. Tenhunen. 1999. Apparent controls on leaf conductance by soil water availability and via light-acclimation on foliage structural and physiological properties in a mixed deciduous, temperate forest. *Int. J. Plant Sci.* 160:707-721.
- Nilsen, E.T., M.R. Sharifi, and P.W. Rundel. 1987. Leaf dynamics in an evergreen and a deciduous species with even-aged leaf cohorts from different environments. *Am. Midl. Nat.* 118:46-55.
- 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. *Scientia Hort.* 110:352-358.
- Niu, G., D.S. Rodriguez, and T. Starman. 2010. Response of bedding plants to saline water irrigation. *Hortscience* 45:628-636.
- Niu, G., D.S. Rodriguez, and L. Aguiniga. 2007. Growth and landscape performance of ten herbaceous species in response to saline water irrigation. *J. Environ. Hort.* 25:204-210.
- Ortuño, M.F., W. Conejero, F. Moreno, A. Moriana, D.S. Intrigliolo, C. Biel, C.D. Mellisho, A. Pérez-Pastor, R. Domingo, M.C. Ruíz-Sánchez, J. Casadesus, J. Bonany, and A. Torrecillas. 2010. Could trunk diameter sensors be used in woody crops for irrigation

- scheduling? A review of current knowledge and future perspectives. *Agric. Water Manag.* 97:1-11.
- Parkerson, C.H. 1990. P & P: A new field-type nursery operation. *Proc. Inter. Plant Prop. Soc.* 40:417-419.
- Pearcy, R.W. 1998. Acclimation to sun and shade, p. 250-263. In: A.S. Raghavendra (ed.). *Photosynthesis: A comprehensive treatise*. Cambridge Press, Cambridge.
- Pearcy, R.W. and D.A. Sims. 1994. Photosynthetic acclimation to changing environments: scaling from the leaf to the whole plant. In: Caldwell, M.M., Pearcy, R.W. (Eds.), *Exploitation of Environmental Heterogeneity by Plants: Ecophysiological Processes Above and Below Ground*. Academic Press, San Diego, pp. 145-174.
- Reich, P.B., M.B. Walters, and D.S. Ellsworth. 1992. Leaf lifespan in relation to leaf, plant and stand characteristics among diverse ecosystems. *Ecol. Monogr.* 62:365-392.
- Rhizopoulou, S., M.S. Meletiou-Christou, and S. Diamantoglou. 1991. Water relations for sun and shade leaves of four Mediterranean evergreen sclerophylls. *J. Exp. Bot.* 42:627-635.
- Rubio, J.S., F. Rubio, V. Martínez, and F. García-Sánchez. 2010. Amelioration of salt stress by irrigation management in pepper plants grown in coconut coir dust. *Agric. Water Manag.* 97:1695-1702
- Ruter, J.M. 1998a. Fertilizer rate and pot-in-pot production increases 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.
- Shannon, M.C. and C.M. Grieve. 1999. Tolerance of vegetable crops to salinity. *Sci. Hort.* 78:5-38.
- Shock, C.C., E.B.G. Feibert, and L.D. Saunders. 1998a. Onion yield and quality affected by soil water potential as irrigation threshold. *HortScience* 33:1188-1191.
- Shock, C.C., E.B.G. Feibert, and L.D. Saunders. 1998b. Potato yield and quality response to deficit irrigation. *HortScience* 33:655-659.
- Singh, K.N. and R. Chatrath. 2001. Salinity tolerance. In: Reynolds, M.P., I. Ortiz-Monasterio, and A. McNab (Eds.), *Application of Physiology in Wheat Breeding*. CIMMYT, Mexico, pp. 101-110.
- Sonneveldt, C. 1988. The salt tolerance of greenhouse crops. *Neth. J. Agric. Sci.* 36:63-73.
- Taber, H.G., V. Lawson, B. Smith, and D. Shogren. 2002. Scheduling microirrigation with tensiometers or Watermarks. *Int. Water Irrig.* 22:22-26.

- Tester, M. and R. Davenport. 2003. Na⁺ tolerance and Na⁺ transport in higher plants. *Ann. Bot.* 91:503-527.
- Thompson, R.B., M. Gallardo, T. Agüera, L.C. Valdez, and M.D. Fernández, M.D. 2006. Evaluation of the Watermark sensor for use with drip irrigated vegetable crops. *Irrig. Sci.* 24:185-202.
- Tilt, K.M., J.D. Williams, C.C. Montgomery, B.K. Behe, and M.K. Gaylor. 1994. Pot-in-pot production of nursery crops and Christmas trees, Auburn University, Agric. And Nat. Res. Cir. 893.
- Topp, G.C. 2003. State of the art of measuring soil water content. *Hydrol. Process.* 17:2993-2996.
- Valladares, F. and R.W. Pearcy. 1998. The functional ecology of shoot architecture in sun and shade plants of *Heteromeles arbutifolia* M. Roem., a Californian chaparral shrub. *Oecologia* 114:1-10.
- Villar-Salvador, P., R. Planelles, E. Enríquez, J. Peñuelas Rubira. 2004. Nursery cultivation regimes, plant functional attributes, and field performance relationships in the Mediterranean oak *Quercus ilex* L. *For. Ecol. Manage.* 196:257-266
- Wayne, P.M. and F.A. Bazzaz. 1993. Birch seedling responses to daily time courses of light in experimental forest gaps and shadehouses. *Ecology* 74:1500-1515.
- Wild, A. 1988. *Russell's Soil Conditions and Plant Growth*, 11th ed. Longman, Harlow.
- 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.
- Zweifel, R., H. Item, and R. Häsler. 2000. Stem radius changes and their relation to stored water in stems of young Norway spruce trees. *Trees* 15:50-57.

Chapter 1

Above-ground and pot-in-pot production systems for *Myrtus communis* L.

Authors: J. Miralles¹, P.A. Nortes², M.J. Sánchez-Blanco³, J.J. Martínez-Sánchez^{1,4} and S. Bañón^{1,4}

¹Dpto. de Producción Vegetal, ETSIA, Universidad Politécnica de Cartagena (UPCT), Paseo Alfonso XIII, 48. 30203 Cartagena (Murcia), Spain

²Dpto de Ingeniería de los Alimentos y del Equipamiento Agrícola, ETSIA, Universidad Politécnica de Cartagena (UPCT), Paseo Alfonso XIII, 48. 30203 Cartagena (Murcia), Spain

³Dpto de Riego, CEBAS-CSIC, C/ Campus Universitario, 14, 30100, Murcia, Spain

⁴Unidad Asociada al CSIC de Horticultura Sostenible en Zonas Áridas (UPCT-CEBAS), Paseo Alfonso XIII, 48. 30203 Cartagena (Murcia), Spain

Submitted for review in May 2008 as manuscript number SW 7513; approved for publication by the Soil & Water Division of ASABE in November 2008.

Article reference:

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.

Year 2009:

Impact factor: 0.889

Category: Agricultural Engineering

Category ranking 2008: (8/11).

Quartile in category: Q3

Abstract

Pot cultivation has the disadvantage of favoring extreme temperatures in the substrate, which can damage roots. To reduce this problem, the pot-in-pot (PIP) cultivation system has been developed. To determine the influence of PIP systems vs. above-ground pot (AGP) systems in native myrtle (*Myrtus communis* L.) production, an experimental field site was established where substrate temperature, soil matric potential (SMP), plant growth, water status, and gas exchange were measured. During the one-year experimental period, the PIP system showed mean monthly maximum substrate temperatures that were 6.3 °C to 8.6 °C lower than the AGP system, while mean monthly minimum temperatures were between 2 °C and 5 °C higher. Daily minimum SMP values were more negative in AGP, so the irrigation requirements were lower for the PIP system. In general, differences in gas exchange and water potential between both systems were very slight, which demonstrated the absence of drought conditions as a result of the high irrigation frequency. The AGP system produced greater plant height (16%) and shoot dry weight (11%) than PIP, while the PIP system increased root dry weight (14%), which was presumably responsible for the lower shoot/root index in the PIP cultivated plants. Such behavior is related to substrate temperature differences between both cultivation systems. The PIP system produced plants that were better able to survive two weeks without irrigation at the end of the experiment. These results suggest that the PIP system encourages greater root development and a higher SMP, which enables plants to better survive water stress conditions.

Keywords: Gas exchange, Leaf water potential, Pot-in-pot, Pot plant, Root temperature, Soil water potential.

1. Introduction

In the nursery, plant cultivation in pots or containers is a very common practice. On the one hand, this is to satisfy market demands for herbal plants and shrubs, and on the other because of its many advantages compared with ground cultivation. These advantages include lower stress for plants during transport and manipulation, space reduction in the nursery, the increased possibility of mechanization, a longer supply period, and greater transplantation success (Davidson et al., 1988). However, container cultivation is more affected by environmental conditions in the substrate-root complex, where extreme temperatures can negatively influence root development. The climatic season (winter or summer) will

determine thermal stress through cold or heat. Container characteristics (material, color, form, drainage holes, etc.) also influence temperature in the root system, and many studies have looked at the use of different container types (Franco et al., 2006).

In contrast to traditional cultivation in above-ground pots (AGP), pot-in-pot (PIP) is an alternative system that consists of cultivating plants in pots that are themselves inserted in other pots buried in the ground. The PIP system was first developed in the southeastern U.S. to protect roots from high temperatures in summer but was quickly adapted to northern states because of the advantages perceived for winter plant production (Mathers and Zondag, 2002). This system was quickly extended to trees, ornamental shrubs, fruit trees, and flowering plants.

The PIP system offers many advantages over the AGP system, but it is not free of problems. In a review of the advantages and disadvantages of this system, Zinati (2005) stressed the avoidance of extreme temperatures in the root sphere. The importance of avoiding extreme temperatures in the substrate is well documented, especially because of their effect on root development. Ruter and Ingram (1992) observed that the normal growth of holly trees stopped when temperature exceeded 35°C, whereas in other species this value was lower, at 32°C (Levitt, 1980). Other authors, such as Kuroyanagi and Paulsen (1988), described how wheat plants that receive high temperatures in the root sphere suffer chlorophyll loss and show lower protein production in shoots. Mathers (2003) observed that roots on the west sides of pots are usually injured or destroyed by high temperatures. Zhu et al. (2004) suggested that the moderating of root temperature by PIP prevents the death of *Acer rubrum* roots in contact with the pot wall, whether in winter or summer.

Moderation of the substrate temperature influences the crop water balance, reducing evapotranspiration in the substrate-plant system (Ruter, 1998) and, as a consequence, maintaining higher substrate moisture content (Fain et al., 1998; Ruter, 1997). Less extreme temperatures at root level as well as good water availability in the substrate can accelerate and improve plant growth and development. Indeed, one of the most interesting and practical aspects of PIP for nursery growers is plant growth magnification (Ruter, 1997; Martin et al., 1999). Ruter (1995) related the moderation of temperature with increased root biomass, although the extent of the effect varied according to the species (Ruter, 1993). Zinati (2005) observed root biomass increases of 50% in PIP grown plants compared with 20% in plants grown in AGP.

A well developed root system is especially important in landscape and gardening projects to ensure success in transplanting and establishment, although it is also important to

choose the species that can best adapt to adverse conditions. Among nursery-grown species, native plants represent an incipient and promising market in the ornamental and landscaping plant sector. This kind of plant favors biodiversity conservation and shows better adaptation to the environment, potentially being more resistant to environmental stresses (Martínez-Sánchez et al., 2003; Franco et al., 2001). Native plants, which are more used to growing in the ground, are more susceptible to the thermal disadvantages of AGP systems. For example, *Myrtus communis* is a sclerophyllous evergreen shrub of the Mediterranean area, very important due to its ornamental and medical uses (Romani et al., 2004). However, it can suffer stress by drought and high solar radiation (Mendes et al., 2001; Gucci et al., 1991).

It is well known that one of the most important aspects for improving water irrigation efficiency is the correct choice of cultivation technique. Techniques that improve root function and minimize water consumption are considered key factors for improving pot cultivation (Mathers, 2003). In this example, a PIP system may help improve water management efficiency in nursery production, which is especially important in drought conditions and where the water is of low quality, such as in arid and semiarid areas of the planet.

The objective of this research was to determine, during one year, differences in substrate temperature and water content in native myrtle cultivated in two systems: PIP and AGP. We also studied how each system affected growth and development, and water status and gas exchange parameters.

2. Material and methods

2.1. Plant material and growing conditions

Seedlings of two year old myrtle (*Myrtus communis* L.) grown in 45 multi-pot forest trays (Plasnor S.A., Gipuzkoa, Spain) were used. The seeds were from natural populations of myrtle growing in southeast Spain; they were collected in November 2003 and stored dry at 5 °C. The pots were arranged in a 9 × 5 configuration, had an inverted pyramid form, and measured 60 × 30 × 17 cm (240 cm³ volume). The plants were transplanted to black PVC pots (cultivation pot) of 2.5 L volume, 16 cm upper external diameter, and 15 cm height. The pots

were filled with a mixture of white peat (40%), clay loam soil (30%), and sand (30%). After transplantation, all the plants were cut back to approximately 15 cm height.

The experiment was performed in an open-air plot of 70 m² at the Tomás Ferro Experimental Agro-Food Station of the Polytechnic University of Cartagena (UPCT) (37° 35' N, 0° 59' W). Transplantation of seedlings to cultivation pots was performed on 15 March 2006, and the experiment finished on 18 June 2007. Weather conditions were taken from a meteorological station sited 100 m from the experimental plot. The mean hourly values of temperature, relative humidity, and solar radiation were registered.

A drip irrigation system was installed, with one dripper per plant (2 L h⁻¹) connected to two spaghetti tubes (one each side of every pot). Local irrigation water (pH 7.2; electric conductivity 1.7 dS m⁻¹) was used, containing Ca²⁺ (95 mg L⁻¹), Mg²⁺ (69 mg L⁻¹), Na⁺ (145 mg L⁻¹), Cl⁻ (232 mg L⁻¹), and HCO₃⁻ (110 mg L⁻¹). Both treatments were irrigated between 12:00 and 14:00 h with the same frequency and volume of water. Irrigation frequency was set so that soil matric potential (SMP) reached values of -60 and -120 kPa in AGP. To meet this criterion, irrigation frequency varied according to the season: one irrigation per week in winter, two irrigations per week in autumn and spring, and three irrigations per week in summer. Irrigation amounts were programmed to obtain leaching of 15% to 20% in AGP, which produced irrigation water volumes between 400 and 700 mL per pot. Greater volumes of water were applied in summer and when the time between irrigations was greater (e.g., after the weekend). The leachate in PIP was not collected. At the end of the experiment (June), irrigation was stopped for two weeks to study plant survival and response to high water stress.

2.2. Experimental design and statistical analysis

The PIP system consisted of placing cultivation pots in pots already buried in the ground. The buried pots were made of black PVC and contained many small drainage holes to ensure drainage (5.5 L volume, 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 m⁻²; Projar S.A., Valencia, Spain), which was covered with a 4 cm layer of gravel (~2 cm dia.) (Fig. 1). A total of 220 cultivation pots were placed in 10 rows, 60 cm apart, so that each row had 22 cultivation pots (Picture 1). These were placed 55 cm apart, buried pots (PIP) alternating with above-ground pots (AGP). A CR1000 datalogger and an AM16/32 multiplexer (Campbell Scientific, Logan,

UT) were installed in the center of the plot connected to eight temperature probes (Termistor 107, Campbell Scientific S.L., Barcelona, Spain) and 12 watermark probes (model 253 Irrometer Company, Riverside, CA). The data were analyzed using a one-way ANOVA. A significance level of $\leq 5\%$ was accepted. Where appropriate, the least statistical difference (LSD 5%) was calculated to indicate differences between treatment means. The statistical analysis was performed using Statgraphics Plus 5.1 software (StatPoint Technologies, Warrenton, VA).

Fig. 1. Pot-in-Pot general design.

Picture 1. Experimental plot, pot-in-pot (PIP) and above ground pot (AGP) and datalogger in the center of the plot.

2.3. Growth and development

On five occasions during the experiment (March, June, and October 2006, and January and May 2007), the number of base shoots (N), plant height (H), plant length (L), and crown width (A) were measured with a tape measure. The crown area was calculated (Eq. 1). At the end of the experiment, the dry weight of six plants was determined, separating shoots and roots. To determine root dry weight, roots were washed with pressurized water using a hose with flat tip before being introduced in a dryer. The survival percentage was determined at the end of the experiment after two weeks without irrigation. A plant was considered to have died when, after watering for two weeks and after the imposed drought period, all its leaves were dry and no new shoots were evident.

Eq. 1. Crown area was calculated as follows:

$$CA = \left(\frac{A + L}{2} \right)^2 \times \frac{\pi}{4}$$

where CA is crown area (cm²), A is crown width (cm), and L is crown length (cm).

2.4. Soil matric potential (SMP) and temperature

The soil matric potential (SMP) was registered using six watermark probes and four substrate temperature probes per treatment to perform the SMP corrections due to temperature (Thompson et al., 2006). The devices were connected to the datalogger and multiplexer, which were programmed to register data every minute and to save the hourly mean value. The watermark and temperature probes were installed in random pots, in a southerly orientation and 5 cm deep. SMP was estimated using the equation of Shock et al. (1998), which is the best way of fitting the studied interval, as described by Thompson et al. (2006).

2.5. Leaf water potential and gas exchange

Leaf water potential (Ψ_l) was determined using a pressure chamber (Soil Moisture Equipment Corp; Santa Barbara, Cal.) according to Scholander et al. (1965). The stomatal conductance and net photosynthesis were measured using a portable photosynthesis system (LI-6200, Licor, Inc., Lincoln, Neb.). All measurements were taken at midday in six plants per treatment and on three occasions in each of the following months: April, August, November, and March.

3. Results and Discussion

3.1. SMP and plant water relations

The mean monthly environmental temperatures during the experimental period ranged between 11 °C and 26 °C, and solar radiation ranged between 8.64 and 23.59 MJ m⁻²d⁻¹ (Fig. 2). Mean monthly maximum values were 16 °C to 30 °C and 326 to 900 Wm⁻², respectively (Fig. 3), and mean monthly minimum temperatures varied between 6 °C and 20 °C (Fig. 4). The number of days with temperatures higher than 30 °C, 32.5 °C, and 35 °C were 57, 16, and 0, respectively, with no day showing a minimum temperature below 0 °C.

Fig. 2. Mean monthly temperature and SMP evolution in substrate of PIP and AGP treatments, and mean monthly environmental temperature and solar radiation. Error bars are standard errors ($n = 4$). Values marked with an asterisk (*) are statistically significant at $P < 0.05$.

Fig. 3. Mean monthly maximum temperature and SMP evolution in substrate of PIP and AGP treatments, and mean monthly maximum environmental temperature and solar radiation. Error bars are standard errors ($n = 4$). Values marked with an asterisk (*) are statistically significant at $P < 0.05$.

Fig. 4. Mean monthly minimum temperature and SMP evolution in substrate of PIP and AGP treatments, and mean monthly minimum environmental temperature and solar radiation. Error bars are standard errors ($n = 4$). Values marked with an asterisk (*) are statistically significant at $P < 0.05$.

The mean monthly substrate temperatures in all the experimental months were similar in PIP and AGP, ranging between 11 °C and 30 °C. The AGP system showed higher mean monthly maximum substrate temperatures than PIP (Fig. 3), with the thermal differences between both systems ranging from 6.3 °C to 8.6 °C. Young and Bachman (1996) and Ruter (1993) described how, on the hottest days of summer, PIP substrates for different species were 2.3 °C and 6 °C lower, respectively, than AGP temperatures. As shown in figure 3, PIP moderated substrate temperature increases from June to September, preventing mean monthly maximum temperatures >34 °C, unlike in AGP, where 45 °C was reached. In winter, PIP did

not show mean monthly maximum temperatures $>15^{\circ}\text{C}$, whereas temperatures reached 22°C in AGP. Such thermal differences can influence plant growth and development, but the magnitude of this effect will depend on the species (Akinnifesi et al., 2004).

Mean monthly minimum substrate temperatures showed the opposite behavior to maximum temperatures (Fig. 4), with PIP reaching higher temperatures than AGP. The thermal differences between both systems ranged from 2°C to 5°C , although the temperature differences between both systems were lower than the corresponding maximum values. Young and Bachman (1996) and Ruter (1993) found that, on the coolest winter days, PIP substrates were 1.1°C and 3°C warmer, respectively, than the corresponding AGP values. This behavior can be explained by the ground effect, which slowed the temperature loss at night. In our case, PIP presented daily temperatures $>6^{\circ}\text{C}$, whereas the AGP substrate reached 1°C (Fig. 7).

Mean monthly temperatures were similar in both systems (Fig. 2) because AGP reached higher daily temperatures than PIP (Fig. 3) but lower temperatures at night (Fig. 4), the one compensating the other.

The daily maximum and minimum substrate temperatures during a representative period of each season are shown in figures 5 through 8, where the high variations of maximum or minimum substrate temperatures in AGP contrast with the low variations in PIP. These results, together with the monthly temperature register, confirm that PIP significantly moderated low and high substrate temperatures, particularly when temperatures were at their most extreme. Similar results were obtained by London et al. (1998) for other species.

Fig. 5. Representative 31-day period for summer, showing daily maximum and minimum substrate temperature in PIP and AGP systems and daily minimum substrate SMP registers in PIP and AGP systems. Error bars are standard errors ($n = 4$ in temperature, and $n = 6$ in SMP). For clarity, only every fifth standard error value is shown.

Fig. 6. Representative 31-day period for autumn, showing daily maximum and minimum substrate temperature in PIP and AGP systems and daily minimum substrate SMP registers in PIP and AGP systems. Error bars are standard errors ($n = 4$ in temperature, and $n = 6$ in SMP). For clarity, only every fifth standard error value is shown.

Fig. 7. Representative 31-day period for winter, showing daily maximum and minimum substrate temperature in PIP and AGP systems and daily minimum substrate SMP registers in PIP and AGP systems. Error bars are standard errors ($n = 4$ in temperature, and $n = 6$ in SMP). For clarity, only every fifth standard error value is shown.

Fig. 8. Representative 31-day period for spring, showing daily maximum and minimum substrate temperature in PIP and AGP systems and daily minimum substrate SMP registers in PIP and AGP systems. Error bars are standard errors ($n = 4$ in temperature, and $n = 6$ in SMP). For clarity, only every fifth standard error value is shown.

Mean monthly soil matric potential (SMP) for PIP and AGP systems were similar from June to March, varying between -21 and -72 kPa, although the AGP values tended to be more negative than PIP (Fig. 2). The high irrigation frequency prevented large substrate water content variations, and only at the end of the experiment (April and May) did PIP show higher SMP values than AGP. Similar behavior was observed for the mean monthly maximum SMP values (Fig. 3). These differences in SMP at the end of the experiment could have been due to the higher water consumption of plants in AGP following growth activation during the winter-spring transition. This may have been caused by higher maximum substrate temperature, together with more developed plants in the AGP system.

More evident differences between PIP and AGP were observed for the mean monthly minimum SMP (Fig. 4). In the first three months registered, there were no significant

differences, possibly because plants were not sufficiently developed enough for the pot size. In September and October, the mean monthly minimum SMP values were more negative in AGP. These results are related with the minimum daily SMP of a representative period in summer and autumn. Regarding figure 5 (summer), the high number of oscillations in daily minimum SMP is due to greater substrate drying; however, the differences between both systems were barely significant. These low differences in summer SMP between PIP and AGP may be explained by greater evaporation because of the higher radiation that the AGP pots received, and the higher transpiration in PIP influenced by higher stomatal conductance values (Fig. 9).

Fig. 9. Mean stomatal conductance values in PIP and AGP. Value marked with an asterisk (*) is statistically significant at $P < 0.05$.

Fig. 10. Mean leaf water potential values in PIP and AGP. Values marked with an asterisk (*) are statistically significant at $P < 0.05$.

Fig. 11. Mean photosynthesis values in PIP and AGP. Values marked with an asterisk (*) are statistically significant at $P < 0.05$.

In autumn (Fig. 6), when the irrigation frequency was lower, AGP reached more negative SMP values than PIP, possibly because transpiration rates leveled out due to similar stomatal conductance levels (Fig. 9). Moreover, after summer, some roots from PIP plants entered the air chamber between the two pots of the PIP system, which may mean that transpired water did not come totally from the substrate, as occurred in AGP. These differences between PIP and AGP agree with experiments performed by Martin et al. (1999) using *Acacia smallii* and *Cercidium floridum* in which AGP needed extra irrigation, as well as programmed irrigation, to keep moisture tensions for all rooting substrates between -0.005 and -0.01 MPa; AGP needed 5.3 L weekly per pot, and PIP needed 3.2 L per pot.

In November, December, and January, the mean monthly minimum SMP was similar in both systems (Fig. 4), which could be a consequence of lower plant growth due to a decrease in temperature and solar radiation. Daily minimum SMP during a representative winter period (Fig. 7) showed less negative values, which were very similar in both systems, reflecting very low irrigation frequency. These registers showed that AGP reached more negative SMP before PIP, which suggests that PIP has lower irrigation requirements.

In February, as the temperature began to increase, the mean monthly minimum SMP in AGP became more negative than the corresponding values in PIP (Fig. 4), and in spring, with this season's better environmental conditions for plants, these differences increased (Fig. 4). This is reflected in the results shown in figure 8 (spring), where a greater number of SMP variations as a result of increasing water needs can be appreciated. Furthermore, AGP clearly reached more negative values than PIP, whose substrate conditions remained better. Some records in AGP reached $SMP < -100$ kPa (Fig. 8), which could have caused water stress.

Nevertheless, such values were isolated, and the average leaf water potential (Fig. 10), in general terms, pointed to no water stress. Indeed, in well developed plants, no leaf water potentials under -1.0 MPa were recorded in either system, the values being greater than those recorded for leaf water potential registered in other experiments with myrtle plants subjected to moderate water stress (Vicente et al., 2006).

Figures 12 and 13 shows hourly temperatures and SMP during 10 days of a hot and cold period. In the hot period (Fig. 12), after irrigation, SMP was similar in PIP and AGP, and remained so at night. As the sun rose, environmental temperatures increased, causing a strong increase in substrate temperature until midday in both treatments, but the increase was especially pronounced in AGP. This different temperature evolution in PIP and AGP resulted in the SMP curves tending to separate, with AGP reaching more negative values than PIP, particularly when the time between irrigations was three days or when temperature and vapor pressure deficit (VPD) were higher (Fig. 12). From midday, substrate temperature decreased in both systems, particularly in AGP, where it became equal to the environmental temperature at night, while the PIP substrate temperature remained higher than environmental. However, this fact did not prevent the SMP curves from separating.

Fig. 12. Hourly environmental temperature, VPD, substrate temperature, and SMP evolution in PIP and AGP systems during a 10-day period in summer. Arrows indicate irrigation events. Error bars in temperatures are standard errors ($n = 4$). For clarity, only every fifth standard error value is shown. Vertical bar show LSD at $P < 0.05$.

Figure 13. Hourly environmental temperature, VPD, substrate temperature, and SMP evolution in PIP and AGP systems during a 10-day period in winter. Arrows indicate irrigation events. Error bars in temperatures are standard errors ($n = 4$). For clarity, only every fifth standard error value is shown. Vertical bar show LSD at $P < 0.05$.

Figure 13 shows a representative 10-day cooler period where SMP in both PIP and AGP were similar after irrigation. Although substrate temperature oscillations in AGP were greater than in PIP, the temperatures ranges were still moderate considering it was winter (Fig. 13). This caused SMP to remain stable in both systems for six days after irrigation (156 h), after which SMP in AGP decreased gradually, but kept more or less constant in PIP.

Before dawn, there was a slight increase in SMP in AGP due to the presence of dew (minimum VPD of 0.07 kPa), after which the potential decreased. The fall in SMP at midday and increase at night became more pronounced as the number of days without irrigation increased. With the following irrigation, SMP became similar in both treatments.

At the beginning of cultivation (April), plants in PIP and AGP showed similar net photosynthesis values (Fig. 11), which can explain the lack of difference in shoot growth (Figs. 14 through 16). In August (summer), the differences observed in SMP had no effect on leaf water potential, and similar values were observed in both systems. However, the $SMP < -80$ kPa registered in summer in AGP could have activated the messages sent by roots, perhaps of a chemical kind, which might have lowered stomatal conductance rates and, as a consequence, led to lower photosynthetic activity. Such behavior probably helped leaf water potential not to fall in these plants. Mendes et al. (2001) found a high sensitivity to stomatal closure in myrtle affected by water deficit. This fact, linked to high solar radiation in this period, could also influence this stomatal conductance behavior. Niinemets et al. (1999) described how stomatal sensitivity was intensified by high solar radiation.

3.2. Growth and development

Shoot evolution in PIP and AGP was similar during the experimental period, increasing from 3.4 to 9.2 shoots per plant from transplanting to the end of cultivation (Fig. 14). The majority of new shoots in both systems (55%) appeared between June and October. Ruter (1993) observed no differences in shoot production in *Lagerstroemia indica* x *fauriei*. However, in *Magnolia* x *soulangiana*, the same author found that PIP plants had more shoots than their AGP counterparts.

Fig. 14. Number of shoots evolution in PIP and AGP systems. Error bars are standard errors ($n = 110$).

Fig. 15. Crown area evolution in PIP and AGP systems. Error bars are standard error ($n = 110$).

Fig. 16. Plant height evolution in PIP and AGP systems. Error bars are standard error ($n = 110$). Value marked with an asterisk (*) is statistically significant at $P < 0.05$.

Crown area seems to be a good horizontal growth index of plants. This parameter was characterized by a strong increase during the first months of spring in both cultivation systems. During the other months, shoot growth was less intense in both systems. Final values indicate that the cultivation system had no significant effect on crown area (Fig. 15). However, other authors observed a clear increase in the width in PIP plants compared with AGP plants of several species (Martin et al., 1999). The height of PIP plants developed in a similar way to AGP plants from transplantation in March to January, after which the AGP plants grew in height more than PIP plants until the end of the experiment, when they were 16% taller (Fig. 16, Picture 2). This effect agrees with Ruter (1993), who found that *L. indica* x *fauriei* plants were taller when they were cultivated in AGP, although the same author found no differences in other species. In our experiment, due to environmental conditions characterized by hot summers and mild winters (Figs. 2 through 4), it might be expected that, in summer, PIP plants would present higher growth because of the moderating effect on substrate temperature (Figs. 3 and 5). However, such behavior did not occur, perhaps due to the physiological characteristics of myrtle, which rests at high temperatures (Brosse, 1979). The reduced temperature in PIP was not sufficient to activate shoot growth in this plant. Moreover, the influence of high solar radiation (Figs. 2 through 4) must be taken into consideration, since this was received by plant shoots of both systems and could activate adaptation mechanisms in myrtle (Tattini et al., 2006). Whatever the case, the influence of PIP on summer growth may depend on the species. For example, during the summer

cultivation of *A. smallii*, Martin et al. (1999) observed that PIP increased plant height by 80% in contrast with AGP, but had no effect on *C. floridum*.

From January to May, plants in AGP became taller than those in PIP, which could be related with the higher substrate temperature during the day in winter. The greater exposure to solar radiation of AGP could explain this phenomenon, despite the environmental temperature being the same for all plants. In this sense, Berninger (1979) described that longitudinal shoot growth in gerbera was more linked to soil temperature than air temperature. DIF (day-night temperature differential) in substrate was always higher in AGP, especially in January (data not shown). In ornamental plant greenhouses, it is common to control DIF to increase or reduce height, since the lower the DIF, the lower the plant height growth (Warner and Edwing, 2001). This would also explain the response found in this experiment.

Picture 2. Above-ground-pot (AGP) and pot-in-pot (PIP) myrtle plants after one year of experimentation.

Table 1 shows the greater root growth in PIP, which resulted in a 14% increase in root dry weight compared with AGP. In *L. indica* x *fauriei* and *Magnolia* x *soulangiana*, Ruter (1993) observed greater root dry weight (47% and 70%, respectively) when the plants were cultivated in PIP. Later works by the same author showed similar behavior in *Magnolia grandiflora* (Ruter, 1995). In *A. smallii*, Martin et al. (1999) found a higher root dry weight in

PIP (167 g) compared with AGP (97 g), although no differences for *C. floridum*. Furthermore, Young and Bachman (1996) recorded an increase of 26% in root dry weight when *Ilex x attenuate* was cultivated in PIP. The larger containers, greater plant development, and different environmental conditions of these experiments, as compared with our experiment, might have contributed to this greater effect of PIP system on root growth.

Table 1. Dry weight production after 12 months.

Parameter	PIP	AGP	Significance ^[a]
Root (g)	32.45	27.90	*
Shoot (g)	51.30	57.60	*
Plant (g)	83.75	85.50	ns
Shoot/Root index (g/g)	1.58	2.06	*

^[a]* = Statistically significant at $P < 0.05$; ns = Not significant.

Myrtle plants in PIP presented 11% less shoot dry weight compared with AGP (Table 1). This opposite behavior of shoot growth to root growth meant that total dry weight did not alter significantly between PIP and AGP, despite the shoot/root ratio being significantly higher in AGP than in PIP. In contrast, Ruter (1993) described that *L. indica x fauriei* cultivation in PIP caused a substantial increase in the shoot/root ratio, although Martin et al. (1999) did not observe this difference in *A. smalli* or *C. floridum*. It has been suggested that a diminishing shoot/root index lowers the relative transpiration capacity, unlike water and nutrient absorption (Bernier et al., 1995). Mathers (2000) described that slight reductions in the shoot/root ratio gives plants greater water stress resistance during nursery production and transplantation, which accelerates plant establishment in the field (Owings, 2005). As mentioned by Guarnaschelli et al. (2006), these effects are more patent in drought conditions, so this change in biomass distribution in plants could improve survival after transplantation. Indeed, after two weeks without irrigation at the end of the experiment, the PIP system led to 90% plant survival, compared with 62% for AGP (Picture 3). The presence of roots in the air chamber between the two pots must be evaluated to know to what extent this factor influenced survival, because relative humidity was very high in this chamber. Indeed, this might be an important criterion to be taken into account for PIP system design.

Picture 3. Experimental plot after two weeks without irrigation at the end of the experiment

4. Conclusion

The most relevant conclusions of this experiment are four. Firstly, the PIP system used in myrtle moderated extreme substrate temperatures. Secondly, PIP maintained a higher substrate SMP, which suggests lower irrigation needs than in AGP. Thirdly, PIP led to lower shoot growth than AGP, because diurnal substrate temperatures in winter were lower than in AGP. Lastly, the PIP system favored greater root development due to moderation of the substrate temperature in summer, which translated into a lower shoot/root ratio, favoring survival in severe water stress situations.

Acknowledgements

The authors thank J. A. Egea, F. Solano, and P. Mateo for irrigating and supervising the myrtle plants while the experiment took place. The authors also gratefully acknowledge G. Egea for his cooperation in providing technical assistance. This work was supported by

CICYT projects AGL 2005-05588-C02-1, AGL 2005-05588-C02-2, and Fundación Séneca Región de Murcia 02991 PI05.

References

- Akinnifesi, F. K., E. C. Rowe, S. J. Livesley, F. R. Kwesiga, B. Vanlauwe, and J. C. Alegre. 2004. Tree root architecture. In *Below-Ground Interactions in Tropical Agroecosystems: Concepts and Models with Multiple Plant Components*, 61-82. M. Noordwijk, G. Cadisch, and C. K. Ong, eds. Wallingford, U.K.: CABI Publishing.
- Bernier, P. Y., J. D. Stewart, and A. Gonzalez. 1995. Effects of the physical properties of Sphagnum peat on water stress in container *Picea mariana* seedlings under simulated field conditions. *Scandinavian J. Forest Res.* 10:184-198.
- Berninger, E. 1979. Effects of air and soil temperatures on the growth of gerbera. *Scientia Hort.* 10:271-276.
- Brosse, J. 1979. *Atlas of Shrubs and Lianes*. Paris, France: Bordas Editions.
- Davidson, H., R. Mecklenburg, and C. Peterson. 1988. *Nursery Management Administration and Culture*. Englewood, Cliffs, N.J.: Prentice Hall.
- Fain, G. B., K. M. Tilt, C. H. Gilliam, H. G. Ponder, and J. L. Sibley. 1998. Effects of cyclic micro irrigation and substrate in pot-in-pot production. *J. Environ. Hort.* 16:215-218.
- Franco, J. A., S. Bañón, J. A. Fernández, and D. I. Leskovar. 2001. Effect of nursery regimes and establishment irrigation on root development of *Lotus creticus* seedlings following transplanting. *J. Hort. Sci. Biotech.* 76:174-179.
- Franco, J. A., J. J. Martínez-Sánchez, J. A. Fernández, and S. Bañón. 2006. Selection and nursery production of ornamental plants for landscaping and xerogardening in semi-arid environments. *J. Hort. Sci. Biotech.* 8:3-17.
- Guarnaschelli, A. B., P. Prystupa, and J. H. Lemcoff. 2006. Drought conditioning improves water status, stomatal conductance, and survival of *Eucalyptus globulus* subsp *bicostata* seedlings. *Annals Forest Sci.* 63:941-950.
- 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. Plantarum* 83:497-505.
- Kuroyanagi, T., and G. M. Paulsen. 1988. Mediation of high-temperature injury by roots and shoots during reproductive growth of wheat. *Plant Cell Environ.* 11:517-523.

- Levitt, J. 1980. Responses of Plants to Environmental Stresses. New York, N.Y.: Academic Press.
- London, J. B., R. T. Fernandez, R. E. Young, and G. D. Christenbury. 1998. Media temperatures and plant growth in above-ground and in-ground pot-in-pot container systems. HortScience 33:512.
- 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.
- Martínez-Sánchez, J. J., P. Ferrandis, L. Trabaud, R. Galindo, J. A. Franco, and J. M. Herranz. 2003. Comparative root system structure of post-fire *Pinus halepensis* Mill. and *Cistus monspeliensis* L. saplings. Plant Ecol. 168:309-320.
- Mathers, H. M. 2000. Overwintering container nursery stock: Part. 1. Acclimation and covering. The Buckeye (Dec.): 14, 16, and 18.
- Mathers, H. M. 2003. Summary of temperature stress issues in nursery containers and current methods of protection. HortTech. 13:617-624.
- Mathers, H. M., and R. Zondag. 2002. "Out-and-About" with Hannah. Nursery stock production using the pot-in-pot technique. Buckeye 20:14-25.
- Mendes, M. M., L. C. Gazarini, and M. L. Rodrigues. 2001. Acclimation of *Myrtus communis* to contrasting Mediterranean light environments: Effects on structure and chemical composition of foliage and plant water relations. Environ. Exp. Botany 45:165-178.
- Niinemets, Ü., A. Söber, O. Kull, W. Hartung, and J. D. Tenhunen. 1999. Apparent controls on leaf conductance by soil water availability and via light-acclimation on foliage structural and physiological properties in a mixed deciduous, temperate forest. Intl. J. Plant Sci. 160:707-721.
- Owings, A. D. 2005. Pot-in-pot nursery production. Louisiana Agric. 48:20.
- Romani, A., R. Coinu, S. Carta, P. Pinelli, C. Galardi, F. F. Vicieri, and F. Franconi. 2004. Evaluation of antioxidant effect of different extracts of *Myrtus communis* L. Free Radical Res. 38:97-103.
- Ruter, J. M. 1993. Growth and landscape performance of three landscape plants produced in conventional and pot-in-pot production systems. J. Environ. Hort. 11:124-127.
- Ruter, J. M. 1995. Growth of southern magnolia in pot-in-pot and above-ground production systems. In Proc. SNA Research Conf., 40:138-139. Atlanta, Ga.: Southern Nursery Association.

- Ruter, J. M. 1997. The practicality of pot-in-pot. *American Nurseryman* 8:32-37.
- Ruter, J. M. 1998. Fertilizer rate and pot-in-pot production increase growth of Heritage River birch. *J. Environ. Hort.* 16:135-138.
- Ruter, J. M., and D. L. Ingram. 1992. High root-zone temperatures influence RuBisCo activity and pigment accumulation in leaves of 'Rotundifolia' holly. *J. American Soc. Hort. Sci.* 117:154-157.
- Scholander, P., H. T. Hammel, E. D. Bradstreet, and E. A. Hemmingeen. 1965. Sap pressure in vascular plants. *Science* 148:339-346.
- Shock, C. C., J. M. Barnum, and M. Seddigh. 1998. Calibration of watermark soil moisture sensors for irrigation management. In *Proc. Intl. Irrig. Show*:139-146. Falls Church, Va.: Irrigation Association.
- Tattini, M., D. Remorini, P. Pinelli, G. Agati, E. Saracini, M. L. Travesi, and R. Massai. 2006. Morpho-anatomical, physiological, and biochemical adjustments in response to root zone salinity stress and high solar radiation in two Mediterranean evergreen shrubs, *Myrtus communis* and *Pistacia lentiscus*. *New Phytologist* 170:779-794.
- Thompson, R. B., M. Gallardo, T. Agüera, L. C. Valdez, and M. D. Fernández. 2006. Evaluation of the Watermark sensor for use with drip-irrigated vegetable crops. *Irrig. Sci.* 24:185-202.
- Vicente, M. J., J. J. Martínez-Sánchez, E. Conesa, S. Bañón, A. Navarro, M. J. Sánchez-Blanco. 2006. Studio della modificazione dello sviluppo e dello stato idrico di piantine di *Myrtus communis* causati da deficit idrico e paclobutrazol. In *Proc. III Convegno Nazionale Piante Mediterranee: Le Piante Mediterranee nelle Scelte Strategiche per l'Agricoltura e l'Ambiente*:194. Bari, Italy: Università degli Studi di Bari.
- Warner, R. M., and J. E. Erwin. 2001. Temperature. In *Tips on Regulating Growth of Floriculture Crops*:10-17. C. A. Buck and S. A. Carver, eds. Columbus, Ohio: Ohio State University.
- Young, R. E. and G. E. Bachman. 1996. Temperature distribution in large, pot-in-pot nursery containers. *J. Environ. Hort.* 14:170-176.
- Zhu, H., C. R. Krause, R. C. Derksen, R. D. Brazee, R. Zondag, and N. R. Fausey. 2004. Real-time measurement of drainage from pot-in-pot container nurseries. *Trans. ASAE* 47:1973-1979.

Zinati, G. 2005. Pot-in-pot nursery production system: What you need to know before establishment. Pub. No. FS519. New Brunswick, N.J.: Rutgers Cooperative Research and Extension. Available at: <http://njaes.rutgers.edu/pubs/publication.asp?pid=FS519>. Accessed 17 January 2008.

Chapter 2

Comparison of stem diameter variations in three small ornamental shrubs under water stress

Authors: J. Miralles¹, A. Navarro², M.J. Sánchez-Blanco², J.J. Martínez-Sánchez^{1,3}, J.A. Franco^{1,3} and S. Bañón^{1,3}

¹Dpto. de Producción Vegetal, ETSIA, Universidad Politécnica de Cartagena (UPCT), Paseo Alfonso XIII, 48. 30203 Cartagena (Murcia), Spain

²Dpto de Riego, CEBAS-CSIC, C/ Campus Universitario, 14, 30100, Murcia, Spain

³Unidad Asociada al CSIC de Horticultura Sostenible en Zonas Áridas (UPCT-CEBAS), Paseo Alfonso XIII, 48. 30203 Cartagena (Murcia), Spain

Received for publication 27 Apr. 2010. Accepted for publication 8 Aug. 2010.

Article reference:

Miralles, J., M.J. Sánchez-Blanco, A. Navarro, J.J. Martínez-Sánchez, J.A. Franco, and S. Bañón. 2010. Comparison of stem diameter variations in three small ornamental shrubs under water stress. Hortscience 45:1681-1689.

Year 2009:

Impact factor: 0.696

Category: Horticulture

Category ranking 2008: (15/30).

Quartile in category: Q3

Abstract

The dendrometer has been proposed as a sensitive plant water indicator based on stem growth. However studies including dendrometers have been mainly focused on fruit trees and less attention has been paid to ornamental shrubs (small plants). In the study described here, stem dendrometers were used to ascertain whether there is any relationship between water status and dendrometric indices in potted ornamental shrubs (1-2 cm diameter). For this purpose three Mediterranean shrubs (*Pittosporum tobira*, *Callistemon citrinus* and *Rhamnus alaternus*) were studied under water stress-recovery conditions in winter, spring and early summer. At the end of the experiment, an extreme water stress treatment, which resulted in plant death (August) was also studied. Stem diameter variations [maximum and minimum daily stem values (MXDS and MNDS, respectively), maximum daily shrinkage (MDS), and stem growth rate (SGR)], daily evapotranspiration (daily plant ET) and leaf water potential (Ψ_{leaf}) parameters were considered throughout the experiment. A regression analysis between dendrometric indices and daily plant ET showed that MXDS and MNDS were sensitive under water stress-recovery conditions, especially, in severe environmental conditions (spring and summer). The SGR in *C. citrinus*, the MDS in *P. tobira* and both indices in *R. alaternus* were seen to be sensitive during the stress to death period. Although more studies are needed, the results confirm that the use of dendrometers in small plants may be useful to provide continuous and automated registers of the plant water status under different substrate water content and climatic conditions. However, the response of these indices may imply a moderate water stress.

Keywords: Pot plant, maximum daily shrinkage, minimum daily stem diameter, maximum stem daily diameter, stem growth rate, evapotranspiration

1. Introduction

Irrigation water is in increasingly short supply, which is a grave problem for the nursery sector. There is clear awareness in this sector that high quality production levels need to be maintained, while improving water use efficiency. To attain this, the selection of appropriate species and efficient irrigation scheduling are the nurseryman's best tools. It is therefore necessary to know the water status of a given species so that irrigation scheduling can be adapted to the real water requirements of species (Nemali and van Iersel, 2006).

In irrigation scheduling, it is very common to use soil water sensors because of their good precision (Burnett and van Iersel, 2008). However, in pot crops, the efficient use of such sensors depends on the physical characteristics of the substrate, minimum soil moisture content and the control of electrical conductivity (EC) in the substrate solution. In recent years, efforts directed at irrigation scheduling based on plant water status have acquired increasing importance, because, as is well known, a plant is the best indicator of its own water status. Besides, water status indicators based on plant measurements have the advantage of being directly related with crop productivity (Goldhamer and Fereres, 2001; Goldhamer et al., 2003). Several parameters, including leaf and stem water potential, leaf conductance and photosynthesis, are used as plant water status indicators. However, most of these are only used in research, because they are expensive and difficult to operate in real field conditions. Therefore, studies have suggested that one of the most sensitive water indicators is plant growth and, more specifically, trunk diameter growth. One of the consequences of this has been the proposed use of the dendrometer, an electronic sensor which measures the stem diameter variations (SDV) originated by water changes in the trunk tissues (Molz and Klepper, 1973). The high frequency measurements obtained with this sensor usually point to a maximum diameter value just before sunrise (MXDS) and a minimum diameter value at sometime during the afternoon (MNDS), the difference between them being the maximum daily shrinkage (MDS). It has been suggested that the MDS is a key indicator of the plant water status (Cohen et al., 2001; Ortuño et al., 2006), and numerous works have studied the relationship between dendrometric indices, plant water status indicators, especially the leaf water potential (Ψ_{leaf}) (Intrigilo and Castel, 2004; Li et al., 1989; Yatapanage and So, 2001) and environmental variables such as vapor pressure deficit (Ortuño et al., 2006). However, other important indicators such as evapotranspiration (ET) have been less studied because of the difficulty involved in its precise determination in soil cultivated trees (Sivyer et al. 1997). When a plant is cultivated in a pot, plant ET can be measured with accuracy (Argo and Biernbaum, 1994; Navarro et al. 2007), and, in this case, the irrigation volume should at least replace the losses occurring through plant transpiration and substrate evaporation.

The use of dendrometers to ascertain the plant water status has mainly focused on fruit trees, where they have found practical application in irrigation scheduling (Fereres and Goldhamer, 2003; García-Orellana et al., 2007; Nortes et al., 2005). Fewer studies in this respect have been carried out in ornamental shrubs, especially those of small size and cultivated in pots (nursery crops). It is due to the original design of the dendrometer, which was from the beginning adapted to large trunk diameters (usual in fruit trees). Also because of

the characteristics of pot cultivation, in which the plant water response may differ from that of the soil, since environmental conditions have a much stronger influence on the pot substrate than on soil, mainly because of temperature changes in the roots (Mathers, 2003; Miralles et al. 2009). In this sense, some authors have suggested that MDS behaves differently in pot-cultivated and soil-cultivated plants under water stress (Gallardo et al., 2006).

In gardening and revegetation in Mediterranean areas of Spain, it is very common to use ornamental plants adapted to the Mediterranean climate, particularly shrubs. Among this group predominate traditional shrubs (autochthonous and allochthonous), widely known and offered in commercial nurseries. Secondly, foreign Mediterranean shrubs, mainly Australian or South African flora, which have been introduced in recent years because of their rapid growth, spectacular flowering and colors, and great variety of forms and volumes. And lastly, native shrubs which have opened an incipient and promising market in the ornamental sector, reflecting present tendencies as regards biodiversity conservation, and, of course, providing greater adaptability to the environment and stronger resistance to stress. In this work we study one species of each group: traditional, foreign Mediterranean and native shrubs.

The objective of this study was to know whether the dendrometric indices could be used as plant water status indicator in well irrigated and water stress conditions in different ornamental shrubs grown in pots. The response of stem diameter variations and their relationship with the daily evapotranspiration throughout the growing season were compared in the three species.

2. Materials and methods

2.1. Plant material and growing conditions

Three different 1.5 - 2 year old Mediterranean shrubs of ornamental and landscaping interest were used, one of them native (*Rhamnus alaternus* L.), one representative of Australian flora (*Callistemon citrinus* [Curtis]. Skeels.cv. Splendens) and the other a very popular commercial shrub from China and Japan (*Pittosporum tobira* [Thunb.] Ait. cv. Nana). The first two species have a columnar form, while the last one is short and wide.

The plants were transplanted during the first week of November 2007 into black PVC pots which contained a substrate of white peat (40%), coco fiber (40%) and perlite (20%), amended with 2 g·L⁻¹ of a controlled release fertilizer (osmocote plus 10N-4.8P-14.9K-

1,2Mg+ microelements; release time 5-6 months at 21 °C). *R. alaternus* and *C. citrinus* were transplanted to square pots (2.8 L volume and 15 × 15 cm at the top and 11 × 11 cm at the base, 20 cm high). *P. tobira* was transplanted, first to a circular pot of 14 cm upper external diameter (1.5 L volume and 12.5 cm high), and later, in the second week of March to a circular pot of 16 cm upper external diameter (2.5 L volume and 15 cm high). The experiment took place in a 100 m² greenhouse with semicircular cover (12.5 m length × 8 m width, 3.5 m height at the side and 5.5 m at the centre), covered totally with polycarbonate sheets and located at the Tomás Ferro Experimental Agro-Food Station of the Polytechnic University of Cartagena (UPCT) in “La Palma” (Cartagena-Spain) (37° 35′ N, 0° 59′ W).

The pots were placed on metal tables (1.5 × 3 × 0.9 m) provided with a system to collect the water leached per half table. On the tables the plants were set in a 33 × 33 cm frame, so that there were 20 plants per half table. The plants were irrigated at 10:00 h using computer-controlled automated programmable equipment (irrigation head and fertilization Xilema NX300 Hidroponic, Novedades Agrícolas, S.A, Spain) and each pot had one emitter (2 L·h⁻¹) connected to two spaghetti tubes (one each side of the pot). During the common irrigation period, the doses and frequency differed for each species. The irrigation volume was determined by noting when the leaching fraction reached 15-20%, and the irrigation frequency by taking into account when pots had lost between 70% and 90% of easily available water in the substrate (~19% of pot volume), determined by weight. Irrigation after a drought and recovery period was made until field capacity was reached. One month after transplanting, when plants were established, fertilization was provided at constant concentrations in the irrigation water, which contained 100N-13.1P-99.6K-42.9Ca (ppm) and, had a pH of 6. This nutrient solution was made by mixing KNO₃, Ca(NO₃)₂, NH₄(NO₃), K(HPO₄) and nitric acid (HNO₃) (Antonio Fuentes Méndez, S.A., Totana, Spain) which were kept in five individual 500 L tanks at a concentration of 6 kg·1000 L⁻¹ for fertilizers and 1 kg·1000 L⁻¹ for nitric acid. The mixture was made up using different injection times that were programmed using the above mentioned automated programmable equipment. The fertilizers increased the EC of the irrigation water by approximately 0.7 dS·m⁻¹, which, before fertilization, had an EC of 1.2 dS·m⁻¹ and a pH of 7.3.

The water stress periods involved withdrawing irrigation in winter (from 26 January to 4 February), spring (from 8 May to 14 May) and early summer (from 11 June to 16 June), while the extreme water stress period was applied from 1 to 31 August, which led to plant death.

The temperature and relative humidity were recorded continuously by a datalogger (HOBO (H08-004-02, MicroDAQ.com, Ltd., USA.). Calculations of the vapor pressure deficit (VPD) were made using the equation derived by Snyder et al. (1986).

2.2. Plant water relations

Midday leaf water potential (Ψ_{leaf}) was estimated according to the method described by Scholander et al. (1965), using a pressure chamber (Soil Moisture Equipment Co., Santa Barbara, CA, USA), for which leaves were sealed in the chamber within 20 s of collection and pressurized at a rate of $0.02 \text{ MPa}\cdot\text{s}^{-1}$ (Turner, 1988). Five samples were used for each species, and measurements were taken in drought periods (described at the previous paragraph). The measurements were taken if possible every day except in summer because of a problem with the pressure chamber. So, in the summer drought and recovery period only the first day and the last day of drought before and after irrigation were registered. In the summer drought to death, measurements were made every two days. This methodology was not appropriate for *P. tobira* because it presented a Ψ_{leaf} that was difficult to explain, with very high values during the whole period, despite symptoms of withering. In this sense and, as in other species, the determination of the end point is complicated by resin exudation from the pith and cortex or ducts in the xylem (Ritchie and Hinckley, 1975).

The bulk modulus of elasticity (ϵ) was estimated via pressure-volume curves as outlined by Wilson et al. (1979), using the formula: $\epsilon = (\text{RWC}_{\text{tlp}} \times \Psi_{\text{os}}) / (100 - \text{RWC}_{\text{tlp}})$, where ϵ is expressed in MPa, Ψ_{os} is the osmotic potential at full turgor (MPa) and RWC_{tlp} is the relative water content at the turgor loss point. Pressure-volume curves were obtained before the application of water stress in the first week of June in three leaves per plant and five plants per species.

The leaf dehydration kinetic was studied for each species using the same balance-programmer system described above. To study each species, three balances (Analytical Sartorius, Model 5201. Capacity 5.2 kg and readability of 0.01 g) inside the greenhouse were used in the first week of June. For each species, 12 leaves per plant were taken from the central part of each plant, avoiding young and old leaves. The leaf petioles were immersed in distilled water for two days (in plastic bottles sealed with parafilm) and kept in darkness at 5-6 °C. The saturated leaves were then dried with absorbent paper and placed on the balances, where their weight was registered every 5 minutes. The leaves remained on the balances until the weight was constant, which occurred in less than 48 hours in all cases. After that, the

leaves were introduced in an oven at 60 °C until they reached constant weight, thus providing the dry weight.

2.3. Determination of stem diameter variations

In the second week of December 2007, the dendrometers were installed on the stems of the three species to determine the stem diameter variations (SDV). The dendrometers (Ecomatic DD, Munich, Germany) were installed four cm from the substrate on the main stem of each plant (four per species) facing northwest (picture 1). The measurements were registered every 30 minutes by a datalogger (Ulogger Ecomatik, Munich, Germany). The SDV had 12 bit resolution and an error of 7 μm . The MDS was calculated from the SDV as the difference in diameter between the maximum, reached just before sunrise (MXDS), and the minimum (MNDS), reached sometime during the afternoon. The stem growth rate (SGR) was calculated as the difference between two consecutive maximum daily trunk diameters (Ortuño et al. 2010). To facilitate the comparison of the MXDS and MNDS data among species, the maximum values were set at zero so that any losses were registered as negative values.

The SDV measurements were registered from 1 January to 1 September, during which time three drought periods were scheduled (winter, spring and early summer), followed by a fourth one during which the plants died (August). To confirm plant death, it was irrigated for two weeks and when it was seen that the leaves remained dry and produced no new shoots, it was considered dead.

Picture 1. Detail of dendrometer and balance installed at *C. citrinus*.

2.4. Evapotranspiration determination

The plant ET was measured gravimetrically by balances (Analytical Sartorius, Model 5201, Capacity 5.2 kg and readability of 0.01 g) with a MITRA programmer that recorded the weight and temperature every hour. Balances (three for each species) were installed at the same time as the dendrometers. On each balance, a PVC tray slightly inclined to one side and with drainage holes was placed so that the leaching could be collected. This tray also protected balances from water damage and prevented extra weight from being recorded through water leaching.

2.5. Treatments, design and statistical analysis

The plant ET and SDV of the three species were studied during irrigation and water stress periods with a randomized complete block design. There were four blocks of 20 plants per species which were set on one half of a cultivation table. The data were analyzed using a simple ANOVA with a $P \leq 0.05$. Where appropriate, the least statistical difference (LSD $P \leq 0.05$) was calculated to indicate differences between treatments. Regression analyses were also determined between SDV indices and daily plant ET, and Ψ_{leaf} . The statistical analysis was performed using Statgraphics Plus 5.1 software and the regression analysis was performed using SigmaPlot 10.0 software.

3. Results

3.1. Study of the indices derived by the stem diameter variations (SDV)

To interpret the SDV data, the MNDS, MXDS and MDS were studied for each species and representative 25 day period (Fig. 1). In winter, a water stress period of nine days was applied. *R. alaternus* showed an increasing diameter growth tendency before the stress period (Fig. 1A), but during the stress both MNDS and MXDS decreased until they reached their lowest values by the end of the water stress period; however, no changes in MDS were found. Four days after suspending the water stress, the diameter quickly recovered in terms of growth and stabilized. *C. citrinus* behaved in a very similar way, although the trends were less pronounced (more noise, Fig. 1B). However, *P. tobira* showed a great loss in MNDS by the

eighth water stress day, resulting in a high MDS; and after the first day of irrigation, both the MNDS and MXDS recovered, leading to high value of MDS.

In spring and summer during the seven and six days of water stress, respectively, only *P. tobira* showed higher MDS in the summer. *R. alaternus* behaved similarly in both seasons (Fig. 1D and G). *C. citrinus* showed a greater fall in MNDS in spring (Fig. 1E), but a less pronounced decrease in summer (Fig. 1H); the MDS response was smoother. *P. tobira* again showed the highest MNDS decrease by the end of the water stress period and sharpest recovery after irrigation, except in spring, when the MNDS did not fall significantly although MDS remained high because the MXDS continued to increase (Fig. 1F and I).

In the severe 31 day water stress period (Fig. 2), the stem growth rate (SGR) started to register negative values (diameter decreasing) in all the plants until it reached a minimum, after which it tended to increase to zero and stabilize at the end as a result of plant death (after about 14, 15 and 15 days for *R. alaternus*, *C. citrinus* and *P. tobira*, respectively). However, *C. citrinus* showed a more irregular tendency than the other two species (Fig. 2A) and it suffered a sudden decrease in SGR and an increase in MDS after 14 days of water stress (Fig. 2B). The MDS response for *R. alaternus* and *P. tobira* was a peak during the first days of water stress (just the contrary to the SGR). *C. citrinus* only showed a gradual decrease during the water stress period except for the sudden change described.

Fig. 1. Daily evolution during 25 days of MXDS, MNDS and MDS for winter, spring and summer for drought and recovery period. Drought period is between the two dotted lines (no irrigation in this period). Vertical bars show LSD at $P \leq 0.05$.

Fig. 2. Daily evolution during 31 days of SGR and MDS for drought to death in summer. Drought period starts at the dotted line (irrigation was stopped at this moment). Arrows shows the day the plants died. The large arrow indicates last irrigation before drought. Vertical bars show LSD at $P \leq 0.05$.

3.2. Evapotranspiration

Higher plant ET levels are associated with high VPD, long days and large plants (Montero et al. 2001). In general terms, daily plant ET decreased when water stress was applied and when VPD was lower (Fig. 3 and 4). In winter, the differences between the water stress period and irrigation period were slight and *C. citrinus* showed greater daily plant ET (Fig. 3A). In spring, *C. citrinus* showed the sharpest fall in daily plant ET, while *P. tobira* seemed unaffected by water stress (Fig. 3B). In all three species, the sharpest fall in plant ET, as well as the sharpest increase after irrigation was observed in summer but the daily plant ET rate was the lowest in *R. alaternus* (Fig. 3C). In severe water stress conditions a strong decrease in daily plant ET was observed, although a constant residual daily plant ET was still evident after the plants of the three species had died.

Fig. 3. Figures A, B and C show daily plant ET evolution during 25 days of *R. alaternus*, *C. citrinus* and *P. tobira* for winter, spring and summer with drought and recovery period. Drought period is between the two dotted lines (no irrigation in this period). Vertical bars show LSD at $P \leq 0.05$. D, E and F show average daily VPD values during the same period.

Fig. 4. Figure A shows daily plant ET evolution during 31 days of *R. alaternus*, *C. citrinus* and *P. tobira* for drought to death in summer. Drought period starts at the dotted line (irrigation was stopped at this moment). Vertical bar show LSD at $P \leq 0.05$. Figure B shows average daily VPD values during the same period.

3.3. Plant water relations

In the winter drought recovery period, leaf water potential (Ψ_{leaf}) decreased in *R. alaternus* and *C. citrinus* until it reached values of -2.59 MPa and -2.67 MPa, respectively. After irrigation Ψ_{leaf} increased to -1.56 MPa and -1.0 MPa for *R. alaternus* and *C. citrinus*, respectively (Fig. 5), values which can be considered reference values for shrubs without water stress (De Herralde et al., 1998; Munné-Bosch et al., 1999).

In the spring and summer drought-recovery periods, Ψ_{leaf} reached more negative values than in winter in both species (Fig. 5B and C). In the drought to death conditions, the Ψ_{leaf} just before the beginning of the water stress period was -2.55 MPa and -1.29 MPa, in *R. alaternus* and *C. citrinus*, respectively (Fig. 5D). As the drought progressed, both species reached very negative values of about -4.00 MPa and remained at this level until the leaves were dry, except in the last register of *C. citrinus*, when it was higher. As regards to the elasticity bulk modulus (ϵ , derived from P-V curves), *R. alaternus* and *C. citrinus* had high ϵ values 12.17 MPa and 15.51 MPa, respectively).

3.4. Regression analysis between daily plant ET and stem diameter indices

The regression analyses for the water stress period fitted quadratic curves, except for MDS and *R. alaternus* in spring and for MNDS and *C. citrinus* in summer, which were linear (Table 1). The curves for MXDS and MNDS were directly related to daily plant ET. However, the MDS curves showed two different daily plant ET values for the same MDS value in spring and, especially, in summer: when the daily plant ET started to decrease as a result of water stress, the MDS first increased and then decreased, showing an inverted parabola. In winter, all the regression analyses were quadratic and in *R. alaternus* only ET-MDS was significant (Table 1). In *C. citrinus*, MXDS was also significant and showed the highest R^2 for this period, while in *P. tobira* the regressions had $R^2 < 0.30$ (Table 1). However, despite the fact that some regressions were significant, the daily plant ET was fairly constant and there was no clear relationship because the curves were nearly parallel to the X axis.

Fig. 5. Leaf water potential evolution for drought and recovery periods winter (A), spring (B) and summer (C) and drought to death period (D). Arrows show irrigation moments. Horizontal bars are standard error (n=5).

Table 1. Regression analysis between daily ET and stem diameter indices during water stress and recovery periods.

	<i>R. alaternus</i>	<i>C. citrinus</i>	<i>P. tobira</i>
Winter			
ET/MXDS	ns	$y=0.096x^2-8.595x+189.2$ $R^2=0.50^*$	$y=-0.232x^2+20.36x-352.7$ $R^2=0.29^*$
ET/MNDS	ns	ns	$y=0.106x^2-3.100x-157.1$ $R^2=0.22^*$
ET/MDS	$y=-0.126x^2+8.331x-28.75$ $R^2=0.40^*$	$y=0.045x^2-3.450x+157.8$ $R^2=0.42^*$	$y=-0.339x^2+23.46x-195.6$ $R^2=0.28^*$
Spring			
ET/MXDS	$y=-0.020x^2+5.166x-138.3$ $R^2=0.65^*$	$y=-0.040x^2+9.712x-551.3$ $R^2=0.60^*$	$y = -0.194x^2 + 52.19x - 3393$ $R^2 = 0.20^*$
ET/MNDS	$y=-0.020x^2+5.859x-346.2$ $R^2=0.48^*$	$y=-0.063x^2+15.81x-1050$ $R^2=0.98^*$	$y = -0.095x^2 + 27.32x - 2045.$ $R^2 = 0.23^*$
ET/MDS	$y=-0.580x+200.9$ $R^2=0.55^*$	$y=0.022x^2-6.103x+498.6$ $R^2=0.48^*$	$y = -0.097x^2 + 24.47x - 1321.$ $R^2 = 0.11^*$
Summer			
ET/MXDS	$y=-0.001x^2+0.503x+129.6$ $R^2=0.63^*$	$y=-0.001x^2+0.777x-58.27$ $R^2=0.56^*$	$y=-0.002x^2+1.345x-5.847$ $R^2=0.85^*$
ET/MNDS	ns	$y=0.599x-227.2$ $R^2=0.90^*$	$y=0.003x^2-1.000x-46.56$ $R^2=0.87^*$
ET/MDS	$y=-0.001x^2+0.486x+112.6$ $R^2=0.30^*$	$y=-0.001x^2+0.219x+165.1$ $R^2=0.63^*$	$y=-0.005x^2+2.346x+40.71$ $R^2=0.87^*$

The number of data used for each regression were n=36 (winter) n=28 (spring) and n=24 (summer).

*Statistically significant at $P \leq 0.05$, ns (not significant).

In spring and, especially, in summer, all the R^2 increased. The highest R^2 for *R. alaternus* in both periods was ET-MXDS and for *C. citrinus* ET-MNDS with $R^2 \geq 0.90$ (Table 1). *P. tobira* showed good correlations for both indices in summer, but for spring the correlations were very weak $R^2 < 0.24$ and showed a constant daily plant ET rate (Table 1).

In the irrigation period all the regressions became linear (Table 2). In general, they showed a positive relation between daily plant ET and stem indices (the higher the daily plant ET, the more the growth) except in winter for MXDS and MNDS in *C. citrinus* and *P. tobira*, when they were inversely correlated. The R^2 were generally < 0.50 , except in summer, when *R. alaternus* and *C. citrinus* had a greater R^2 for ET-MXDS (Table 2).

Table 2. Regression analysis between daily ET and stem diameter indices during irrigation periods.

	<i>R. alaternus</i>	<i>C. citrinus</i>	<i>P. tobira</i>
Winter			
ET/MXDS	$y=1.712x+21.56$; $R^2=0.46^*$	ns	$y=-2.310x+183.3$; $R^2=0.28^*$
ET/MNDS	$y=1.306x-41.85$; $R^2=0.36^*$	$y=-0.774x+30.72$; $R^2=0.37^*$	$y=-3.903x+112.4$; $R^2=0.40^*$
ET/MDS	$y=0.406x+63.41$; $R^2=0.16^*$	$y=0.545x+47.02$; $R^2=0.29^*$	$y=1.593x+70.91$; $R^2=0.39^*$
Spring			
ET/MXDS	ns	$y=1.169x+10.89$; $R^2=0.37^*$	$y=1.623x-42.41$; $R^2=0.14^*$
ET/MNDS	ns	$y=0.830x-49.17$; $R^2=0.44^*$	$y=1.212x-142.8$; $R^2=0.17^*$
ET/MDS	$y=0.425x+29.51$; $R^2=0.49^*$	ns	ns
Summer			
ET/MXDS	$y=0.250x+7.176$; $R^2=0.76^*$	$y=0.426x-27.71$; $R^2=0.64^*$	$y=0.405x+93.98$; $R^2=0.39^*$
ET/MNDS	ns	$y=0.168x-101.7$; $R^2=0.20^*$	$y=0.521x-90.77$; $R^2=0.34^*$
ET/MDS	$y=0.226x+49.01$; $R^2=0.47^*$	$y=0.258x+73.99$; $R^2=0.41^*$	ns

The number of data used for each regression were $n=124$. *Statistically significant at $P \leq 0.05$, ns (not significant).

The regressions in the extreme water stress for ET-SGR were inverted peak functions. All the species showed a fairly constant SGR because the SGR inverted peak was produced when plant ET decreased to 100-130 mL·day⁻¹ (Fig. 6A). *R. alaternus* and *P. tobira* had moderate correlations ET-SGR and *C. citrinus* was weak. The ET-MDS regressions were quadratic, with $R^2 > 0.70$, except in *C. citrinus*, which showed the lowest R^2 (Fig. 6B) and also the most open parabola of the three. So, in *R. alaternus* and *P. tobira* the MDS tended to increase at the beginning of the water stress, but, when the daily plant ET rate reached about 200 mL·day⁻¹ in *R. alaternus* and 300 mL·day⁻¹ in *P. tobira*, the MDS started to decrease (Fig. 6B). However, in *C. citrinus*, because of its open parabola, it was difficult to see any tendency, and the MDS tended to be more or less constant until the daily plant ET had decreased to rates below 100 mL·day⁻¹. The SGR- Ψ_{leaf} correlations were three grade polynomial, which mainly described an inverted peak (more closed for *R. alaternus*) whose minimum point was between -4.00 and -3.00 MPa (Fig. 6C). The MDS- Ψ_{leaf} was only significant for *R. alaternus* and similar to the ET-MDS correlation, but with the highest point at about -3.00 MPa (Fig. 6D).

Fig. 6. Analysis of regression between daily ET-SGR (A), daily ET- MDS (B), Ψ_{hmd} -SGR (C) and Ψ_{hmd} -MDS (D) during drought to death period. The regression analysis equations are: ET-SGR $y = -177 / (1 + ((x - 100.1) / 15.65)^2)$ $R^2 = 0.48^*$ for *R. alaternus*, $y = -88.32 / (1 + ((x - 122.07) / 50.20)^2)$ $R^2 = 0.33^*$ for *C. citrinus* and $y = -5.661 - 106.6 \cdot e^{(-0.5 \cdot ((x - 125.5) / 41.03)^2)}$ $R^2 = 0.52^*$ for *P. tobira*. ET-MDS $y = -0.002x^2 + 1.343x + 74.47$ $R^2 = 0.71^*$ for *R. alaternus*, $y = -0.001x^2 + 0.748x + 122.4$ $R^2 = 0.37^*$ for *C. citrinus* and $y = -0.003x^2 + 1.899x + 42.49$ $R^2 = 0.83^*$ for *P. tobira*. Ψ_{hmd} -SGR $y = -9426.3 - 9292.03x - 3002.1x^2 - 316.8x^3$ $R^2 = 0.37$ for *R. alaternus*, $y = -227,09 - 348.21x - 174,47x^2 - 24,6x^3$ $R^2 = 0.42$ for *C. citrinus*. Ψ_{hmd} -MDS $y = -654.06 - 569.76x - 94.76x^2$ $R^2 = 0.42$ for *R. alaternus*, not significant in *C. citrinus*. * Statistically significant at $P \leq 0.05$.

4. Discussion

The stem dendrometer indices showed a response to water stress. This was especially clear in *P. tobira*, which is of great interest because of the difficulty involved in obtaining Ψ_{leaf} , suggesting that this may be an easy way to ascertain the water status of this plant. Previous studies in peach and plum found a more sensitive response of SDV to different

irrigation treatments than stem water potential (Remorini and Massai, 2003; Intrigliolo and Castel, 2004). The strongest responses were found for MXDS and MNDS in all the water stress and recovery periods, but especially in high water demanding conditions (spring and summer). This behavior could be related to the greater tissue water loss by the higher daily plant ET rates (Cohen et al., 1993; Alarcón et al., 2000; Nicolás, 2003). In the drought to death, *C. citrinus* suffered a strong decrease in diameter after 14 days (Fig. 2B), which could reflect a sudden collapse of stem tissues, leading to plant death due to strong leaf dehydration as reflected by the leaf dehydration kinetic (data not shown). This fact can explain the value of Ψ_{leaf} one day before the collapse (Fig. 5D) because only a few young leaves remained alive.

In general, MXDS had a similar response to MNDS and the diameter growth decreased under water stress, which was also observed by Wei et al. (2007) in conifers. Nevertheless, in *P. tobira* during the spring water stress period, MXDS continued to increase (Fig. 1F), while MNDS decreased slightly (Fig. 1E and 1D). The transplantation of *P. tobira* to a bigger pot in spring would have decreased the plant/substrate ratio, favoring greater water availability and maintaining daily plant ET rates constant (Fig. 3B), and the greater water availability did not permit a significant decrease in MNDS. Gallardo et al. (2006) in pepper also observed the absence of any response of the MDS to drought when the plants were cultivated in the field rather than in pots because of the greater water availability.

MDS increased in fruit trees subjected to moderate water deficit (Huguet et al., 1992). However, in this experiment this behavior was only clear in *P. tobira* for spring and summer. On other hand, in the drought to death period, *R. alaternus* showed an increasing MDS, which after suffering moderate stress ($\Psi_{\text{leaf}} < -3.00$ MPa), started to decrease again (Fig. 6D). This change in MDS behavior may be attributed to the end of water recruitment from the bark, including phloem and cambium, when the water stored in the stem is reduced (Garnier and Berger 1986; Huguet et al. 1992; Remorini and Massai, 2003). After plant death, MDS and daily plant ET were constant, probably as a result of hydration at night due to the presence of dew and the dehydration of wood and substrate during the day. Furthermore, the dendrometer could also be affected by temperature (Link et al. 1998), which would contribute to the residual MDS. In winter, none of the studied species showed any MDS response and *C. citrinus* did not show any MDS response in any period. Perhaps, the low vegetative activity and VPD in winter were not sufficient to produce an MDS response (Huguet et al. 1992; Ginestar and Castel, 1996). The lack of a clear MDS response in *C. citrinus* may be related with its hydraulic architecture, as Moriana et al. (2003) found in olive trees.

The regression analysis of these indices and the daily plant ET confirmed that there is a relationship between plant water status and dendrometric indices, as has been also reported in several studies (Ortuño et al., 2010). However, in our experiment, when daily plant ET rates were low (in winter) the correlations were weak or not significant ($R^2 \leq 50$). The regression analyses in drought to death showed SGR response to low daily plant ET and Ψ_{leaf} values, which confirmed the usefulness of this index to provide information on plant water status as reported by Moriana and Fereres (2002) in olive trees. However, in *R. alaternus* and *P. tobira* the MDS fit was only useful with daily plant ET and in *R. alaternus* with Ψ_{leaf} . Furthermore, MDS use is more suited to humid soils (Huguet et al. 1992; Ortuño et al. 2004) compared with SGR, which in this study, responded to lower daily plant ET and Ψ_{leaf} values than MDS (Fig. 6).

The irrigation period fits showed that when there was no water restriction the transpiration was greater in days with higher water demand (Nicolás, 2003). The dendrometric indices were directly related to the daily plant ET rate, except MNDS and MXDS in winter for *C. citrinus* and *P. tobira*, which may be related to no diameter growth where just higher water demand reduces the diameter because of water uptake from the stem tissues. Downes et al. (1999) described this behavior as different interactions of cambium activities and environmental conditions in two species of *Eucalyptus*.

The results described for the different periods of drought reflected different responses that depended on the environmental conditions and the species. For example, in winter, MNDS needed more days to decrease significantly than in spring and summer. However, even though the indices responded more quickly in severe environmental conditions, the Ψ_{leaf} response was usually faster (one or two days after the drought started). Under drought to death conditions, the Ψ_{leaf} was more sensitive than the dendrometric indices, as reported in the results section (SGR or MDS did not respond until $\Psi_{\text{leaf}} < -3.00$ MPa). This was contrary to the findings of other authors, e.g. Ortuño et al. 2010. This different behavior could be related with the speed at which water is used; this may activate a regulation mechanism that increases the stomatal resistance when the water reserves of the cultivation media are decreasing and affect the Ψ_{leaf} (Bañón et al. 2003). Moreover, the use of pots implies that the substrate water is used in a more uniform way compared with soil, where variability increases as the stress becomes more pronounced (Russo et al., 1994).

5. Conclusion

The dendrometric indices are sensitive to water stress in small ornamental shrubs. MXDS and MNDS showed a good response in the studied species, especially under severe environmental conditions, although Ψ_{leaf} was more sensitive to water stress. The SGR and the MDS in the drought to death period were sensitive to daily plant ET ratios between 130 and 300 mL·day⁻¹, respectively, and to $\Psi_{\text{leaf}} < -3.00$ MPa. *C. citrinus* showed no correlations with MDS- Ψ_{leaf} , and Ψ_{leaf} could not be determined in *P. tobira*; on the other hand *P. tobira* had the best R^2 for the MDS and SGR-daily plant ET correlations. The correlations obtained for the irrigation periods confirmed the different behaviors of the dendrometric indices under water stress. These results suggest that irrigation could be based on the evolution of these indices, especially SGR in *C. citrinus*, MDS in *P. tobira* and both in *R. alaternus*, although a moderate stress may result. This condition may not be a problem for ornamental shrubs, because although it may affect plant growth and quality it can also be useful for plant hardening and growth control (Bañón et al., 2003). Many different irrigation protocols involving moderate stress have been studied in fruit trees; for example, Ortuño et al. (2009) applied the protocol proposed by Goldhamer and Fereres (2004) to adult lemon trees. The application of this protocol to fruit trees may lead to a loss of production since it submits the trees to a constant deficit between irrigations, which makes application of this criterion more interesting for use in ornamental crops than in fruit trees. However, before irrigation scheduling based on SGR or MDS can be developed for ornamental shrubs, it will be necessary to compare well-irrigated plants with stressed plants and to ensure that the minimum irrigation frequency is at least every other day.

Acknowledgements

The authors thank Raquel Valdes for irrigating and supervising the shrubs while the experiment took place. This work was supported by projects CICYT (AGL 2005-05588-C02-1-2) and Fundación Séneca Región de Murcia (02991/ PI/05 and 05660/PI/07).

References

- Alarcón, J. J., R. Domingo, S. Green, M.J. Sánchez-Blanco, P. Rodríguez, and A. Torrecillas. 2000. Sap flow as an indicator of transpiration and the water status of young apricot trees. *Plant Soil* 227:77-85.
- Argo, W. R. and J.A. Biernbaum. 1994. Irrigation requirements, root-medium pH, and nutrient concentrations of Easter lilies grown in five peat-based media with and without an evaporation barrier. *J. Am. Soc. Hortic. Sci.* 119:1151-1156.
- Bañón, S., J. Ochoa, J.A. Franco, M.J. Sánchez-Blanco, and J.J. Alarcón. 2003. Influence of water deficit and low air humidity in the nursery on survival of *Rhamnus alaternus* seedlings following planting. *J. Hortic. Sci. Biotechnol.* 78:518-522.
- Burnett, S.E. and M.W. van Iersel. 2008. Morphology and irrigation efficiency of *Gaura lindheimeri* grown with capacitance sensor-controlled irrigation. *HortScience* 43:1555-1560.
- Cohen, M., T. Ameglio, P. Cruiziat, P. Archer, C. Valancogne, and L. Ferré. 1993. Las relaciones hídricas en el nogal: consumo de agua e indicadores de estrés hídrico. I Simposio Hispano-Portugués de Relaciones hídricas en las Plantas. Palma de Mallorca:93-96.
- Cohen, M., D. Goldhamer, E. Fereres, J. Girona, and M. Mata. 2001. Assessment of peach trees responses to irrigation water deficits by continuous monitoring of trunk diameter changes. *J. Hortic. Sci. Biotechnol.* 76:55-60.
- De Herralde, F., C. Biel, R. Savé, M.A. Morales, A. Torrecillas, J.J. Alarcón and M.J. Sánchez-Blanco. 1998. Effect of water and salt stresses on the growth, gas exchange and water relations in *Argyranthemum coronopifolium* plants. *Plant Sci.* 139:9-17.
- Downes, G., C. Beadle, and D. Worledge. 1999. Daily stem growth patterns in irrigated *Eucalyptus globulus* and *E. nitens* in relation to climate. *Trees* 14:102-111
- Fereres, E. and D. Goldhamer. 2003. Suitability of stem diameter variation and water potential as indicators for irrigation scheduling of almond trees. *J. Hortic. Sci. Biotechnol.* 78:139-144.
- Gallardo, M., R.B. Thompson, L.C. Valdez, and M.D. Fernández. 2006. Response of stem diameter variations to water stress in greenhouse-grown vegetables crops. *J. Hortic. Sci. Biotechnol.* 81:483-495.

- García-Orellana, Y., M.C. Ruíz-Sánchez, J.J. Alarcón, W. Conejero, M.F. Ortuño, E. Nicolás, and A. Torrecillas. 2007. Preliminary assessment of the feasibility of using maximum daily trunk shrinkage for irrigation scheduling in lemon trees. *Agric. Water Manag.* 89:167-171.
- Garnier, E. and A. Berger. 1986. Effect of water stress on stem diameter changes of peach trees growing in the field. *J. Appl. Ecol.* 23:193-209.
- Ginestar, C. and J.R. Castel. 1996. Use of stem dendrometers as indicators of water stress in drip-irrigated citrus trees. *Acta Hort.* 421:209-219.
- Goldhamer, D. and E. Fereres. 2001. Irrigation scheduling protocols using continuously recorded trunk diameter measurements. *Irrig. Sci.* 20:115-125.
- Goldhamer, D. and E. Fereres, E. 2004. Irrigation scheduling of almond trees with trunk diameter sensors. *Irrig. Sci.* 23:11-19.
- Goldhamer, D., E. Fereres, and M. Salinas. 2003. Can almond trees directly dictate their irrigation? *Calif. Agric.* 57:138-144.
- Huguet, J., S. Li, J. Lorendeau, and G. Pelloux. 1992. Specific micromorphometric reactions of fruit trees to water stress and irrigation scheduling automation. *J. Hortic. Sci.* 67:631-640.
- Intrigliolo, D. S. and J.R. Castel. 2004. Continuous measurement of plant and soil water status for irrigation scheduling in plum. *Irrig. Sci.* 23:93-102.
- Li, S., J.G. Huguet, and C. Bussi. 1989. Irrigation scheduling in a mature peach orchard using tensiometers and dendrometers. *Irrig. and Drainage Syst.* 3:1-12
- Link, S., M. Thiede, and M. van Bavel. 1998. An improved strain-gauge device for continuous field measurement of stem and fruit diameter. *J. Exp. Bot.* 49:1583-1587
- 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.
- Molz, F. J. and B. Klepper. 1973. On the mechanism of water-stress-induced stem deformation. *Agron. J.* 65:304-306.
- Montero, J. I., A. Antón, P. Muñoz, and P. Lorenzo. 2001. Transpiration from geranium grown under high temperature and low humidity in green houses. *Agric. For. Meteorol.* 107:323-332.

- Moriana, A. and E. Fereres. 2002. Plant indicators for scheduling irrigation of young olive trees. *Irrig. Sci.* 21:83-90
- Moriana, A., F. Orgaz, E. Fereres, and M. Pastor. 2003. Yield responses of a mature olive orchard to water deficits. *J. Amer. Soc. Hortic. Sci.* 128:425-431.
- Munné-Bosch, S., S. Nogués, and L. Alegre. 1999. Diurnal variations of photosynthesis and dew absorption by leaves in two evergreen shrubs growing in Mediterranean field conditions. *New Phytol.* 144:109-119.
- Navarro, A., S. Bañón, 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.
- 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.
- Nicolás, E. 2003. Utilización de las medidas de flujo de savia para el estudio de las relaciones hídricas del albaricoquero (*Prunus armeniaca* L.). Tesis Doctoral. Universidad Politécnica de Cartagena:262.
- Nortes, P. A., A. Pérez-Pastor, G. Egea, W. Conejero, and R. Domingo. 2005. Comparison of changes in stem diameter and water potential values for detecting water stress in young almond trees. *Agric. Water Manag.* 77:296-307.
- Ortuño, M. F., J.J. Alarcón, E. Nicolás, and A. Torrecillas. 2004. Interpreting trunk diameter changes in young lemon trees under deficit irrigation. *Plant. Sci.* 227:275-280.
- Ortuño, M.F., W. Conejero, F. Moreno, A. Moriana, D.S. Intrigliolo, C. Biel, C.D. Mellisho, A. Pérez-Pastor, R. Domingo, M.C. Ruíz-Sánchez, J. Casadesus, J. Bonany, and A. Torrecillas. 2010. Could trunk diameter sensors be used in woody crops for irrigation scheduling? A review of current knowledge and future perspectives. *Agric. Water Manag.* 97:1-11.
- Ortuño, M. F., Y. García-Orellana, W. Conejero, F. Pérez-Sarmiento, and A. Torrecillas. 2009. Assessment of maximum daily trunk shrinkage signal intensity threshold values for deficit irrigation in lemon trees. *Agric. Water Manag.* 96:80-86.
- Ortuño, M. F., Y. García-Orellana, W. Conejero, M.C. Ruíz-Sánchez, J.J. Alarcón, and A. Torrecillas. 2006. Stem and leaf water potentials, gas exchange, sap flow, and trunk diameter fluctuations for detecting water stress in lemon trees. *Trees* 20:1-8.
- Remorini, D. and R. Massai. 2003. Comparison of water status indicators for young peach trees. *Irrig. Sci.* 22:39-46.

- Ritchie, G. A. and T.M. Hinckley. 1975. The pressure chamber as an instrument for ecological research. *Av. Ecol. Res.* 9:165-254.
- Russo, D., J. Zaidel, and A. Laufer. 1994. Stochastic analysis of solute transport in partially saturated heterogeneous soil 1. Numerical experiments. *Water Resour. Res.* 30:769-779.
- Scholander, P., H.T. Hammel, E.D. Bradstreet, and E.A. Hemmingeen. 1965. Sap pressure in vascular plants. *Science*, 148:339-346.
- Sivyer, D., J.R. Harris, N. Persaud, and B. Appleton. 1997. Evaluation of a pan evaporation model for estimating post-planting street tree irrigation requirements. *J. Arboricult.* 23:250-256.
- Snyder, R., R.H. Shaw, and K.T. Dawld. 1986. Vapour pressure deficit and other psychrometric properties of air from temperature and relative humidity. *Agric. Res.* 2:183-192.
- Turner, N. C. 1988. Measurement of plant water status by the pressure chamber technique. *Irrig. Sci.* 9:289-308.
- Wei, X., W. Yanhui, Y. Pengtao, L. Hailong, S. Zhongjie, and G. Wei. 2007. Growth in stem diameter of *Larix principis-rupprechtii* and its response to meteorological factors in the south of Liupan Mountain, China. *Acta Ecol. Sin.* 27:432-441.
- Wilson, J. R., M.J. Fisher, E.D. Schulze, G.R. Dolby, and M.M. Ludlow. 1979. Comparison between pressure-volume and dewpoint-hygrometry technique for determining the water relations characteristics of grass and legume leaves. *Oecologia* 41:77-88.
- Yatapanage, K.G. and H.B. So. 2001. The Relationship between Leaf Water Potential and Stem Diameter in Sorghum. *Agron. J.* 93:1341-1343.

Chapter 3

***Rhamnus alaternus* growth under four simulated shade environments: Morphological, anatomical and physiological responses**

Authors: J. Miralles¹, J.J. Martínez-Sánchez^{1,2}, J.A. Franco^{1,2} and S. Bañón^{1,2}

¹Dpto. de Producción Vegetal, ETSIA, Universidad Politécnica de Cartagena (UPCT), Paseo Alfonso XIII, 48. 30203 Cartagena (Murcia), Spain

²Unidad Asociada al CSIC de Horticultura Sostenible en Zonas Áridas (UPCT-CEBAS), Paseo Alfonso XIII, 48. 30203 Cartagena (Murcia), Spain

Received for publication 6 August 2010. Received in revised form 25 October 2010.
Accepted for publication 9 December 2010.

Article reference:

Miralles, J., J.J. Martínez-Sánchez, J.A. Franco, and S. Bañón. 2011. *Rhamnus alaternus* growth under four simulated shade environments: Morphological, anatomical and physiological responses. *Scientia Hort.* 127:562-570.

Year 2009:

Impact factor: 1.197

Category: Horticulture

Category ranking 2008: (8/30).

Quartile in category: Q2

Abstract

Light management and species light plasticity are critical factors for proper crop production in nurseries. So, to assess the light necessities of *Rhamnus alaternus*, which is a promising native evergreen Mediterranean shrub of ornamental and ecological interest, it was exposed to five shade levels (0%, 32%, 48%, 84% and 93%) for one year (0% of shade received annual mean maximum photosynthetic photon flux density [PPFD] of $1293 \pm 415 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ with a summer mean maximum PPFD of $1541 \pm 225 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ [mean $\pm$ s.d.]). The treatments providing 84% and 94% shading produced less compact plants, with greater height and less stem diameter, greater specific leaf area and higher shoot/root index. In general, the shading produced dark green leaves except the 94% treatment, in which the leaves were lighter because the relative chlorophyll content was reduced. Leaf thickness was reduced in all the shading treatments compared with the control. The stomatal density and pore area were reduced with the 84% and 93% shading treatments, as was photosystem II (PSII) efficiency and the leaf soluble sugar content (falling to 60% of the control level); in contrast, non-photochemical quenching increased. The 93% treatment caused irreversible damages in PSII because dark-adapted PSII efficiency was reduced. The results show that solar radiation can be used as a tool to improve plant quality during the nursery period. We conclude that shading screens providing more than 84% shade are not recommendable in terms of photochemical activity, soluble sugar content and overall plant quality. Shading screens of 32% and 48% improve *R. alaternus* quality in spring and summer, respectively. As regards its interest as an understory species for reforestation programs, we also conclude that this shrub can be adapted to a wide range of shade intensities, although its proper development would be compromised in very strong shading conditions.

Keywords: pot plant, chlorophyll fluorescence, water relations, soluble sugars, leaf anatomy.

1. Introduction

The acquisition and effective use of light are crucial for seedling establishment in the forest understory. Light varies greatly both in time and space in the Mediterranean environment, and this phenomenon intermittently hampers or accelerates plant growth (Montgomery and Chazdon, 2002). It is therefore necessary to know the shade plasticity of any species for both nursery production and reforestation success because, as reported by Villar-Salvador (2004), low quality plants might exhibit morphological and physiological

characteristics that impair their performance under stressful conditions. Appropriate conditioning techniques used in the nursery during seedling production are crucial for the establishment, survival and subsequent growth of plants after transplanting (Franco et al., 2006). Though it is not the only way for plants to acclimate to a changing environment, morphological plasticity in seedlings is thought to be an important adaptation to shade (Messier and Nikinmaa, 2000) and has received much attention in forest ecology literature (e.g. DeLucia et al., 1998).

Rhamnus alaternus L. is a perennial dioecious shrub distributed throughout the Mediterranean Basin, which flowers during late winter and early spring, with a peak in mid-February. It produces fleshy fruits that ripen in late spring and early summer, and thus represents an important water and nutrient source for birds and small mammals (Gulias et al., 2004). These characteristics make *R. alaternus* an important understory species of the Mediterranean area, where it is well adapted to high temperature, high solar radiation and vapor pressure deficit (VPD) (Martínez-Sánchez et al., 2008). Besides, it has a high ornamental value because of its bright green leathery foliage, which, with its small red fruits, creates a very pleasing effect. It is also well adapted to growing in pots. However, there is still uncertainty as regards the shade tolerance classification of *R. alaternus* and its physiological traits in response to light stress. As reported by Poorter (1999), species classified as shade tolerant are able to germinate, grow and survive in low light environments (1-5% of daylight), whereas shade intolerant species require high light environments (>50% of daylight) to germinate and grow. However, characterization of inter- and intra-specific variations in shade tolerance is poor because the physiological basis for these variations is not well defined (Carter and Klinka, 1992).

Both the light level and the species in question can lead to differences in photosynthetic pigments, electron transport, photosynthesis rates, etc. (Boardman, 1977). These differences result in morphological and biochemical adaptive changes in response to these conditions (Lichtenthaler et al., 2007). The changes that result from greater shading are thinner leaves with greater leaf area and different colors, this last aspect affected by higher concentrations of chlorophyll per unit dry weight (Naidu and De Lucia, 1998; Niinemets, 1999).

At the physiological level, light changes affect growth and productivity of plants, due to its influence on carbon assimilation (Cannell et al., 1987). In general, sunny leaves have greater efficiency in the use of quantum and thus high photosynthetic capacity per leaf area (Niinemets and Tenhunen, 1997; Wayne and Bazzaz, 1993). This may contribute to

explaining why switching high-light grown leaves to low irradiance conditions has been found to be associated with substantial decreases in foliar levels of total non-structural carbohydrates (Veneklaas and den Ouden, 2005).

The amount of chlorophyll fluorescence provides quantitative information on the stabilized photosynthesis state and on the plant protective mechanisms against stress induced by excess radiation (Meng et al., 2001), which makes it an important tool for evaluating the effect of light. Evapotranspiration (ET) can also be affected by shade, as mentioned by Rhizopoulou et al. (1991), who observed that sunny leaves under severe evaporation reduce water loss through lower leaf size and stomatal conductance, and increased thickness. Leaf water potential is less affected by leaves under shade (Mendes et al., 2001). So, the ET will influence water consumption and plant water status, which is a very important issue in semi-arid Mediterranean conditions, where water is a limiting factor. Therefore, shade can have a positive effect on irrigation scheduling (Sorrentino et al., 1997) to modify the water use efficiency (WUE) because this variable is influenced by both photosynthesis and stomatal conductance (Donovan, 1994).

The aim of this work was to study the shade plasticity of *R. alaternus* to evaluate its potential use in reforestation and the best light level for its nursery production. The effects of five illumination levels were evaluated on morphological (plant architecture), anatomical (distribution of biomass, leaf anatomy, leaf area and leaf color) and physiological (photosynthesis, chlorophyll, stomatal conductance, fluorescence and leaf water potential) parameters of *R. alaternus* plants.

2. Material and methods

2.1. Plant material and crop conditions

One year old seedlings of *R. alaternus* from wild seeds were used, grown in 60 multi-pot forest trays measuring 60 cm (length) $\times$ 30 cm (width) $\times$ 17 cm (height) (240 cm³ volume) with an inverted pyramid form (Plasnor S.A. Legazpi, Spain). The pots were arranged in a 10 $\times$ 6 configuration and the trays were placed in the greenhouse with a 50% shading screen during its first year. Fifty percent shading during this nursery period is used by the forest nursery (Viveros Ajauque S.L., Fortuna, Spain) to germinate and grow forest crops in forest trays. Their experience with *R. alaternus* is considered acceptable, because they have had

good success after field-transplantation in reforestation programmes. The seedlings consisted of a main vertical shoot of about 25-30 cm with nearly no secondary shoots and simple leaves inserted in the shoot. In April 2007 the seedlings were transplanted to pots with a square section on the upper surface of 15×15 cm, 11×11 cm at the base and 20 cm high (2.8 L volume). The substrate consisted of black peat, coco fiber and perlite (40/40/20, v/v/v), and was amended with $2 \text{ g}\cdot\text{L}^{-1}$ of a slow release fertilizer (Osmocote 10-11-18 +2 + plus microelements; release time between 5 and 6 months at 21°C ; The Scotts Company LLC, OH). A total of 300 seedlings were transplanted to the pots (one seedling per pot), for establishment. The plants were grown in a greenhouse for two months in full sunlight, after which, 180 plants were selected for the experiment (the most homogeneous). These were pinched at 20 cm height, and placed on tables in the different shading conditions (Fig. 1). The experiment started at this time (the first week of June 2007) and finished the second week of October 2008 (one year, four months and two weeks later).

Fig. 1. *R. alaternus* after pinching at 20 cm of height in 0% shading treatment.

The experiment was conducted at the Tomas Ferro Experimental Agro-food Station of the Technical University of Cartagena (UPCT) on the Mediterranean coast of Spain (lat. $37^\circ 36'52'' \text{N}$, long. $0^\circ 58'07'' \text{W}$). On two outside concrete platform of 90 m^2 where three repetitions of five metal crop tables were placed and covered with shading screens (black mesh windbreak to UV stabilized, monofilament composed of polyethylene and high density

polyethylene raffia; PROJAR SA, Valencia, Spain) with a different percentage of shade provided by the supplier: 25% (S25), 50% (S50), 65% (S65) and 80% (S80). The control tables had no shading screens (0% shade S0). Environmental climatic conditions (Fig. 2) were recorded from August 2007 to October 2008 for each of the treatments by three micro-HOBO (HOBO Micro Station Data Logger H21-002 MicroDAQ.com, Ltd., Contoocook, NH) which recorded the hourly average values of temperature ($^{\circ}\text{C}$), humidity (%) and PAR on each of the tables (Temperature/RH Smart Sensor S-THB-M008, MicroDAQ.com, Ltd., Contoocook, NH; HOBO Photosynthetic Light (PAR) sensor S-LIA-M003 Smart Sensor MicroDAQ.com, Ltd., Contoocook, NH). This data showed that the shading percentage provided by the supplier did not correspond with percentage obtained from the real PPFD measurements, so real shading percentages for each treatment were: $32\pm 8\%$ for S25, $48\pm 7\%$ for S50, $84\pm 6\%$ for S65 and $93\pm 2\%$ for S80 (mean $\pm$ s.d.). The real PPFD data were obtained by averaging the percentage of PPFD reduction for each day and shading treatment. The percentage of reduction was calculated as: $((\text{PPFD no shade} - \text{PPFD shade}) / \text{PPFD no shade}) \times 100$, where PPFD no shade is the real PPFD for the no shade treatment S0 and PPFD shade is the real PPFD for each shading treatment with its respective shading net S25, S50, S65 and S80.

Fig. 2. Mean maximum daily photosynthetic photon flux density (PPFD) in the different shading treatments (from 1 August 2007 to 31 October 2008).

The crop table dimensions were 3 m long, 1.30 m wide and 0.80 m high. On each of the tables, 12 pots were placed and arranged in two rows of six separated by 1 m. Plants in the same row were 0.4 m apart. The described plant arrangement ensured that plants did not shade each other. The shade screens were held up by rectangular, 1.80 m long and 0.30 m wide supports (two supports on opposite sides of the table). The supports were attached to the table by steel wire of 0.5 mm diameter. Between the supports, plastic wire of 2 mm diameter was fixed to support the shading screen. Finally, to achieve greater tension in the mesh and wind resistance, it was clamped to the table with steel crocodile clips. The distance from the table base to the screen top was about 1 m, which ensured that the plants could develop properly without touching the screen (Picture 1).

Picture 1. Experimental table design, 0% of shading (front) and 32% of shading (behind).

The plants were irrigated using two arrow-drippers installed on opposite sides of the pot which were connected to an emitter of $2 \text{ L} \cdot \text{h}^{-1}$ with two microtubules. The amount of water applied was set to produce 15-20% leaching in the control treatment and irrigation frequency was fixed to avoid excessive drying of the substrate, which would affect its rehydration. This volume changed in each season, but the water supplied to the plants in each season was the same in all treatments. Thus, in fall and winter each irrigation lasted about 10

minutes, but about 15 minutes in spring and summer. The average contribution in fall was 400 mL·pot⁻¹ three times a week, in winter 475 mL·pot⁻¹ twice a week, in spring 560 mL pot⁻¹ three times a week, and in summer 570 mL·pot⁻¹ three times a week.

2.2. Measurements of DW, leaf area and growth indexes

In the first week of October 2008, leaf area and dry weight (DW) of roots, stem and leaves was determined in six plants per treatment (two from each replicate). The leaf area 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, 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 series precision balance (sensitivity of 10 mg and up to 1200 g, Gram Precision SL, Barcelona, Spain).

The growth indexes determined were the shoot DW/root DW (S/R) and the specific leaf area (SLA) (leaf area/leaf DW).

2.3. Measurements of water evapotranspired, leaching and water use efficiency

The water applied to the pots during the experiment was determined by irrigation time, and was verified using plastic graduated beakers which collected the water from a single dripper. Drainage was recorded in three plants per treatment (one per repetition) placing the plants on a tray made of polyethylene (38 cm wide x 53 cm long x 9 cm height) and then measured in 1000 mL graduated beakers. Both, irrigation volume and leaching were performed once a week throughout from October 2007 to September 2008. The water evapotranspired was determined as the difference between water supplied and leached. The water use efficiency (WUE) was expressed by the ratio of total DW gain/water evapotranspired throughout the experimental period.

2.4. Measurements of leaf color and relative chlorophyll content (RCC)

The color and RCC measurements were made for 12 plants of each treatment (four per repetition) the first week of October 2008. For the determination of both, representative plant

leaves were chosen, taken from south-facing mid-height and mature. 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 (L), tone (hue angle, H) and saturation (chrome, C). The RCC was measured using the same criteria as for color but with a SPAD-502 chlorophyll meter (Konica Minolta Sensing, Inc., Osaka, Japan) which estimates RCC with the light transmitted through the leaf at 650 nm (wave length photosynthetically active) and 940 nm. For each measurement the average of three shots was determined.

2.5. Measurement of gas exchange and leaf water potential

The midday stomatal conductance (g_s) and net photosynthesis rate at midday (P_n) were measured using a CIRAS-2 Portable Photosynthesis System (PP Systems, Amesbury, MA). The flow rate of the chamber was $200 \text{ mL} \cdot \text{min}^{-1}$ with 350 ppm of CO_2 , 100% relative humidity and ambient light (a sunny day during the week that measurements were made was chosen to ensure homogeneity in the measurements).

Leaf water potential at midday (Ψ_{hmd}) was estimated in accordance with the method described by Scholander et al. (1965), using pressure chamber (Soil Moisture-Hammel, mod. 3000, Soil Moisture Equipment Co, Santa Barbara, CA), in which the leaves were placed within 20 seconds of collection and increasing the pressure at $0.02 \text{ MPa} \cdot \text{s}^{-1}$ (Turner, 1988).

The measurements were made in six plants per treatment (two from each repetition) in the last week of September 2007, December 2007, March 2008, June 2008 and September 2008. A sunny day within the week was chosen. The leaves were selected with the same criteria as described for the color measurement.

2.6. Measurement of plant architecture and compactness index

To determine the plan architecture, the following parameters were measured: plant height, shoot number and shoot length (those born from the main stem), and base stem diameter. All the parameters were determined in the same weeks as the gas exchange and Ψ_{hmd} in 12 plants per treatment (four from each repetition), and also in 15 plants for the plant morphology characterization in the first week of June 2007 before the experiment began. The diameter was determined using an electronic gauging device SYLVAC (sensitivity of 0.01

mm and maximum 150 mm, TECMICRO SA, Madrid, Spain) and a yellow flexometer Blister (3 m long, 1 mm precision, Germans Boada S.A., Rubi, Spain) for the shoot length and plant height. The compactness index was determined as: $(\text{total number of shoots}+1)/(\text{shoot length}+\text{height})$. This index was developed in this research and tested for this species, which is more compact when the plants have shorter shoots and a greater number of the same.

2.7. Measurement of leaf cell layers in cross section

The cell layer thickness was determined in six leaves of different plants from the same treatment (two from each replicate) during the second week of October 2008, selecting the leaves using the same criteria as for color measurements. The leaves were picked immediately prior to measurement to prevent dehydration, thus giving the thickness of the cell layers in cell turgid conditions. The layer measurements assessed were: upper cuticle, upper epidermis, palisade parenchyma, spongy parenchyma, lower epidermis and lower cuticle. Cross section samples were obtained using a manual microtome blade with a precision of 10 μm (Shibuya, SH-551, Microciencia S.A., Barcelona, Spain). Subsequently, the sample was placed on a slide with water and covered using a cover glass before observation with an optical microscope Olympus model phases BX41TF (Olympus corporation, Tokyo, Japan) with a magnification of 200. The thicknesses were measured using the lens strip.

2.8. Measurement and extraction of soluble sugar contents

To determine the concentration of leaf soluble sugars (glucose, sucrose and fructose) 10-20 leaves per plant (depending on leaf size) were collected the second week of October 2008. Mature leaves from mid height were chosen. A total of six samples from each treatment (two from each repetition) were collected for the extraction. The leaves of each sample were cleaned with ethanol in the laboratory, cut by scissors into little pieces and 1 g was weighed on a GRAM ST Series Accuracy scales (sensitivity of 10 mg up to 1200 g, Gram Precision SL, Barcelona, Spain). Then the 1 g of pieces was introduced into a 100 mL beaker with 50 mL of ethanol 80%. The mixture was blended with an Ultraturrax T-25 (ITC. SL, Barcelona, Spain) for 1 min at 10,000 rpm. The beaker was then introduced in a 80 °C water bath for 15 min using an analogue magnetic stirrer model ACS-100 C / C "(ITC, SL, Barcelona, Spain), introducing a magnetic stirrer bar in the water to maintain temperature homogeneity. The mixture was filtered using a DP 145 110 filter paper (Albet-Hahnemuehle S.L., Barcelona,

Spain) and a funnel, and collected in a round based flask. To wash the sample, another 50 mL of 80% ethanol was added. After a second filtering, the flask was placed at a rotary evaporator R-210/215 (BÜCHI Labortechnik AG, Flawil, Switzerland) with a water temperature of 50 °C and a vacuum pump RD model (DVP Vacuum Technology SLR, San Pietro in Casale, Italy). Once all the ethanol had evaporated, the sugars were collected by shaking 2 mL of water five times to dissolve the sugars stuck to the wall. The 10 mL solution was filtered through 0.45 µm Millipore Millex-HN nylon microfilters (Millipore Corporation, MA) and 2 mL were introduced in a test tube using a micropipette. Finally, 8 mL of distilled water were added to obtain 10 mL for sugar determination by ion-exclusion liquid chromatography, as described by Chinnici et al. (2005).

2.9. Stomatal density and pore size

Stomatal characterization involved observing and counting the stomata on images made by scanning electron microscope (SEM). Three pieces from the upper and three pieces from the lower surfaces, which were cut from the right middle part of six leaves per treatment (two from each replicate, one for upper and other for lower), were collected in the second week of October 2008, following the criteria described for color. The collected leaves were cleaned with ethanol prior to being cut into pieces. The samples (pieces) were dehydrated in graded acetone (30%, 50%, 70%, 90%, 100%, 15 min each), critical point dried with a critical point dryer (Leica Microsystemas S.L.U., Barcelona, Spain) and attached to aluminium stubs. The specimens were coated with gold during 60 seconds at 2.2 kV and 15 mA (Metals Bio-Rad Polaron Division 200A, Quorum Technologies Ltd., East Grinstead, UK) and viewed in a SEM (Hitachi S3500N, 0.5-30 kW, Hitachi Ltd., Tokyo, Japan) operating at 5 kV. Digital photos were taken at 500-2000 increments to determine stomatal density and pore size, respectively. Measurements were performed using the image analysis software ImageJ ver. 1.43 (McGill University, Montreal, QC, Canada).

To calculate the single pore area the ellipse area formula was used: $\text{area} = \pi (a \times b)$ (a and b are the semiaxes of the ellipse, i.e. half the vertical and horizontal diameters). For each sample, the average of six single pores was determined. To calculate the individual leaf pore area, and plant pore area, the following formulas were used: 'individual leaf pore area' $\text{mm}^2 = (\text{'individual leaf area' mm}^2 \times \text{'stomatal density' stomas} \cdot \text{mm}^{-2}) \times (\text{'single pore area' mm}^2)$; 'plant pore area' $\text{mm}^2 = \text{'individual leaf pore area' mm}^2 \times \text{'plant leaf area' mm}^2$.

2.10. Measurement of chlorophyll fluorescence

The chlorophyll fluorescence was measured in the last week of September 2008 in six leaves per treatment (two per replicate) using a Pulse Modulated Fluorimeter FMS-2 (Gomensoro Scientific Instrumentation, SA, Madrid, Spain). The leaves measured were chosen following the criteria described for color. The method and parameter determined were the same as described by Sheng et al. (2008).

2.11. Statistical analysis

The shading effects were studied with a randomized complete block design. There were three blocks of 12 plants per treatment which were set on a crop table. The data were analyzed using a simple ANOVA with a $P < 0.05$. When the ANOVA indicated significant effects, means were separated using the least significant difference test (LSD), with $P < 0.05$ considered to be statistically significant. The statistical analysis was performed using Statgraphics Plus 5.1 software (Manugistics Inc., Rockville, MD).

3. Results

3.1. Shoot evolution

Shading stimulated longitudinal growth of the primary shoots (Fig. 3A). Three patterns of behavior were observed in: (i) control plants, which developed shorter shoots, (ii) plants grown under S25 and S50 treatments, which developed shoots of intermediate length, and (iii) plants cultivated with the two highest levels of shading, which developed the longest shoots. Four months after pinching, S65 and S80 plants showed lower development as regards the number of shoots. This parameter increased sharply in control plants in winter and increased slowly during the spring and summer (Fig. 3B). S25 and S50 treatments led to almost identical plant evolution but the stronger shading reduced sprouting during spring and summer, which resulted in a lower number of shoots for S65 and S80 plants at the end of the year. Shading greatly favored plant height following pinching at the beginning of fall (Fig. 3C). However, from this point to the end of the experiment, the control plants grew more, although the shading treatments produced taller plants at the end of the experiment. Plants

under S80 in the winter were shorter than in the spring due to shoot fall caused by very long and shoots of poor consistency. The base stem diameter showed an opposite evolution to height, because the shading reduced diameter growth, resulting in greater diameter for the S0, S25 and S50 plants. Spring stimulated diameter growth in all cases (Fig. 3D).

Fig. 3. Seasonal evolution of average shoot length (those born from the main stem) (A), number of shoots per plant (B), plant height (C) and main stem diameter (D). Measurements correspond to the first week of June 2007 (the beginning of experimentation), and to the last week of September 2007, December 2007, March 2008, June 2008 and September 2008. Vertical bars indicate SE (n=12).

3.2. Plant growth and leaf color

Plant DW was only reduced by S80, especially in roots (62% less than in the control). Shoot DW was also affected (27% less than in the control) (Table 1). This fact increased the S/R ratio from 3.7 (control) to 7.4 in S80. S65 also increased its S/R despite there were no statistical differences in DW. S65 and S80 increased plant leaf area to nearly double that of the control. Control plants had the greatest number of leaves and S80 the least (about 40% less); the rest of treatments were similar and showed intermediate values (Table 1). These

results meant that individual leaf area became 1.91 and 2.34 times greater for S65 and S80, respectively, compared with control. The SLA increased with the degree of shading, particularly in S80 (255% compared with the control). The compactness index was reduced by shading, particularly in S65 and S80, with S25 and S50 showing intermediate values (Picture 2).

The colorimetry study showed that control plants had the lowest green tone, S25 and S50 had intermediate values, and S65 and S80 had the highest values. Luminosity was lower in S65 than in S80. The saturation only increased in control plants (Table 1).

Table 1. Effect of shading on growth, development and leaf color.

Parameters	Shading (%)				
	0 (S0)	32 (S25)	48 (S50)	84 (S65)	93 (S80)
Plant DW (g)	143.3 b	159.2 b	162.0 b	146.7 b	93.9 a
Shoot DW (g)	112.8 b	121.5 b	131.9 b	121.6 b	82.2 a
Root DW (g)	30.5 c	37.7 d	30.0 cd	25.1 b	11.6 a
Shoot/root	3.70 a	3.26 a	4.41 ab	4.71 b	7.38 c
Plant leaf area (dm ²)	25.39 a	20.19 a	23.78 a	41.19 b	49.23 b
Leaves per plant	1484 c	957 b	1057 b	1261 b	888 a
Individual leaf area (cm ²)	1.71 a	2.11 a	2.25 a	3.27 b	5.54 c
Specific leaf area (cm ² /g)	43.9 a	57.2 b	62.3 b	97.9 c	156.2 d
Compactness index	0.27 a	0.10 b	0.10 b	0.05 c	0.05 c
Color Tone (H)	106.0 a	109.3 b	111.8 b	115.4 c	116.5 c
Luminosity (L)	35.9 b	35.6 b	35.0 b	32.9 a	37.9 c
Saturation (C)	22.0 b	19.6 a	19.9 a	19.8 a	19.1 a

*Different letters in the same row indicate statistically significant differences between means at $P < 0.05$ according to the least significant difference (LSD) test.

3.3. Leaf soluble sugars concentration

The treatments S25 and S50 did not affect the total soluble sugar concentration in leaves compared with the control, but stronger shading (S65 and S80) reduced it by about 40% (Table 2). Sucrose and fructose were affected in the same way, the former being more concentrated. Glucose concentration was significantly different between treatments, however, this behavior was unrelated with shading (Table 2).

Picture 2. Representative plants after nearly one year of experimentation. Left *R. alaternus* with 0% of shading, right *R. alaternus* with 93% of shading.

Table 2. Effect of shading on leaf soluble sugar concentrations (mg g^{-1} DW).

Parameters	Shading (%)				
	0 (S0)	32 (S25)	48 (S50)	84 (S65)	93 (S80)
Total sugars	120.3 b	112.8 b	115.5 b	71.0 a	66.1 a
Glucose	8.3 c	5.2 b	8.1 c	3.2 a	7.7 c
Fructose	5.0 c	2.2 b	3.9 bc	0.8 a	1.2 a
Sucrose	107.0 b	105.4 b	103.6 b	67.1 a	57.2 a

*Different letters in the same row indicate statistically significant differences between means at $P < 0.05$ according to the least significant difference (LSD) test.

3.4. Leaf blade thickness and stomatal characteristics

Leaf blade thickness fell as shading increased in intensity (Table 3) between 25% (S25) and 62% (S80) compared with the control (Picture 3). The epidermis (upper and lower) was not affected. The upper cuticle thickness was reduced by half in S80. The lower cuticle was affected by all the shading treatments, especially by S80 (57% thinner than the control) (Picture 4). The parenchyma tissues were the thickest layers, especially spongy parenchyma which represented about half leaf thickness. These layers were reduced in thickness by shading of 48% and above. The spongy parenchyma was more strongly reduced, by about 60% in S80 (Picture 3).

Stomatal density was only reduced by S65 and S80 (about 37% compared with the control) (Picture 5). Compared with the control, single pore area increased in S25 and S50, but was reduced by S65 and S80. The individual leaf pore area was only smaller in the control, and the plant leaf pore area was smaller in both control and S25 (Table 3).

Table 3. Effect of shading on transversal leaf section dimensions (μm) and stomatal characteristics.

Parameters	Shading (%)				
	0 (S0)	32 (S25)	48 (S50)	84 (S65)	93 (S80)
Upper cuticle	10.0 a	8.8 ab	8.1 ab	6.9 bc	5.0 c
Upper Epidermis	18.8 a	15.0 a	15.0 a	13.8 a	11.9 a
Palisade parenchyma	170.0 a	145.0 a	88.8 b	101.8 b	48.8 c
Spongy parenchyma	253.8 a	173.8 a	125.0 b	115.6 bc	100.0 c
Lower epidermis	15.6 a	12.5 a	12.5 a	10.6 a	14.4 a
Lower cuticle	16.3 a	7.5 b	8.8 b	8.1 b	5.6 b
Stomatal density (stomas/ mm^2)	197.0 b	197.0 b	189.0 b	165.0 a	135.0 a
Single pore area (μm^2)	19.3 b	25.4 c	25.5 c	17.6 a	17.2 a
Individual leaf pore area (mm^2)	0.65 a	1.05 b	1.08 b	0.95 ab	1.29 b
Plant pore area (mm^2)	965 a	1009 a	1145 b	1193 b	1145 b

*Different letters in the same row indicate statistically significant differences between means at $P < 0.05$

according to the least significant difference (LSD) test.

Picture 3. Images obtained with optical microscopy. Cell layers thickness of treatments 0% of shading (left) and 93% of shading (right).

Picture 4. Images obtained with optical microscopy. Cuticle thickness of treatments 0% of shading (left) and 93% of shading (right).

Picture 5. Images obtained with electron microscopy. Stomatal density of treatments with 0% of shading (left) and 93% of shading (right).

3.5. Irrigation and leaching

The average amount of water evapotranspired during the experiment was about 1 L·pot⁻¹ and week, except in S80 which used about 30% less (Fig. 4). Water use efficiency (WUE) was the same for all the treatments (data not shown). The irrigation leaching evolution (Fig. 4) showed that in fall S65 and S80 produced the highest leaching (45% and 70%, respectively). This behavior was similar in winter, although S65 had the same leaching as the

control. In spring, leaching was similar in all treatments, but was increased in summer by shading (Fig. 4).

Fig. 4. Average seasonal irrigation and leaching in the different shading treatments. Data was calculated as the mean of 13 weeks per season of the tree repetitions. Vertical bars indicate SE (n=39).

3.6. Photosynthesis, stomatal conductance and leaf water potential

S65 and S80 led to more constant and lower photosynthesis rates during the experimental period. The rest of the treatments showed greater photosynthesis rates during fall and winter, especially the control. Control plants maintained the P_n during fall, but the value of this parameter fell sharply during the winter (Fig. 5A).

The evolution of midday g_s was similar in all the shading treatments, except in S80, which showed erratic behavior. This evolution was similar to P_n for the control plants (Fig. 5B). At the end of spring, all the treatments showed very low g_s rates, which increased strongly at the end of summer, especially in S25, S50 and S65 (Fig. 5B).

Ψ_{hmd} evolution was similar in all the treatments, although the control plants usually had slightly higher values than shaded plants. Ψ_{hmd} was strongly reduced during spring, and showed minimum values at the beginning of summer. At the end of summer Ψ_{hmd} recovered and continued to increase in fall (Fig. 5C).

Fig. 5. Seasonal evolution of (A) photosynthesis (P_n), (B) stomatal conductance (g_s) and (C) leaf water potential (Ψ_{hmd}) in the different shading treatments. Measurements correspond to the last week of September 2007, December 2007, March 2008, June 2008 and September 2008. Vertical bars indicate SE (n=6).

3.7. Photochemical efficiency and relative chlorophyll content (RCC)

Dark-adapted photosystem II efficiency (F_v/F_m) was only reduced in S80. However, light-adapted photosystem II efficiency (ϵPSII) was reduced in S65 and S80. The non-

photochemical quenching (NPQ) response increased in S65 and S80 (opposite to ϵ PSII). The electron transport rate (ETR) response was similar to ϵ PSII (Table 4).

The RCC was lower in both control and S80 (Table 4).

Table 4. Photochemical efficiency and relative chlorophyll content (RCC) study.

Parameters	Shading (%)				
	0 (S0)	32 (S25)	48 (S50)	84 (S65)	93 (S80)
Fv/Fm	0.82 b	0.83 b	0.84 b	0.81 ab	0.78 a
ϵ PSII	0.33 b	0.31 b	0.33 b	0.20 a	0.18 a
NPQ	2.27 a	2.36 a	2.39 a	2.99 b	2.94 b
ETR	56.2 b	52.6 b	55.1 b	34.1 a	29.8 a
RCC	65.3 a	72.8 b	69.6 b	71.3 b	63.5 a

*Different letters in the same row indicate statistically significant differences between means at $P < 0.05$

according to the least significant difference (LSD) test.

4. Discussion

The light level produced clear differences between growth and development in *R. alaternus*, although only the drastic reduction in solar radiation (93%) decreased dry matter (Table 1). The roots were more affected than shoots, as indicated by the increasing S/R ratio. The 84% shading also affected this ratio, although shoot biomass was not affected. Villar-Salvador (2004) also found a significant S/R increase in *Quercus ilex* seedlings with a shading of 45%. These data are in accordance with the theory of "functional equilibrium" (Brouwer 1963), where a decrease in light induces an increasing S/R ratio (Table 1). The reduction in evaporative demand, due to shading, affected the water absorption needs, which possibly diminished root growth.

Shading reduced the number of leaves, as Mendes et al. (2001) found in *Myrtus communis*. Under shade, the SLA increased due to leaf thinning (Tables 1 and 3), which is coherent with the observations of Abrams and Kubiske (1990) in five conifers trees in the understory of Wisconsin forest. Niinemets and Kull (1998) also found in five deciduous temperate woody species (*Populus tremula*, *Fraxinus excelsior*, *Tilia cordata*, *Corylus avellana* and *Fagus sylvatica*) that the SLA was reduced with increasing irradiance because thickness and leaf density increased.

Epidermis thickness was not affected by shading, possibly due to the single cell layer, while the parenchyma (spongy and palisade) was strongly reduced (Table 3). These results are

related with those found by Dimassi-Theriou and Bosadalidis (1997) in kiwifruit, in which parenchyma thickness increased when leaves were subjected to high light radiation. The reduction in parenchyma thickness by shading (thinner leaf) led to better light penetration to the chloroplasts (Evans, 1999). This thickness reduction and the increasing individual leaf area could be a leaf adaptation mechanism to low light conditions, which would have led to greater light capture.

Shading produced taller plants due to shoot growth as they looked for light, indicating the activation of some phototropism responses (Takemiya et al. 2005) to modify plant leaf distribution in order to limit mutual shading. This response would explain the decreased number of leaves and number of shoots as a result of shading (Table 1 and Fig. 3B), as reported by Sorrentino et al. (1997) in lily. Shading also reduced stem diameter growth (Fig. 2D), as reported by Cregg et al. (1993) in loblolly pine, in which reductions in diameter growth were observed almost immediately when the shade treatments (50% and 72%) were imposed. This indicates that the branch cambium was a low priority sink for current photosynthates compared with the growing foliage. This would agree with Kramer and Kozlowski (1979), who reported that under stress, cambium growth is reduced before shoot apical meristems, and Buisson and Lee (1993), who registered less diameter growth in papaya plant developed under artificial shade.

Compactness and leaf color are two important quality criteria in potted plant. *R. alaternus* is not very compact during its first years (Martínez-Sánchez et al., 2008), and shading reduced the compactness index (Table 1). This was because the shoot length increased and the number of shoots decreased. At the end of the experiment, leaf color in control plants was characterized by a green yellowish tone, low saturation and slight darkness. Shading improved leaf color, a greener tone (increasing H), although it was slightly less saturated (less C). Germana et al. (2001) observed that a 70% reduction of PAR produces a darker green color in mandarin leaves, as Brand (1997) observed in *Kalmia latifolia* subjected to shading.

The water supplied was the same for all the treatments, while the least water evapotranspired was by S65 and S80, as seen from the greater amount of leached water collected. In fall and winter, S25 and S50 evapotranspired more (Fig. 4) because the plants were bigger than the control plants (Fig. 3A and 3C). In contrast, S65 and S80 had lower water needs, possibly because shading decreased P_n and g_s (Fig. 5A and 5B). The similar drainage in spring (despite shading) was due to the fact that shaded plants had a greater leaf area than the control, so a similar g_s is translated into greater transpiration, thus compensating

the greater evaporation of the control. The severe climatic conditions of the summer induced plant stomata closure (Fig. 5A and 5B). Under these conditions, substrate evaporation strongly influences water consume in the pot (Alarcón et al., 2006). Thus, the control plants suffered greater substrate evaporation because they were more exposed to wind and light than the other shading treatments (natural *R. alaternus* development exacerbates this effect). As a consequence, water evapotranspired was inversely proportional to shading percentage (Fig. 4).

Although water evapotranspired was clearly affected by shading, WUE was not because shading reduced both water evapotranspired and biomass production. Dai et al. (2009) found similar results in *Tetrastigma hemsleyanum*, although Germana et al. (2001) and Syvetsen (1987) found greater WUE in plants subjected to shading. The last author attributed this behavior to the greater chlorophyll content resulting from shading conditions, which maximize light capture capacity (Kura-Hotta et al., 1987; Lei et al., 1996) and favor photosynthesis (Mao et al., 2007). Other studies in woody plants (Niinemets, 1997) and shrubs (Rhizopoulou et al. 1991; Andersen et al. 1991) showed chlorophyll increasing as a result of shading, as was observed in this experiment (Table 4); however, this did not happen under S80, which points to a degree of photosynthetic damage due to light stress. The increase in chlorophyll could be a plant adaptation to low light levels in order to maintain the photosynthesis rate. Usually, higher chlorophyll content is related with darker green leaves (Newman and Follet, 1988), as occurred in this experiment (Table 1 and 4). Visually, S25, S50 and S65 leaves were darker green than control plants, although S80 produced lighter green leaves. Greater luminosity and greener tone, and lower saturation in S80 (Table 1), would be correlated with this behavior.

Strong shading (S65 and S80) reduced stomatal density and single pore area (Table 3), an effect also observed by Mendes et al. (2001) in myrtle and Israeli et al. (1995) in bananas. Niinemets and Tenhunen (1997) reported that stomatal density improves CO₂ absorption (greater photosynthetic rate), and *R. alaternus* control plants had the greatest P_n and g_s under non-stressful solar radiation (in fall and winter Figs. 5A and 5B). However, the DW results (Table 1) showed similar biomass production, except for S80. This behavior can be explained by the plant leaf pore area (Table 3), which increased at shading percentages of ≥48% compared with control and would compensate the lower P_n rates, except for S80 where plant growth was affected. On the other hand, the P_n in control plants during spring and summer was lower than in S25 and S50, probably because these environmental conditions at midday were stressful, which hindered the absorption of CO₂ and produced photorespiration

(Sorrentino *et al.*, 1997). The fall in g_s and Ψ_{hmd} at the end of spring in all the treatments (Fig. 5B and 5C) confirmed the stressful environmental conditions, which caused stomatal closure in an attempt to avoid water loss (Dai *et al.*, 2009; Tenhunen *et al.*, 1984). At the end of summer, the climatic conditions were less stressful and shading softens them even further, which resulted in greater g_s in all shading treatments except for S80 than in control plants (Fig. 5B). Sofo *et al.* (2009) found that a reduction in sun light in summer increased P_n in olive trees, as in our experiment in S50 and S65. However, strong shading (93%) negatively affected the P_n , which remained low throughout the crop year (Fig. 5A), implying photosynthetic damage.

The plants subjected to 48%, 84% and 93% shading had a worse water status than the control, according to the Ψ_{hmd} values (Fig. 5C). This behavior was contrary to that found by Mendes *et al.* (2001) in myrtle, in which Ψ_{hmd} was cushioned by shading in august. The thick cuticle of control plants may have reduced water loss (Jenks *et al.*, 2007), thus maintaining greater Ψ_{hmd} than in shaded plants. Similarly, the content of solutes might have had an effect, the lower soluble sugars content at the end of the experiment in leaves subjected to strong shading (Table 2), in theory, not helping to maintain the Ψ_{hmd} .

Chlorophyll fluorescence analysis studies the photosynthetic activity of plants. Such an analysis was performed to verify the damage produced by shading described in the preceding paragraphs. The values of F_v/F_m and $\epsilon PSII$ (Table 4) showed that strong shading (84% but especially 93%) reduced the photochemical activity of PSII, reducing the photosynthetic capacity of the plant. The 93% shading reduced F_v/F_m , which indicate irreversible damages in PSII (Correia *et al.*, 1992). S65 and S80 increased their NPQ and, as also reported by Maxwell and Johnson (2000), an increase in NPQ may be the result of the plant developing a system of protection against light excess or intrinsic damage in the leaf. Therefore, S65 and S80 plants had low $\epsilon PSII$ (Table 4) and P_n (Fig. 5A), which confirms light energy dissipation and malfunction in reaction centers (Araus *et al.*, 1998). Any other damage related to photorespiration or disorders in electron transport was dismissed because the lower ETR corresponded to lower P_n values (Fryer *et al.*, 1998).

According to Melis (1999), a decreasing ETR and P_n , and increasing NPQ indicate plants damage by excess of light. However, in this study these conditions in ETR and P_n appeared under strong shading, which indicates that both light excess and light deficiency are harmful. Other authors reported that the adaptation to low solar radiation improves PSII efficiency (Anderson and Osmond, 1987; Demming-Adams, 1998), but, in this case, a slight light reduction did not affect the PSII, which indicates that *R. alaternus* presents plasticity to

lower levels of light. On the other hand, as reported by Flowers et al. (2007), a decreasing ETR can produce photoinhibition and chlorophyll loss. The results in Table 4 confirm that S80 did not reduce leaf RCC levels compared with the control, which makes it difficult to relate chlorophyll loss with a reducing ETR in this species.

5. Conclusion

R. alaternus is a light-adapted species with plasticity to lower light environments. However, strong light reduction (84% shading) reduced the photochemical efficiency of photosystem II and photosynthesis. When the light reduction was drastic (93% shading), photosystem II suffered irreversible damage, and both P_n and F_v/F_m had lower values. So, this species is interesting as an understory species in Mediterranean forests, where shade is not drastically reduced, because of its adaptation mechanisms to light changes. One of these facts is the capacity of increasing leaf area to compensate lower gas exchange rates per unit area. From a nursery point of view, shading $\geq 84\%$ would produce low quality plants, with a low compactness index and high S/R index. As reported in our experimental results, shading between 32% and 48% could be applied in spring and summer, respectively, in the hours of strongest radiation (by means of an automatic shading screen). This would optimize photosynthesis and improve leaf color.

Acknowledgements

The authors thank R. Valdes and R. Espín for irrigating and supervising the shrubs while the experiment took place. This work was supported by projects CICYT (AGL 2005-05588-C02-1-2) and Fundación Séneca Región de Murcia (02991/ PI/05 and 05660/PI/07).

References

Abrams, M.D., M.E. Kubiske. 1990. Leaf structural characteristics of 31 hardwood and conifer tree species in central Wisconsin: influence of light regime and shade-tolerance rank. *Forest Ecol. Manage.* 31:245-253.

- Alarcón, J.J., M.F. Ortuño, E. Nicolás, A. Navarro, and A. Torrecillas. 2006. Improving water-use efficiency of young lemon trees by shading with aluminised-plastic nets. *Agric. Water Mgt.* 82:387-398.
- Andersen, P.C., J.G. Norcini, and G.W. Knox. 1991. Influence of irradiance on leaf physiology and plant growth characteristics of *Rhododendron* x 'Pink Ruffles'. *J. Amer. Soc. Hort. Sci.* 116:881-887.
- Anderson, J.M. and C.B. Osmond. 1987. Shade-sun responses: compromises between acclimation and photoinhibition. In: Kyle, D.J., Osmond, C.B.(Eds.), *Photoinhibition*. Elsevier, Amsterdam, pp. 1-38.
- Araus, J.L., T. Amaro, J. Voltas, H. Nakkoul, M.M. Nachit. 1998. Chlorophyll fluorescence as a selection criterion for grain yield in durum wheat under Mediterranean conditions. *Field Crops Res.* 55:209-223.
- Boardman, N.K. 1977. Comparative photosynthesis of sun and shade plants. *Annu. Rev. Plant Physiol.* 28:355-377.
- Brand, M.H. 1997. Shade influences plant growth, leaf colour, and chlorophyll content of *Kalmia latifolia* L. cultivars. *HortScience* 32:206-208.
- Brouwer, R. 1963. Some aspects of the equilibrium between overground and underground plant parts. - Meded. Inst. Biol. Scheik. Onderz. Landb. Gewas. 213:31-39.
- Buisson, D. and D.W. Lee. 1993. The developmental responses of papaya leaves to simulated canopy shade. *Am. J. Bot.* 80:947-952.
- Cannell, M.G.R., R. Milne, L.J. Sheppard, and M.H. Unsworth. 1987. Radiation interception and productivity of willow. *Appl. Ecol.* 24:261-278.
- Carter, R.E. and K. Klinka. 1992. Variation in shade tolerance of Douglas fir, western hemlock, and western red cedar in coastal British Columbia. *Forest Ecol. Manage.* 55:87-105.
- Chinnici, F., U. Spinabelli, C. Riponi, and A. Amati. 2005. Optimization of the determination of organic acids and sugars in fruit juices by ion-exclusion liquid chromatography. *J. Food Comp. Anal.* 18:121-130.
- Cregg, B.M., R.O. Teskey, and P.M. Dougherty. 1993. Effect of shade stress on growth, morphology, and carbon dynamics of loblolly pine branches. *Trees* 7:208-213.
- Correia, M.J., M.M.C. Chaves, and J.S. Pereira. 1992. Afternoon depression photosynthesis in grapevine leaves - evidence for high light stress effect. *J. Exp. Bot.* 41:417-426.

- Dai, Y., Z. Shen, Y. Liu, L. Wang, D. Hannaway, and H. Lu. 2009. Effects of shade treatments on the photosynthetic capacity, chlorophyll fluorescence, and chlorophyll content of *Tetrastigma hemsleyanum* diels et Gilg. *Environ. Exp. Bot.* 65:177-182.
- DeLucia, E.H., T.W. Sipe, J. Herrick, and H. Maherali. 1998. Sapling biomass allocation and growth in the understory of a deciduous hardwood forest. *Am. J. Bot.* 85:955-963.
- Demming-Adams, B. 1998. Survey of thermal energy dissipation and pigment composition in sun and shade leaves. *Plant Cell Physiol.* 143:93-104.
- Dimassi-Theriou, K. and A.M. Bosabalidis. 1997. Effects of light, magnesium and sucrose on leaf anatomy, photosynthesis, starch and total sugar accumulation, in kiwifruit cultured *in vitro*. *Plant Cell Tiss. Org. Cult.* 47:127-134.
- Donovan, L.A. and J.R. Ehleringer. 1994. Carbon isotope discrimination, water-use efficiency, growth, and mortality in a natural shrub population. *Oecologia* 100:347-354.
- Evans, J.R. 1999. Leaf anatomy enables more equal access to light and CO₂ between chloroplast. *New Phytol.* 143:93-104.
- Flowers, M.D., E.L. Fiscus, K.O. Burkey, F.L. Booker, and J.J.B. Dubois. 2007. Photosynthesis, chlorophyll fluorescence, and yield of snap bean (*Phaseolus vulgaris* L.) genotypes differing in sensitivity to ozone. *Environ. Exp. Bot.* 61:190-198.
- Franco, J. A., J.J. Martínez-Sánchez, J.A. Fernández, and S. Bañón. 2006. Selection and nursery production of ornamental plants for landscaping and xerogardening in semi-arid environments. *J. Hort. Sci. Biotechnol.* 81:3-17.
- Fryer, M.J., J.R. Andrews, K. Oxborough, D.A. Blowers, and N.R. Baker. 1998. Relationship between CO₂ assimilation, photosynthetic electron transport, and active O₂ metabolism in leaves of maize in the field during periods of low temperature. *Plant Physiol.* 116:571-580.
- Germana, C., A. Continella, and E. Tribulato. 2001. Bio-agronomic effects of net shading on "primosole" mandarin. *Acta Hort.* 559:293-295.
- Gulias, J., A. Traveset, N. Riera, and M. Mus. 2004. Critical stages in the recruitment process of *Rhamnus alaternus* L. *Ann. Bot.* 93:723-731.
- Israeli, Y., Z. Plaut, and A. Schwartz. 1995. Effect of shade on banana morphology, growth and production. *Sci. Hort.* 62:45-56.
- Jenks, M.A., P.M. Hasagewa, and S.M. Jain. 2007. Eco-physiological and molecular-genetic determinants of plant cuticle function in drought and salt stress tolerance. In: Jenks, M.A., et al. (Eds.), *Advances in Molecular Breeding Toward Drought and Salt Tolerant Crops*. Springer Netherlands, pp. 91-120.

- Kramer, P.J. and T.T. Kozlowski. 1979. Physiology of woody plants. Academic Press, New York.
- Kura-Hotta, R., K. Satoh, and S. Kato. 1987. Chlorophyll concentration and its changes in leaves of spinach raised under different light levels. *Plant Cell Physiol.* 87:12-19.
- Lei, T.T., R. Tabuchi, M. Kitao, and T. Koike. 1996. The functional relationship between chlorophyll content, leaf reflectance, and light capturing efficiency of Japanese forest species under natural shade and open light regimes. *Physiol. Planta.* 96:411-418.
- Lichtenthaler, H.K., A. Ac, M.V. Marek, J. Kalina, and O. Urban. 2007. Differences in pigment composition, photosynthetic rates and chlorophyll fluorescence images of sun and shade leaves of four tree species. *Plant Physiol. Biochem.* 45:577-588.
- Mao, L.Z., H.F. Lu, Q. Wang, and M.M. Cai. 2007. Comparative photosynthesis characteristics of *Calycanthus chinensis* and *Chimonanthus praecox*. *Photosynthetica* 45:601-605.
- Martínez-Sánchez, J.J., J.A. Franco, M.J. Vicente, M. Muñoz, S. Bañón, E. Conesa, J.A. Fernández, R. Valdés, J. Miralles, J. Ochoa, M. Aguado, J. Esteva, J. López, and L. Aznar. 2008. Especies silvestres mediterráneas con valor ornamental. Selección, producción viverística y utilización en jardinería. Dirección General de Patrimonio Natural y Biodiversidad. Consejería de Agricultura y Agua. Región de Murcia, pp. 224.
- Maxwell, K. and G.N. Johnson. 2000. Chlorophyll fluorescence -a practical guide. *J. Exp. Bot.* 51:659-668.
- Melis, A. 1999. Photosystem-II damage and repair cycle in chloroplast: what modulates the rate of photodamage in vivo? *Trends Plant Sci.* 4 :130-135.
- Mendes, M.M., L.C. Gazarine, and M.L. Rodrigues. 2001. Acclimation of *Myrtus communis* to contrasting Mediterranean light environments - effects on structure and chemical composition of foliage and plant water relations. *Environ. Exp. Bot.* 45:165- 178.
- Meng, Q., K. Siebke, P. Lippert, B. Baur, U. Mukherjee, and E. Weis. 2001. Sink-source transition in tobacco leaves visualized using chlorophyll fluorescence imaging. *New Phytol.* 151:585-595.
- Messier, C. and E. Nikinmaa. 2000. Effects of light availability and sapling size on the growth, biomass allocation, and crown morphology of understory sugar maple, yellow birch, and beech. *Ecoscience* 7, 345-356.
- Montgomery, R.A. and R.L. Chazdon. 2002. Light gradient partitioning by tropical tree seedlings in the absence of canopy gaps. *Oecologia* 131:165-174.

- Naidu, S.L. and E.H. DeLucia. 1998. Physiological and morphological acclimation of shade-grown tree seedlings to late season canopy gap formation. *Plant Ecol.* 138:27-40.
- Newman, S.E. and M.W. Follett. 1988. Irrigation frequency and shading influences on water relations and growth of container-grown *Euonymus japonica* 'Aureo-marginata'. *J. Environ. Hort.* 6:96-100.
- Niinemets, Ü. 1997. Acclimation to low irradiance in *Picea abies*: influences of past and present light climate on foliage structure and function. *Tree Physiol.* 17:723-732.
- Niinemets, Ü. 1999. Differences in chemical composition relative to functional differentiation between petioles and laminae of *Fraxinus excelsior*. *Tree Physiol.* 19:39-45.
- Niinemets, Ü. and O. Kull. 1998. Stoichiometry of foliar carbon constituents varies along light gradients in temperate woody canopies: implications for foliage morphological plasticity. *Tree Physiol.* 18:467-479.
- Niinemets, Ü. and J.D. Tenhunen. 1997. A model separating leaf structural and physiological effects on carbon gain along light gradients for the shade-tolerant species *Acer saccharum*. *Plant Cell Environ.* 20:845-866.
- Poorter, L. 1999. Growth responses of 15 rain-forest tree species to a light gradient: the relative importance of morphological and physiological traits. *Funct. Ecol.* 13:396-400.
- Rhizopoulou, S., M.S. Meletiou-Christou, and S. Diamantoglou. 1991. Water relations for sun and shade leaves of four Mediterranean evergreen sclerophylls. *J. Exp. Bot.* 42:627-635.
- Scholander, P., H.T. Hammel, E.D. Bradstreet, and E.A. Hemmingen. 1965. Sap pressure in vascular plants. *Science* 148:339-346.
- Sheng, M., M. Tang, H. Chen, B. Yang, F. Zhang, and Y. Huang. 2008. Influence of arbuscular mycorrhizae on photosynthesis and water status of maize plants under salt stress. *Mycorrhiza* 18:287-296.
- Sofo, A., B. Dichio, G. Montanaro, and C. Xiloyannis. 2009. Shade effect on photosynthesis and photoinhibition in olive during drought and rewatering. *Agric. Water Mgt.* 96:1201-1206.
- Sorrentino, G., L. Cerio, and A. Alvino. 1997. Effects of shading and air temperature on leaf photosynthesis, fluorescence and growth in lily plants. *Sci. Hort.* 69:259-273.
- Syvetsen, J.P. 1987. Nitrogen content and CO₂ assimilation characteristics of *Citrus* leaves. *HortScience* 22:289-291.
- Takemiya, A., S. Inoue, M. Doib, T. Kinoshita, and K. Shimazaki. 2005. Phototropins promote plant growth in response to blue light in low light environments. *Plant Cell* 17:1120-1127.

- Tenhunen, J.D., H.P. Meister, M.M. Caldwell, and O.L. Lange. 1984. Environmental constraints on productivity of the Mediterranean sclerophyll Shrub *Quercus coccifera*. *Options Medit.* 1:33-53.
- Turner, N.C. 1988. Measurement of plant water status by the pressure chamber technique. *Irrig. Sci.* 9:289-308.
- Veneklaas, E. and F. den Ouden. 2005. Dynamics of non-structural carbohydrates in two *Ficus* species after transfer to deep shade. *Environ. Exp. Bot.* 54:148-154.
- Villar-Salvador, P., R. Planelles, E. Enríquez, and J. Peñuelas Rubira. 2004. Nursery cultivation regimes, plant functional attributes, and field performance relationships in the Mediterranean oak *Quercus ilex* L. *Forest Ecol. Manage.* 196:257-266.
- Wayne, P.M. and F.A. Bazzaz. 1993. Birch seedling responses to daily time courses of light in experimental forest gaps and shade houses. *Ecology* 74:1500-1515.

Chapter 4

A calibrated TDT soil moisture sensor can be used for precise automated irrigation of container-grown Plants

Authors: J. Miralles¹ and M.W. van Iersel²

¹Dpto. de Producción Vegetal, ETSIA, Universidad Politécnica de Cartagena (UPCT), Paseo Alfonso XIII, 48. 30203 Cartagena (Murcia), Spain

²Department of Horticulture, 1111 Plant Sciences Bldg., University of Georgia, Athens, GA 30602-7273, USA

Accepted for publication in HortScience since 20 April 2011.

Year 2009:

Impact factor: 0.696

Category: Horticulture

Category ranking 2008: (15/30)

Quartile in category: Q3

Abstract

Irrigation control systems that irrigate container-grown plants based on crop water needs can reduce water and fertilizer use and increase the sustainability of ornamental crop production. The use of soil moisture sensors to determine when to irrigate is a viable option. We tested a commercially available irrigation controller (CS3500, Acclima, Meridian, ID), which uses time domain transmissometry (TDT) sensors to measure soil volumetric water content (θ). The objectives of this study were: a) to test the accuracy of TDT sensors in soilless substrate, b) to quantify the ability of the Acclima CS3500 irrigation controller to maintain stable θ readings during the production of container-grown begonia (*Begonia semperflorens* L.), by turning a drip irrigation system on and off as needed, and c) to study the growth and photosynthetic physiology of begonia at six θ levels. Calibration of the TDT sensors in pots filled with substrate (but without plants) showed that the θ determined by the TDT sensors had a very close relationship ($R^2=0.99$) with the gravimetrically determined θ , but the TDT sensors underestimated θ by about $0.08 \text{ m}^3\cdot\text{m}^{-3}$. Therefore, a custom calibration of the TDT sensors for the soilless substrate was necessary to get accurate θ data. The irrigation controller was programmed to maintain six θ thresholds, ranging from 0.136 to $0.472 \text{ m}^3\cdot\text{m}^{-3}$ (based on our own sensor calibration) and was able to maintain θ readings within $0.008 \text{ m}^3\cdot\text{m}^{-3}$ of the threshold. Theta and Sigma probes were used to collect comparative θ and bulk electrical conductivity (EC) data, respectively. The results showed a strong correlation with TDT sensor measurements of θ ($R^2=0.92$), but a moderate relationship for bulk EC ($R^2=0.53$). The begonias had similar dry weight at θ levels of $0.348 \text{ m}^3\cdot\text{m}^{-3}$ and higher, while total evapotranspiration increased linearly with the θ threshold. The lowest θ threshold reduced leaf size, net photosynthesis (P_n), and stomatal conductance (g_s). Overall, the TDT sensors can provide accurate measurements of θ in soilless substrate, but need a substrate specific calibration. The Acclima CS3500 controller, using TDT sensors, was able to maintain stable θ readings throughout a production cycle. These results suggest that this irrigation controller may be suitable for production of greenhouse crops, as well as in drought stress research.

Keywords: automated irrigation, *Begonia semperflorens*, greenhouse production, leaching, time domain transmissometry, water use.

1. Introduction

Water conservation through more efficient irrigation has received much attention in recent years, especially in regions suffering from drought and with limited water availability. Population growth and increased urbanization have increased competition for water (Jury and Vaux, 2005). Furthermore, consumers and governments are increasingly interested in agricultural sustainability, resulting in market opportunities for sustainable ornamental crops (Dennis et al., 2010). The ornamentals industry will also need to comply with existing and new laws, regulating the environmental impact of production (Fernandez et al., 2009). Sustainable ornamental crop production must include appropriate irrigation practices. Excessive irrigation results in leaching and runoff of water and fertilizer, which may cause eutrophication and algal blooms (Conover and Poole, 1992; Majsztrik et al., 2011). More efficient irrigation practices can also reduce energy use and CO₂ emissions.

To achieve more efficient irrigation, growers should not use timers or make irrigation decisions based on the visual appearance of the substrate or plants (Nemali et al., 2007). Instead, environmental measurements such as evapotranspiration (Allen et al., 1998) and soil or substrate θ or tension (Shock and Wang, 2011; Topp and Ferre, 2002) can be used to infer plant water needs and to schedule agricultural, municipal, or residential irrigation (Blonquist et al. 2006). Measurements of θ are particularly useful for precise irrigation scheduling in uniform nursery container mixes (Shock and Wang, 2011). Jones (2007) concluded that soil θ is the most valuable environmental or plant variable for irrigation control, allowing for easy automation. Automating irrigation based on soil water tension has been used for decades (see Shock and Wang, 2011), while the use of soil or substrate θ has become more feasible in recent years with the advent of low cost sensors (e.g., Nemali and van Iersel, 2006).

Nemali and van Iersel (2006) developed an automated irrigation system that can maintain substrate θ at specific thresholds. The system was accurate and successfully used in further work to study irrigation efficiency in gaura (*Gaura lindheimeri* Engelm. & Gray ‘Siskiyou Pink’ (Burnett and van Iersel, 2008) and petunia (*Petunia $\times$ hybrida*) (van Iersel et al., 2010). This irrigation system determines θ with capacitance sensors connected to a datalogger. The datalogger can control irrigation by opening and closing solenoid valves as needed. The limitation of this system is that a basic knowledge of programming and wiring dataloggers is required. However, there are commercially available irrigation systems that not only include soil moisture sensors, but also an interface to control irrigation. Cardenas-Lailhacar and Dukes (2010) tested four commercial, sensor-based irrigation systems in a

coarse textured soil (Arredondo fine sand) and concluded that TDT sensors (Acclima) could be used for accurate control of θ in field plots with Bermuda grass [*Cynodon dactylon* (L.) Pers.]. McCready et al. (2009) tested the same TDT sensor in a similar soil (field capacity about 11%) but with three different θ levels (7% dry, 10% medium and 13% wet) and showed that θ levels of 7 and 10% reduce water use, although 7% reduced St. Augustine grass [*Stenotaphrum secundatum* (Walter) Kuntze] quality.

The TDT sensor measures the permittivity of the soil by sending an electromagnetic signal along a waveguide and measuring the propagation time of that wave. This propagation time depends on the soil dielectric properties, which are mainly governed by the water content of the soil surrounding the probes (Blonquist et al., 2005a). The TDT sensor provides accurate apparent permittivity measurements, which can be used to estimate θ (Blonquist et al., 2005a). The distortion parameters of the returned wave are used to determine the bulk EC. The dimensions of the TDT sensor are 20.3 cm $\times$ 5.4 cm $\times$ 1.4 cm (l $\times$ w $\times$ h) and they explore about 200 mL of soil or substrate (Blonquist et al., 2006). These sensors have potential use in the irrigation of ornamental crops, as well as irrigation and drought stress research. However, TDT sensors may need a specific calibration for soilless substrate, since the dielectric properties differ from those of mineral soil for which the standard factory calibration was developed. Using TDT sensors with an irrigation controller (e.g., CS3500, Acclima) can provide similar information as the automated irrigation system described by Nemali and van Iersel (2006). To assess the potential of such irrigation control for use with container-grown plants, the accuracy and performance of the sensors, controller, and irrigation system need to be tested. Thus, the aims of this study were: 1) to evaluate the built-in calibration of TDT sensors in soilless substrate and their accuracy in determining θ , 2) to quantify the ability of the Acclima CS3500 irrigation controller to maintain stable θ readings during the production of container-grown begonia (*Begonia semperflorens* L.), by turning a drip irrigation system on and off as needed, and 3) to study the growth and photosynthetic physiology of begonias at six θ levels.

2. Material and methods

2.1. Sensor calibration

The TDT sensors were calibrated following the protocol described by Nemali et al. (2007). Briefly, different amounts of water were mixed into 3.65 L of substrate (45% peat moss, 15% perlite, 15% vermiculite, and 25% bark; Fafard 3B, Fafard, Agawam, MA) and pots were filled with this substrate. The sensors were inserted into the substrate, while carefully maintaining close contact between the substrate and TDT sensor by slightly compressing the substrate. The TDT sensors were connected to an irrigation controller (CS3500, Acclima), which was used to take sensor readings (Picture 1). These readings provided measurements of θ , using the standard soil calibration built into the irrigation controller software (Irrigation Manager, Acclima). The pots with substrate were then weighed, oven-dried at 60 °C until constant weight was reached, and θ was determined as $[(SW_1 - SW_2)/V]$, where SW_1 is the substrate weight before drying, SW_2 the substrate weight after drying, and V the volume of the pot. The accuracy of the sensors was evaluated by plotting the θ as measured by the irrigation controller versus the θ determined from substrate weight and volume.

Picture 1. Acclima CS3500 irrigation controller (left) and TDT-sensor (right).

2.2. Plant material and crop conditions

Begonia seedlings were obtained from a commercial seedling producer (Speedling, Blairsville, GA). The seedlings were transplanted into 19.5 cm diameter, round pots with a 3.65 L volume, filled with a soilless substrate (Fafard 3B, Fafard) and amended with a slow

release fertilizer (Osmocote Plus 14-14-14; 14N-4.2P-11.6K; The Scotts Co., Marysville, OH) at a rate of 15 g/pot.

Pots were arranged in 12 rows of five plants and irrigation of each row of plants was controlled using a 2.5 cm solenoid valve (Watermaster, Orbit Irrigation Products, Bountiful, UT) connected to an irrigation controller (CS3500, Acclima). A TDT sensor was inserted into the substrate at an approximate 45° angle in one of the five pots in a row, and irrigation of all five pots was based on the θ reading of that sensor. The sensors were connected to the irrigation controller which measured the sensor output every 10 minutes, providing readings of θ (but not calibrated for our substrate) and temperature. Bulk electrical conductivity (bulk EC) was measured twice a day. Each irrigation line also had a flow meter (DLJ50, Daniel L. Jerman Co., Hackensack, NJ) to monitor water use in each experimental unit. Each plant was watered using a pressure-compensated drip emitter (2 lph, Netafim USA, Fresno, CA) with a spaghetti tube going to each pot (Picture 2). To allow establishment of the plants, θ was maintained at $0.429 \text{ m}^3 \cdot \text{m}^{-3}$ for one week before the treatments started.

The irrigation controller was programmed to maintain six θ set points, each replicated twice. Set points programmed into the irrigation controller were 5, 12, 19, 26, 33, and 40%. After applying our substrate-specific calibration to the TDT-sensor readings, these set points corresponded to actual θ levels of 0.136, 0.210, 0.281, 0.348, 0.412 and $0.472 \text{ m}^3 \cdot \text{m}^{-3}$. When the θ dropped below the set point, the irrigation controller opened the corresponding solenoid valve for 15 s, thus applying approximately 8.3 mL of water to each plant. To configure the irrigation system, and to establish the irrigation protocols described, the Irrigation Manager software (Acclima) was used.

The experiment was conducted in a glass-covered greenhouse at the University of Georgia in Athens, GA (lat. 34°N, long. 84°W). Environmental conditions in the greenhouse were monitored using a temperature/RH sensor (HMP50, Vaisala, Woburn, MA) and a photosynthetic photon flux sensor (SQ-100, Apogee Instruments, Logan, UT) connected to a datalogger (CR200, Campbell Sci. Inc., Logan, UT). Sensors were measured every minute to obtain daily averages, minimum and maximum values. Daily light integral (DLI, $\text{mol} \cdot \text{m}^{-2} \cdot \text{d}^{-1}$) was calculated by integrating the photosynthetic photon flux measurements throughout the day. Daily minimum and maximum temperatures were $18.0 \pm 2.3 \text{ }^\circ\text{C}$ and $26.9 \pm 3.2 \text{ }^\circ\text{C}$, daily minimum and maximum relative humidity $46 \pm 18\%$ and $82 \pm 7\%$, and the DLI was $13.7 \pm 6.8 \text{ mol} \cdot \text{m}^{-2} \cdot \text{d}^{-1}$ (mean $\pm$ s.d.) over the 66 d of the experiment.

Since there was no leaching, except for the first five days at the $0.472 \text{ m}^3 \cdot \text{m}^{-3}$ threshold, total evapotranspiration of each group of plants was determined as the sum of the

total amount of water applied, as determined from flow meter readings, plus the change in the amount of water in the substrate during the experiment was determined from the initial and final θ measurements and the volume of the pots $[(\theta_{\text{initial}} - \theta_{\text{final}}) \times 3.65 \text{ L}]$.

Picture 2. Experimental plot. The Acclima CS3500 irrigation controller, Begonias, solenoid valves, flow meters and TDT-sensor can be observed.

2.3. Independent measurements of θ and EC

To obtain independent data for evaluation of the accuracy of the TDT sensors, weekly measurements of θ , and bulk and pore water EC were taken with a Theta probe (Delta-T devices, Cambridge, UK) and Sigma probe (Delta-T devices), respectively. The Sigma probe measures bulk EC and calculates the pore water EC as described by Hilhorst (2000). Bulk EC is a measure of how well the substrate (including air, solution and substrate particles) conducts electricity, while pore water EC determines the electrical conductivity of the solution in the substrate. For Theta probe measurements, a calibration developed specifically for soilless substrates (Nemali et al., 2007) was used. A total of four pots per experimental unit were measured each time.

2.4. Measurements at the end of the experiment

Stomatal conductance (g_s) and net photosynthesis (P_n) were measured at midday using a leaf photosynthesis system (CIRAS-1; PP Systems, Amesbury, MA) and instantaneous water use efficiency was calculated as P_n/g_s . The air flow rate through the cuvette was 200 mL·min⁻¹ with a [CO₂] of 420 µmol·mol⁻¹, an air temperature of 20 °C, a vapor pressure deficit of 1.6 kPa, and a photosynthetic photon flux of 2000 µmol·m⁻²·s⁻¹. The measurements were made on four plants per replication. Chlorophyll fluorescence was also measured on four leaves per replication using a pulse modulated fluorometer (mini-PAM, Walz, Effeltrich, Germany). The method and parameters determined were the same as described by Sheng et al. (2008), but with an actinic light of 510 µmol·m⁻²·s⁻¹ for the light adapted measurements.

At final harvest, the area of one fully-expanded leaf from the upper 1/3 of the canopy of each plant was determined using a leaf area meter (LI-3100, LI-COR Biosciences, Lincoln, NE). Shoot dry weight (DW) was determined after drying the shoots in a convection oven at 60 °C.

2.5. Experimental Design and Statistical analysis

The design was a randomized complete block with six treatments and two replications. Experimental units consisted of groups of five plants, one of which had a TDT sensor in the pot to control irrigation. Data collected at the end of the experiment were subjected to ANOVA (StatGraphics Plus 5.1, StatPoint Technologies, Warrenton, VA). When the ANOVA indicated significant effects ($P < 0.05$), means were separated using Fisher's LSD. Regression analyses were performed using SigmaPlot 10.0 (Systat software Inc., San Jose, CA).

3. Results and discussion

3.1. Calibration of sensors

There was an almost perfect, quadratic relationship between the θ as measured by the TDT sensors and the weight-based measurement of θ of the substrate (Fig. 1). Since the different θ calibration points were obtained with 12 different TDT sensors, these calibration

results also indicate that there are no major differences among different sensors. However, the standard calibration for the TDT sensors was not accurate for the soilless substrate used in our study. The TDT sensors consistently underestimated θ , which resulted in errors of up to $0.08 \text{ m}^3 \cdot \text{m}^{-3}$. Estimation of θ using dielectric sensors is based on the ability of sensors to measure the ‘real’ part of the dielectric permittivity (ϵ), or a property directly related to ϵ . The total ϵ of a soil depends on the ϵ of the air ($\epsilon \approx 1$), soil solids ($\sim 2\text{--}9$) and water (~ 80) in the soil (Blonquist et al., 2006). Since water has a much higher ϵ than air or soil solids, the overall ϵ of a soil is closely related to θ . However, ϵ differs among different soils and substrates, thus affecting the calibration of the sensors. By performing a substrate-specific calibration, we were able to convert the TDT measurements to the actual θ of the soilless substrate. The TDT sensors were not able to measure the θ of very dry substrate ($< 0.08 \text{ m}^3 \cdot \text{m}^{-3}$) since the sensors consistently read $0 \text{ m}^3 \cdot \text{m}^{-3}$ in such dry substrates (results not shown). Blonquist et al. (2005a) concluded that TDT sensors had the accuracy of time domain reflectometry sensors in determining θ under laboratory conditions, and were less affected by bulk EC and temperature than other, lower frequency (50 MHz) sensors (Blonquist et al., 2005b).

Fig. 1. The relationship between the substrate water content (θ) of a bark-based soilless substrate, as measured by TDT sensors using the factory calibration for mineral soil, and the θ determined based on wet and dry weight and volume of the substrate (volumetric θ). At a volumetric θ below $0.08 \text{ m}^3 \cdot \text{m}^{-3}$, the TDT sensors read $0 \text{ m}^3 \cdot \text{m}^{-3}$ and those data were not included in the calibration.

3.2. Substrate Water Content Control

The θ readings of the TDT sensors were close to the threshold θ (average θ levels, based on our calibration, were 0.146, 0.212, 0.285, 0.350, 0.415 and 0.474 $\text{m}^3\cdot\text{m}^{-3}$, 0.002 to 0.010 $\text{m}^3\cdot\text{m}^{-3}$ above the threshold), and with little variation once the threshold θ was reached (Fig. 2). The standard deviation between daily θ means was higher in the driest treatment (0.0044 $\text{m}^3\cdot\text{m}^{-3}$ vs. 0.0020 $\text{m}^3\cdot\text{m}^{-3}$ in the wettest treatment), which is consistent with previous findings (Nemali and van Iersel, 2006; van Iersel et al., 2010). This may be the result of the decreased hydraulic conductivity of peat-based substrates at lower water contents (Naasz et al., 2005), which slows water movement through the substrate and may result in a less uniform water distribution (van Iersel et al., 2010).

Fluctuations in θ were about 13 times smaller than those reported by Nemali and van Iersel (2006). One of the reasons for the very stable θ could be the short irrigation time (15 seconds) used, applying only 8-9 mL per irrigation event, much smaller than the 100 mL applied by Nemali and van Iersel (2006). Van Iersel et al. (2010) reported that reducing the amount of water applied per irrigation event reduced fluctuations in θ .

The θ in the 0.472 $\text{m}^3\cdot\text{m}^{-3}$ treatment increased on day 5 because the substrate had to be slightly compacted to avoid continuous leaching and improve contact between the substrate and sensor. On day 55, there was a small θ decrease in all treatments because of problems with the water supply. This decrease was less evident in the driest treatment, probably due to its lower evapotranspiration.

Fig. 2. Average substrate volumetric water content (θ) readings of the TDT sensors over a 66 d period, during which begonias were grown in 3.65 L pots. A substrate-specific calibration (see Fig. 1) was used to determine θ . Dashed lines indicate the θ threshold at which the containers in the various treatments were irrigated. Error bars indicate the standard deviation ($n=2$).

3.3. Theta and Sigma probe correlations

There was a strong, quadratic relationship between the TDT-sensor readings (using our substrate-specific calibration) and the Theta probe measurements of θ over the course of the experiment ($R^2 = 0.91$) (Fig. 3). Theta probe readings were always lower than those of the TDT sensors, likely because the Theta probe only measures about the top 6 cm of the substrate while the TDT sensors explore nearly the entire depth of the pot and the top part of a substrate normally is drier than the bottom part (Wallach, 2008). Overall, the TDT sensor can be considered a good θ sensor for soilless substrates, because it can be calibrated for such substrates and is responsive to changes in θ . Furthermore, its readings are strongly correlated with those of the Theta probe, which has been reported to be accurate in soilless substrates, with little sensitivity to temperature or EC (Nemali et al., 2007).

The bulk EC measured with the TDT sensor had a weak correlation with pore water EC ($r^2 = 0.30$) and a moderate correlation with the bulk EC ($r^2=0.53$), as measured with the SigmaProbe (Fig. 4). One of the factors causing these poor correlations was that the bulk EC measured by the TDT sensors was almost always 0 dS·m⁻¹ at θ below 0.28 m³·m⁻³. These results suggest a limited usefulness of the TDT sensors for bulk EC measurements in soilless substrate. On the other hand, the use of a controlled release fertilizer may increase variability in the data, because the SigmaProbe measures only a small volume of substrate. This may result in inconsistent reading if the EC of the substrate is not uniform, as may be the case with controlled release fertilizers. The poor correlation between TDT sensor bulk EC measurements and pore water EC is not surprising, since pore water EC depends on substrate properties, the dielectric permittivity (and thus θ), and the bulk EC (Hilhorst, 2000).

Fig. 3. The relationship between substrate volumetric water content (θ) measurements by time domain transmissometry (TDT) sensors (using our substrate-specific calibration) and a Theta probe. Data were collected weekly in pots that were irrigated when θ dropped below one of six different θ thresholds.

Fig. 4. The relationship between bulk electrical conductivity (EC) measurements collected with time domain transmissometry (TDT) sensors and the pore water EC (top) and bulk EC (bottom) as measured using a SigmaProbe.

3.4. Evapotranspiration and plant growth

The evapotranspiration increased linearly as the threshold θ increased ($r^2 = 0.68$) (Fig. 5). Leaching was only noticed during the first five days of the experiment and only in the $0.472 \text{ m}^3 \cdot \text{m}^{-3}$ treatment. Average evapotranspiration over the 66 d experiment was $64 \text{ mL} \cdot \text{d}^{-1}$ in the $0.472 \text{ m}^3 \cdot \text{m}^{-3}$ treatment and only $22 \text{ mL} \cdot \text{d}^{-1}$ in the $0.136 \text{ m}^3 \cdot \text{m}^{-3}$ treatment. Conover (2010) reported an average water use of angel-wing begonias (*Begonia coccinea*) of $68 \text{ mL} \cdot \text{d}^{-1}$, similar to that at our highest θ threshold. Van Iersel et al. (2010) also reported increasing water use with increasing threshold θ , but with a much stronger correlation ($r^2=0.89$). On the other hand, Burnett and van Iersel (2008) saw leaching and excess irrigation at the highest θ

threshold, resulting in a quadratic relationship between θ and water use. The leaching at the beginning of our experiment may explain the high standard deviation in the $0.472 \text{ m}^3 \cdot \text{m}^{-3}$ treatment, but there is no apparent reason for the high variability in the $0.348 \text{ m}^3 \cdot \text{m}^{-3}$ treatment (Fig. 5). This may have been caused by differences in plant size or microclimatic conditions.

Shoot DW increased as the θ threshold increased, but there were no statistical differences in DW at a θ of $0.348 \text{ m}^3 \cdot \text{m}^{-3}$ or above (Table 1). Burnett and van Iersel (2008) found a linear growth response in gaura, and van Iersel et al. (2010) a quadratic response in petunia, with little additional growth above a threshold of $0.25 \text{ m}^3 \cdot \text{m}^{-3}$.

Fig. 5. Cumulative evapotranspiration from treatment initiation until the termination of the experiment (66 d), as a function of the substrate volumetric water content (θ) threshold at which begonias were irrigated.

Table 1. Effect of different volumetric water content (θ) threshold values for irrigation scheduling on stomatal conductance (g_s), net photosynthesis (P_n), instantaneous water use efficiency (WUE_i), area of the fully expanded leaves, and shoot dry weight (DW) of *Begonia semperflorens* after two months of irrigation using an Acclima CS3500 irrigation controller.

$\theta_{\text{threshold}}$ ($\text{m}^3 \cdot \text{m}^{-3}$)	g_s ($\text{mmol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$)	P_n ($\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$)	WUE_i ($\mu\text{mol}/\text{mol}$)	Leaf size (cm^2)	Shoot DW (g)
0.136	73 b*	9.1 c	128 a	37.0 c	2.20 d
0.210	166 a	14.7 a	109 ab	61.0 ab	4.06 c
0.281	168 a	12.4 ab	76 b	55.9 b	4.34 bc
0.348	189 a	14.3 a	80 b	68.3 ab	5.98 ab
0.412	135 a	10.9 bc	82 b	74.8 a	5.99 ab
0.472	174 a	12.2 ab	73 b	68.9 ab	6.50 a

*Means within a column followed by the same letter are not significantly different ($P < 0.05$).

There was a quadratic relationship between evapotranspiration and shoot DW. Shoot DW increased linearly as evapotranspiration increased from 1.3 to 3.1 L (Fig. 6). The slope of the regression line is an estimate of the water use efficiency of the plants, indicating that the plants could produce 3 g of dry matter for every liter of water used. This is similar to the $2.54 \text{ g} \cdot \text{L}^{-1}$ reported for petunia under greenhouse conditions, with θ ranging from 0.05 to 0.40 $\text{m}^3 \cdot \text{m}^{-3}$ (van Iersel et al., 2010). When evapotranspiration exceeded 3.1 L/plant, there was a tendency for a decrease in DW with increasing evapotranspiration. This could be due to overirrigation negatively impacting root activity. Nemali and van Iersel (2006) reported a similar behaviour in four bedding plants species, where the highest threshold θ tended to reduce shoot DW.

The area of the fully-expanded leaf was greatly reduced by low θ (Table 1). Such leaf area reduction is a well-known drought response, reducing the transpiring surface area of many species, e.g., oleander (*Nerium oleander*) (Bañón et al., 2005) and gaura (Burnett and van Iersel, 2008). The area of the fully-expanded leaf was strongly and positively correlated with shoot DW ($r^2 = 0.93$) (data not shown), suggesting that reduced leaf elongation may be partially responsible for growth reductions under drought conditions.

3.5. Photosynthetic activity

The P_n and g_s were reduced and the instantaneous water use efficiency increased at the 0.136 $\text{m}^3 \cdot \text{m}^{-3}$ threshold, but they were similar among the other treatments (Table 1). Earl (2003) also found that P_n and g_s in soybean and cotton were only decreased in the most severe

drought treatment (a substrate water content of 20% of container capacity). There were no treatments differences in maximum quantum yield of photosystem II ($F_v/F_m = 0.80$), photosystem II efficiency ($\phi PS_{II}=0.16$), and non photochemical quenching ($NPQ=2.28$). Similar ϕPS_{II} in all treatments and lower P_n in the lowest θ treatment, suggest that this treatment may have had increased photorespiration, a normal response to drought (Maxwell and Johnson, 2000; Wingler et al., 1999). Cha-um and Kirdmanee (2010) found that F_v/F_m and ϕPS_{II} of eucalyptus (*Eucalyptus camaldulensis*) were not sensitive to drought stress, although P_n and NPQ decreased under severe drought. The F_v/F_m results indicate that photosystem II is not damaged by this level of drought (Maxwell and Johnson, 2000), which is consistent with the suggestion of Fracheboud and Leipner (2003) that chlorophyll fluorescence is not a very useful indicator for drought stress in C3 plants. However, the drought stress resulted in morphological and physiological changes (stomatal closure, reduction of leaf size) in the begonias to reduce water loss, but also reducing growth. The strong correlation between leaf size and shoot DW is consistent with findings by Montesano and van Iersel (2007), who found that growth reductions of tomato (*Solanum lycopersicum* L.) exposed to salinity stress are caused more by effects on leaf elongation than by effects on leaf photosynthesis.

Fig. 6. Shoot dry weight of begonias as a function of total evapotranspiration since the start of the irrigation treatments. The quadratic regression curve was fitted to all data points, while the linear regression includes only

those treatments which did not receive excess water (open symbols). The slope of this regression line is an estimate of the water use efficiency of the begonias.

4. Conclusion

The TDT sensors provide accurate measurements of θ after a substrate-specific calibration. The CS3500 irrigation controller, using TDT sensors, accurately maintained θ readings at different, user-specified levels. Growth of begonias was reduced by a θ threshold below $0.348 \text{ m}^3 \cdot \text{m}^{-3}$. The lowest threshold θ ($0.136 \text{ m}^3 \cdot \text{m}^{-3}$) reduced shoot DW, leaf area, leaf photosynthesis, and stomatal conductance. However, photosystem II was not damaged by the drought stress. Overall, shoot DW was much better correlated with the area of the uppermost fully-expanded leaf than with leaf photosynthesis. This suggests that drought effects on plant growth are caused more by reductions in photosynthetically active surface area than by photosynthesis per unit leaf area.

The CS3500 irrigation controller, using TDT sensors, was able to irrigate plants as needed, suggesting that it can be used for irrigation control of container-grown crops as well as for drought stress research. The CS3500 irrigation controller has the advantage that it is commercially available and easy to install without the need for programming knowledge. Due to the length of the TDT sensors, their use will be limited to pots of at least 20 cm height. Although the TDT sensors also measure bulk substrate EC, further research must be done to test its value. Under our experimental conditions (soilless substrate and slow release fertilizer), the TDT sensor generally measured a bulk EC of $0 \text{ dS} \cdot \text{m}^{-1}$ at θ below $0.28 \text{ m}^3 \cdot \text{m}^{-3}$, which would limit its usefulness.

Acknowledgements

Funding for this research was provided by the Fred C. Gloeckner Foundation and USDA-NIFA-SCRI Award no. 2009-51181-05768 and by projects CICYT (AGL 2005-05588-C02-1-2). We thank Acclima for donating the sensors and irrigation controller, Fafard Inc. for donating the substrate, and Sue Dove for technical assistance.

References

- Allen, R.G., L.S. Pereira, D. Raes, and M. Smith. 1998. Crop evapotranspiration. Guidelines for computing crop water requirements. FAO Irrigation and Drainage Paper 56. FAO, Rome, Italy.
- Bañón, S., J. Ochoa, J.A. Franco, J.J. Alarcón, and M.J. Sánchez-Blanco. 2005. Hardening of oleander seedlings by deficit irrigation and low air humidity. *Environ. Expt. Bot.* 56: 36-43.
- Blonquist Jr, J.M., S.B. Jones, and D.A. Robinson. 2006. Precise irrigation scheduling for turfgrass using a subsurface electromagnetic soil moisture sensor. *Agr. Water Mgt.* 84:153-165.
- Blonquist J.M., S.B. Jones, and D.A. Robinson. 2005a. A time domain transmission sensor with TDR performance characteristics. *J. Hydrol.* 314:235-245.
- Blonquist J.M., S.B. Jones, and D.A. Robinson. 2005b. Standardizing characterization of electromagnetic water content sensors: Part 2 Evaluation of seven sensing systems. *Vadose Zone J.* 4:1059-1069.
- Burnett, S.E. and M.W. van Iersel. 2008. Morphology and irrigation efficiency of *Gaura lindheimeri* grown with capacitance sensor-controlled irrigation. *HortScience* 43:1555-1560.
- Cardenas-Lailhacar, B. and M.D. Dukes. 2010. Precision soil moisture sensor irrigation controllers under field conditions. *Agr. Water Mgt.* 97:666-672
- Cha-um, S. and C. Kirdmanee. 2010. Effects of water stress induced by sodium chloride and mannitol on proline accumulation, photosynthetic abilities and growth characters of eucalyptus. *New Forests* 40:349-360.
- Conover, C.A. 2010. Angel-wing begonia growth and water requirements affected by paclobutrazol. 18 March 2011. <http://mrec.ifas.ufl.edu/foilage/resrpts/rh_94_4.htm>
- Dennis, J.L., R.G. Lopez, B.K. Behe, C.R. Hall, C. Yue, and B.L. Campbell. 2010. Sustainable production practices adopted by greenhouse and nursery plant growers. *HortScience* 45:1232-1237.
- Earl, H.J. 2003. A precise gravimetric method for simulating drought stress in pot experiments. *Crop Sci.* 43:1868-1873.

- Fernandez, R.T., J. Lea-Cox, G. Zinati, C. Hong, R. Cabrera, D. Merhaut, J. Albano, M. van Iersel, T. Yeager, and D. Buhler. 2009. NCDC216: A new multistate group for water management and quality for ornamental crop production and health. *Proc, SNA Res. Conf.* 54:35-38.
- Fracheboud, Y. and Leipner, J., 2003. The application of chlorophyll fluorescence to study light, temperature, and drought stress. p. 125-150. In: J.R. DeEll and P.M.A. Toivonen (eds.). *Practical applications of chlorophyll fluorescence in plant biology*. Kluwer Acad. Pub., Boston.
- Hilhorst, M.A. 2000. A pore water conductivity sensor. *Soil Sci. Soc. Amer. J.* 64:1922-1925.
- Jones, H.G. 2007. Monitoring plant and soil water status: Established and novel methods revisited and their relevance to studies of drought tolerance. *J. Expt. Bot.* 55:2427-2436.
- Jury, W.A. and H. Vaux. 2005. The role of science in solving the world's emerging water problems. *Proc. Nat. Acad. Sci.* 102:15715-15720.
- Majsztrik, J., A.G. Ristvey, and J.D. Lea-Cox. 2011. Water and nutrient management in the production of container-grown ornamentals. *Hort. Rev.* 38:253-296.
- Maxwell, K. and G.N. Johnson. 2000. Chlorophyll fluorescence - a practical guide. *J. Expt. Bot.* 51:659-668.
- McCready, M.S., M.D. Dukes, and G.L. Miller. 2009. Water conservation potential of smart irrigation controllers on St. Augustinegrass. *Agr. Water Mgt.* 96:1623-1632.
- Montesano, F. and M.W. van Iersel. 2007. Calcium can prevent toxic effects of Na^+ on tomato leaf photosynthesis, but does not restore growth. *J. Amer. Soc. Hort. Sci.* 132:310-318.
- Naasz, R., J.-C. Michel, and S. Charpentier. 2005. Measuring hysteretic hydraulic properties of peat and pine bark using a transient method. *Soil Sci. Soc. Amer. J.* 69:13-22.
- 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.
- Nemali, K.S., F. Montesano, S. Dove, and M.W. van Iersel. 2007. Calibration and performance of moisture sensors in soilless substrates: ECH₂O and Theta probes. *Sci. Hort.* 112:227-334.
- Sheng, M., M. Tang, H. Chen, B. Yang, F. Zhang, and Y. Huang. 2008. Influence of arbuscular mycorrhizae on photosynthesis and water status of maize plants under salt stress. *Mycorrhiza* 18:287-296.
- Shock, C.C. and F-X. Wang. 2011. Soil water tension, a powerful measurement for productivity and stewardship. *HortScience* 46:178-185

- Toop, G.G. and T.P.A. Ferre. 2002. Water content, p. 417-545. In: J.H. Dane and G.C. Topp (Eds.), *Methods of soil analysis part 4. Physical methods*. Soil Sci. Soc. Amer. Inc., Madison, WI.
- van Iersel, M.W., S. Dove, J-G. Kang, and S.E. Burnett. 2010. Growth and water use of petunia as affected by substrate water content and daily light integral. *HortScience* 45:277-282.
- Wallach, R. 2008. Physical characteristics of soilless media, p. 41-116. In: M. Raviv and J.H. Lieth (Eds.), *Soilless culture. Theory and practice*. Elsevier, Amsterdam.
- Wingler, A., W.P. Quick, R.A. Bungard, K.J. Bailey, P.J. Lea, and R.C. Leegood. 1999. The role of photorespiration during drought stress: an analysis utilizing barley mutants with reduced activities of photorespiratory enzymes. *Plant Cell Environ.* 22:361-373.

Chapter 5

Pot-in-pot increases salt tolerance in *Euonymus japonicus*: Maintains plant growth and reduces water consumption

Authors: J. Miralles¹, M.J. Sánchez-Blanco^{1,2}, J.J. Martínez-Sánchez^{1,3} and S. Bañón^{1,3}

¹Dpto. de Producción Vegetal, ETSIA, Universidad Politécnica de Cartagena (UPCT), Paseo Alfonso XIII, 48. 30203 Cartagena (Murcia), Spain

²Dpto de Riego, CEBAS-CSIC, C/ Campus Universitario, 14, 30100, Murcia, Spain

³Unidad Asociada al CSIC de Horticultura Sostenible en Zonas Áridas (UPCT-CEBAS), Paseo Alfonso XIII, 48. 30203 Cartagena (Murcia), Spain

Under review in HortScience since 13 February 2011.

Abstract

Irrigation techniques which can improve saline irrigation tolerance are needed to produce good quality plants. The aim of this study was to determine whether the pot-in-pot (PIP) cultivation system can improve the saline irrigation tolerance at $9 \text{ dS}\cdot\text{m}^{-1}$ of *Euonymus japonicus* compared with above ground potting (AGP) in terms of water use efficiency (WUE), irrigated water evapotranspired (IWET), stem diameter variations and photochemical efficiency. A 172 days experiment starting on 6 March with four treatments was set up: AGP and PIP with control irrigation at $1.2 \text{ dS}\cdot\text{m}^{-1}$ (AGP-c and PIP-c, respectively); and AGP and PIP with saline water at $9 \text{ dS}\cdot\text{m}^{-1}$ (AGP-s and PIP-s, respectively). A soil moisture sensor-controlled irrigation system was used to irrigate all the treatments when AGP-c reached a volume water content (θ) of 0.33 or $0.35 \text{ m}^3\cdot\text{m}^{-3}$. The θ gradually increased in saline irrigated plants as the experiment progressed. PIP-s led to a higher θ than AGP-s in general, although the θ was lowest in PIP-c treatment between 12 July to 2 August. The IWET was greater in AGP treatments than in PIP (lowest in PIP-s), and the dry weight (DW) was only reduced in AGP-s. The WUE was lowest in AGP-s and highest in PIP-s. Daily IWET was positively correlated with the daily light integral (DLI) and age in the control irrigation treatments, while under saline irrigation the IWET was only correlated with DLI, presumably as a result of greater stomata regulation and lower plant growth. Stem growth was greater in PIP treatments. AGP-s plants did not grow, while PIP-s stopped growing by the middle of July. The MDS was inversely correlated with θ between 12-July and 2-August only in the irrigation control treatments, particularly PIP-c. Salinity decreased net photosynthesis in AGP-s plants, which suffered photorespiration. The results show that PIP can improve WUE and salinity tolerance of *Euonymus japonicus* because it maintains plant growth.

Keywords: Pot plant, maximum daily shrinkage, stem growth rate, evapotranspiration, water use efficiency, daily light integral, volume water content, automated irrigation, gas exchange.

1. Introduction

The favorable growing conditions of the Mediterranean coastal region in Spain (high solar radiation) contrast sharply with the scarcity of good quality water, which forces growers to use water with moderate or high saline concentrations (Rubio et al., 2010). Further, a high population density, especially in coastal areas, has a great reduction in woodlands and an increase in intensive agriculture (Naveh and Lieberman, 1994). Such conditions have led to

the overexploitation of aquifers, which has resulted in their salinization through sea water intrusion and fertilizer infiltration. In areas like the Spanish Mediterranean, it would be desirable to irrigate plants with saline water or to blend saline with high quality water, as well as to grow selected salt-tolerant crops (Flowers et al., 2005). Besides, there are other factors which may affect salt tolerance, and good management may help produce good quality plants. Niu et al. (2010b) reported that salt tolerance of plants depends on species, climatic conditions, type of substrate or soil, and irrigation method. However, the cultivation system can also influence the salt tolerance.

Pot-in-pot (PIP) production, introduced around 1990 (Parkerson, 1990) in the USA, is a nursery production method that 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, 1998). Limited research has been carried out to determine the potential benefits of PIP production using saline irrigation. Niu and Rodriguez (2006) reported that, although salinity levels of the irrigation water were kept constant, root zone salinity increased with time. Salt accumulation in the root zone is affected by substrate properties, plant size and environmental conditions because these factors influence the substrate moisture content and cation exchange capacity in the root zone (Niu et al., 2010b). Previous research into PIP compared with above ground potting (AGP) determined that PIP reduces root zone temperature stress (Young and Bachman, 1996) and enhances efficient water use by decreasing container evapotranspiration (Martin et al., 1999). These facts can then affect salt accumulation (less evapotranspiration) and root development (lower temperature).

Euonymus japonicus 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. Percival (2005) determined that two cultivars of the same genus species (*Euonymus fortunei*) were within the first four in order of salinity tolerance within 30 woody perennials species studied. However, there is little information concerning the response of this species, in terms of water consumption and growth to saline irrigation.

The volume water content (θ) can be easily monitored with soil moisture sensors and can provide information on water uptake by roots, which is often limited by the osmotic component of salinity (Rodríguez et al., 1997). Furthermore, irrigation can be easily automated with a soil moisture sensor (Jones, 2004), which, under saline irrigation conditions, is very important because leaching plays an important role in salt-tolerance and can be

controlled more accurately with this automated irrigation system. Along with plant growth, trunk diameter growth was studied, because several studies have suggested that is one of the most sensitive water indicators (Ortuño et al., 2010) and also suitable for shrubs of small diameter (Miralles et al., 2010a). Therefore stem diameter variations (SDV) originated by water changes in the trunk tissues were studied by dendrometer (Molz and Klepper, 1973). The high frequency measurements obtained with this electronic sensor usually point to a maximum diameter value just before sunrise (MXDS) and a minimum diameter value sometime during the afternoon (MNDS), the difference between them being the maximum daily shrinkage (MDS) and the difference between two consecutive MXDS the stem growth rate (SGR). It has been suggested that both MDS and the SGR are related with the plant water status (Ortuño et al., 2010), and could be sensitive to both salinity and the cultivation system used.

The objective of this study was to evaluate the potential benefits of the PIP system for reducing saline effects (growth reduction and photosynthetic damages) in *Euonymus japonicus*, and changes in water consumption. For this, plants were irrigated with good quality and saline water in PIP and AGP, and the following points were studied: 1) the effects on θ , dendrometry and photochemical efficiency; and 2) the irrigated water evapotranspired (IWET) and its water use efficiency (WUE).

2. Material and methods

2.1. Plant material and crop conditions

One year old seedlings of commercial *Euonymus japonicus* var. Marieke were grown in 96 multi-pot trays measuring 60 cm (length) $\times$ 30 cm (width) $\times$ 3 cm (height) (Plasnor S.A. Legazpi, Spain). The pots were arranged in a 12 $\times$ 8 configuration, had an inverted cube form, and a volume of 56 cm³. The seedlings were transplanted in January 2010 to black PVC pots (cultivation pot) of 2.5 L volume, 16 cm upper external diameter and 15 cm height. The substrate was a mixture of white peat, coco fiber and perlite (40/40/20, v/v/v), and was amended with 3 g·L⁻¹ of a slow release fertilizer (osmocote plus 14-14-14; 14N-4.2P-11.6K; release time 2-3 months at 21 °C; The Scotts Company LLC, Marysville, OH). A total of 250 seedlings were transplanted to the pots (one seedling per pot). For establishment, the plants

were grown in a greenhouse for two months, after which 220 plants were transferred to the experimental plot (Picture 1).

Picture 1. Experimental plot at the end of the experiment.

The experiment was conducted in an open air plot of 70 m² at the “Tomás Ferro” Experimental Agro-Food Station of the Polytechnic University of Cartagena (UPCT), (37°35' N, 0°59' W), starting on 6 March 2010 and ending on 24 August 2010 (172 d). Weather conditions were taken from a Meteorological Station sited 100 m from the experimental plot. The mean, maximum and minimum daily values of temperature and relative humidity, solar radiation data [daily light integral (DLI)], and daily precipitation were registered. Calculations of the vapor pressure deficit (VPD) were made using the equation derived by Snyder et al. (1986).

2.2. Experimental design

The PIP system consisted of placing cultivation pots in pots already buried in the ground. The buried pots were made of black PVC 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 gr/m² PROJAR S.A., Valencia, Spain), which was covered with a 4 cm layer of gravel (~2 cm Ø). 220 cultivation pots were placed in 10 rows, 60 cm apart so that each row had 22 cultivation pots. These were placed 55 cm apart, buried pots (PIP) alternating with above ground pots (AGP). A drip irrigation system was installed with one auto-compensated 2 L·h⁻¹ dripper (Netafim USA, Fresno, CA) per plant connected to one spaghetti tube which finished in an arrow. Each row of 22 pots received the same quality of water during the experiment, so five rows received saline water (9 dS·m⁻¹) and the other five good quality water (about 1-2 dS·m⁻¹). This resulted in a total of four treatments with five blocks of 11 plants each: AGP with good water (AGP-c), PIP with good water (PIP-c), AGP with saline water (AGP-s) and PIP with saline water (PIP-s). 7.5 grams per pot of slow release fertilizer (described above) was applied on 1 March and 1 June.

A datalogger 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 S.L., Barcelona, Spain) per treatment, volume water content (θ) with five soil moisture sensors (Decagon Devices, Ltd., Pullman, WA) per treatment and dendrometry with five LVDT dendrometers (Ecomatik DD, Munich, Germany) per treatment (Picture 2). Both, soil moisture sensors and LVDT sensors were connected to the CR1000 through two multiplexers (one with 16 LVDT sensors and the other with 20 soil moisture sensors and four LVDT sensors). The CR1000 was programmed to collect data every 10 minutes and to calculate average θ and its standard error 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 maximum and minimum daily diameter and the number of irrigation events on a daily basis. The maximum daily shrinkage (MDS) was calculated from the stem diameter variations (SDV) as the difference in diameter between the maximum, reached just before sunrise (MXDS), and the minimum (MNDS), reached some time during the afternoon. The stem growth rate (SGR) was calculated as the difference between two consecutive maximum daily trunk diameters (Ortuño et al., 2010). The accumulated stem growth was determined by adding each SGR to the previous measurement.

Picture 2. Detail of plant with dendrometer and soil moisture sensor (only the wire is visible).

Irrigation was applied when the average θ of the AGP-c treatment reached the threshold of $0.33 \text{ m}^3\text{m}^{-3}$ from 6 March to 7 June. After this date, the threshold was increased to $0.35 \text{ m}^3\text{m}^{-3}$ (because of an error in the CR1000 program), until the 24 August. 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 good water and saline water pumps through an Agronic 3000 irrigation controller (Sistemas Electronics Progres, S.A., Lerida, Spain). The saline solution of $9 \text{ dS}\cdot\text{m}^{-1}$ was obtained by adding about $4 \text{ g}\cdot\text{L}^{-1}$ of sodium chloride to the irrigation water in a 1000 L water tank. Each time an irrigation event took place, all the plants were irrigated for the same time (about 15 minutes and 550 ml) which led to average leaching of $\sim 32\%$ ($\sim 180 \text{ mL}$) for AGP-c. Leaching (32%) was the double the fraction recommended for irrigation with good water in the area, in order to ensure that no substrate salinity would affect the good water irrigation treatments. This irrigation criterion was established because previous studies with the PIP system concluded that it saves water (Miralles et al., 2009), while Navarro et al. (2007) observed a significant increase in θ , as the salinity stress increased, so the highest water needs were associated with the AGP-c treatment.

2.3. Irrigation water evapotranspired and water use efficiency

Samples of irrigation water and leachate were collected once a week in bottles and plastic bags, respectively. The bottles were placed inside the buried pots with one dripper in the bottle to collect the irrigation volume (three replicates were used for each type of irrigation water). The bags were placed inside the empty chamber in five replicates of the PIP system or inside buried pots below five replicates of the AGP treatments, where pot-grown plants were held in place by a mesh structure (Picture 3). When two or three irrigation events were registered in the same week the irrigation and leachate were averaged. The irrigation water evapotranspired (IWET) between two irrigation events was calculated as the difference between the amount of irrigation water applied and the leachate of the second irrigation event if no precipitation was registered in this period. The accumulated irrigation and IWET, regardless of precipitation, were calculated adding each irrigation and IWET to the previous measurement. The daily mean IWET within a period without precipitation was determined as: $IWET_b/D$, where D is the number of days between two consecutive IWET values with no precipitation between them, and $IWET_b$ is the second IWET datum registered in that period.

The water use efficiency (WUE) was calculated as the total dry weight (DW) harvested on 30 July divided by the total IWET from 13 April to 30 July. To calculate the DW, five plants per treatment (shoots 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 series precision balance (sensitivity of 10 mg and up to 1200 g, Gram Precision SL, Barcelona, Spain).

2.4. Gas exchange and chlorophyll fluorescence

The midday stomatal conductance (g_s) and net photosynthesis rate at midday (P_n) were measured using a CIRAS-2 Portable Photosynthesis System (PP Systems, Amesbury, MA). The flow rate of the chamber was 200 mL·min⁻¹ with 350 ppm of CO₂, 100% relative humidity and 1500 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of photosynthetic photon flux density (PPFD). The measurements were made in five plants per treatment.

The chlorophyll fluorescence was measured in five leaves per replicate using a Pulse Modulated Fluorimeter FMS-2 (Hansatech Instruments Ltd, Pentney, England). The method

used and parameters determined were the same as described by Sheng et al. (2008) but using an actinic light of 400 PPFD for the light adapted measurements.

Gas exchange and chlorophyll fluorescence were measured on 20/May, 21/June and 20/July.

Picture 3. Detail of bags for leaching collection. AGP (left) with the mesh structure to support the plant, PIP (right) with the bag inside the space between pots.

2.5. Study of correlations

Correlations between mean DLI, mean VPD and days after first irrigation (DAFI) vs. daily mean IWET were studied. To calculate mean DLI and mean VPD, the daily mean registers were averaged for the same days as for the daily mean IWET. The DAFI were 134 days, day 1 being 13 April and day 134 the 24 August.

Correlations between MDS vs. minimum daily θ were also determined for the period 12 July and 2 August.

2.6. Statistical analysis

The design was a randomized complete block. There were five blocks of 11 plants per treatment, which were set in the same row. A total of five replicates (one per block) were used, except in the case of substrate temperature for which there were two replicates. Variability in θ between replications is shown as the least statistical difference (LSD 5%) to indicate differences between treatment means. The accumulated IWET and stem growth variability are shown as the square root of the sum of squares of standard deviations. Data for the measurements of gas exchange, chlorophyll fluorescence and water use efficiency were subjected to a two-way ANOVA with a $P < 0.05$ using the software Statgraphics Plus 5.1 (StatPoint Technologies Inc., Warrenton, VA). The regression analyses were performed with the software sigmaplot 10.0 (Systat software Inc., San Jose, CA).

3. Results and discussion

3.1. Substrate water content and stomatal conductance

The automated irrigation system was generally able to maintain AGP-c θ above the thresholds established (Fig. 1). However, irrigation on 13 April and 4 June were triggered at a lower θ than the $\theta_{\text{threshold}}$ for AGP-c because on the first date, the pumps were not properly connected, and on the second date there was an electricity cut (the CR1000 continued to work because it had a battery). For the first 38 days of the experiment, plants were not irrigated because of their small size, frequent precipitation and mild environmental conditions (DLI and VPD, not high, Fig. 2). However, as environmental conditions became warmer, irrigation frequency started to increase (once a week at the beginning and three or four times a week at the end of experiment). The marked increase of θ on 14 June and, to a lesser extent, on 13 August reflects stronger rainfall, allowing a more uniform water distribution than with the dripper. De Rijck and Schrevens (1998) reported that the solution supplied to a rockwool slab by dripper irrigation had a tendency to flow down rather than to spread horizontally, which would explain the greater θ increases when the precipitation occurred in our experiment. At the end of the experiment, the θ data showed a tendency for the saline treatments to keep more humidity in the substrate than control irrigation, especially the PIP-s treatment. This

result agrees with that obtained by Navarro et al. (2007) in *Arbutus unedo* seedlings treated for six months with 5.45 and 9.45 dS·m⁻¹.

Fig. 1. Average volume water content (θ) of each treatment. Horizontal dashed lines indicate the θ threshold to irrigate when AGP-c reached 0.33 m³·m⁻³ from 6 March to the 7 June and 0.35 m³·m⁻³ from 7 June until 24 August (the end of experiment). Vertical dashed lines indicate the period during which θ was correlated with maximum daily shrinkage (MDS) (12 July to 2 August). Vertical bar shows LSD at $P < 0.05$.

Under control irrigation conditions, θ in PIP-c was similar to AGP-c until the end of June, and lower from 23 July to the 3 August, after which θ values in PIP-c were higher than AGP-c (Fig. 1). Miralles et al. (2009) found hardly any differences in soil matric potential between PIP and AGP in summer in *Myrtus communis*. This may be explained by the higher transpiration in PIP, influenced by the higher stomatal conductance, which would compensate the greater substrate evaporation observed in AGP. In this experiment, no differences in g_s between both systems were found (Fig. 3B), possibly because the gas exchange measurements on the 20th of July were taken with similar θ in control irrigation treatments (both AGP and PIP). In addition, the environmental conditions of summer (high VPD and low θ , Fig. 1 and 2)

could cause stomatal closure in order to avoid water loss at midday (Dai et al., 2009; Tenhunen et al., 1984). On the other hand, higher previous θ in PIP-c could have delayed the stomatal closure, producing a greater θ decrease. Saline irrigation treatments had higher g_s at the end of experimentation (Fig. 3B), and higher θ (Fig. 1), which may be due to low stomatal regulation at midday. In *Sophora secundiflora* irrigated with saline water at $3.0 \text{ dS}\cdot\text{m}^{-1}$ Niu et al. (2010a) reported a 38% lower g_s than in the control. Lloyd and Howie (1991) also reported lower values of g_s in the morning in orange trees treated with $20 \text{ mol}\cdot\text{m}^{-3}$ NaCl, due to the inability of the stomata to function. So, although midday g_s was the same, or greater, in saline irrigation as in the control irrigation, the daily g_s must have been lower in saline irrigation because θ was higher and IWET was lower (Fig. 1 and 4). The possibility that measurement of g_s with the methodology used may not be representative of the entire plant (van Iersel and Bugbee, 2000) would explain these findings.

Fig. 2. Daily light integral (DLI) and mean daily vapour pressure deficit (VPD) from 6 March 2010 to the 24 August 2010. Vertical dashed lines indicate the period during which θ was correlated with maximum daily shrinkage (MDS) (12 July to 2 August).

Fig. 3. Photosystem II efficiency (ϵ_{PSII}) (A), Stomatal conductance (g_s) (B), and net photosynthesis (P_n) (C). This data were analyzed with a two way-ANOVA, and both ϵ_{PSII} and g_s were only significant for the irrigation factor (control and saline). P_n had a significant interaction, so all the treatments were plotted separately. Error bars indicate the standard error ($n=10$ in ϵ_{PSII} and g_s ; $n=5$ in P_n).

3.2. Water use

The amount of water applied during the experimental period was $19.63 \text{ L} \cdot \text{pot}^{-1}$ in the control irrigation and $21.78 \text{ L} \cdot \text{pot}^{-1}$ in the saline irrigation (Fig. 4) (each saline irrigation event using an average of 55 mL extra of water). This difference was due to the way in which the water pumps worked. The IWET was greater in AGP systems than in PIP, and in the control irrigation compared with saline irrigation (Fig. 4). As a consequence, the mean leaching fraction was about 32% in AGP-c, 40% in PIP-c, 39% in AGP-s and 48% in PIP-s. Martin et al. (1999) reported that *Acacia smallii* and *Cercidium floridum* required 5.3 L weekly per pot in AGP, and 3.2 L per pot in PIP. This can be explained because AGP produced strong substrate evaporation in the pot (Alarcón et al., 2006). On the other hand, PIP reduced water loss because this system protects the pot from solar radiation and has an air chamber between the two pots which provides nearly 100% of relative humidity at the base of the pot (Miralles et al., 2009).

Fig. 4. Volume of accumulated irrigation water (AIW) (control and saline), accumulated irrigation water evapotranspired (AIWET), and daily precipitation. Error bars indicate the square root of the sum of squares of standard deviations.

The IWET curves showed two slopes, one from the beginning to the end of June and another steeper slope until the end of the experiment, especially in the control irrigation treatments. However, after precipitation events (Fig. 4), the IWET stopped because the rainwater had to be consumed. The two slopes observed could be related to the environmental conditions and plant growth changes. So, the regression, DLI (environmental variable) vs. IWET and days after first irrigation (DAFI) (plant size variable) vs. IWET were studied (Table 1). Only IWET vs. DLI was significant for the saline irrigation, while both correlations were significant in the control irrigation, although better for the IWET vs. DAFI. For the cultivation system, both correlations were significant, although IWET vs. DLI was slightly greater.

Table 1. Regression analysis between irrigated water evapotranspired (IWET) and both DLI and days after first irrigation (DAFI).

	IWET vs. DLI	IWET vs. DAFI
Salt	$y = -72.68 + 7.81x$; $R^2 = 0.18^{**}$	ns
Control	$y = 14.34 + 4.89x$; $R^2 = 0.07^*$	$y = 69.02 + 0.73x$; $R^2 = 0.18^{**}$
AGP	$y = -28.34 + 6.77x$; $R^2 = 0.12^{**}$	$y = 86.15 + 0.55x$; $R^2 = 0.09^*$
PIP	$y = -30.00 + 5.93x$; $R^2 = 0.12^*$	$y = 74.32 + 0.43x$; $R^2 = 0.07^*$

The data were analyzed for the factor irrigation water (salt and control) and the factor cultivation system (AGP and PIP) because the interaction was not significant. *Statistically significant at $P < 0.05$, **Statistically significant at $P < 0.005$, ns (not significant).

In Petunia van Iersel et al. (2010) reported that irrigation volume in all treatments (different θ threshold) tended to increase during the experiment, probably as a result of increased water use by larger plants and changes in DLI. Kim and van Iersel (2009) also developed a similar correlation for abutilon (*Abutilon x hybridum*) and lantana (*Lantana camara*) for DLI and age, and also for the VPD although this was not as strongly correlated. In our study, the VPD was not significant and the R^2 were weaker than those reported in the above works. These differences in results may be related to two factors: 1) the different irrigation systems (absence of leaching), and 2) the different crop conditions (no precipitation). Burnett and van Iersel (2008), reported greater between-replication variations at the two highest θ because of leaching, when studying mean total applied irrigation volumes. Perhaps, different substrate hydration rates by drip irrigation (De Rijck and Schrevens, 1998) produce quite dry zones which could decrease hydraulic conductivity in peat-based substrates (Naasz et al., 2005). This decrease in hydraulic conductivity slows water movement in the substrate and potentially results in less uniform water distribution,

thus increasing variability (van Iersel et al., 2010). So, the IWET estimation when there is leaching is less accurate because irrigation does not moisten the substrate homogenously and does not replace all the water evapotranspired, because leaching is produced before the substrate reaches field capacity. This becomes more important as the substrate becomes dryer, which may explain why salinity treatments at the end of the experiment have a more stable and greater θ than control irrigation (Fig. 1). The non significant correlation between IWET vs. DAFI observed with saline irrigation may be related with the fact that growth stopped in this treatments (no growth in AGP-s at any time and growth cessation by mid July in PIP-s), as shown by accumulated stem growth (Fig. 5A). So, the plants used water for maintenance and not to growth, which made the DAFI component less important. Navarro et al. (2007) also reported greater θ in *Arbutus unedo* plants irrigated with saline water as well as a significant decrease in total biomass, leaf area, plant height and water flowing through the root system.

E. japonicus plants showed lower total DW production and WUE in AGP-s (about 5.39 g and 0.52 g DW·L⁻¹, respectively), while PIP-s had a similar DW as irrigation control treatments (about 10.2 g) and a greater WUE (0.20 g DW·L⁻¹ more than the control at 1.03 g DW·L⁻¹). Geissler et al. (2009) obtained similar results in salt treated *Aster tripolium* because stomatal resistance increased and growth stopped. Karlberg et al. (2006) suggested that WUE becomes lower under saline conditions because the plant allocates relatively more photosynthates to counteract the adverse effects of salinity due to ion toxicity. Thus, fewer assimilates remain for plant growth compared to non-stressed plants. On the other hand, this author also reported that WUE can 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. So, the WUE of the PIP system showed that ion toxicity was avoided, and was only affected by high soil osmotic potential, which caused water stress. Several studies have reported greater WUE under water stress, e.g. Jaleel et al. (2008) in two varieties of *Catharanthus roseus*.

Fig. 5. Accumulated stem growth diameter (A), Stem growth rate (SGR) (B) and maximum daily shrinkage (MDS) (C). Vertical dashed lines indicate the period during which volume water content (θ) and MDS were correlated (12 July to 2 August). Error bars indicate the square root of the sum of squares of standard deviations in the accumulated stem growth diameter (A). In SGR (B) and MDS (C) error bars indicate the standard error (n=5).

3.3. Dendrometry and photochemical study

The SGR was not statistically significant between treatments (Fig. 5B). However, the accumulated stem growth was greater in PIP treatments, and AGP-s plants did not grow (Fig 5A). The MDS was higher in control treatments from 12 July to 2 August (correlation period), especially in PIP and when θ values were the lowest (Fig. 1 and 5C). The results of MDS- θ correlations in that period (Fig. 6) showed no significance in the saline treatments and a weak and modest correlation in AGP-c and PIP-c, respectively. These correlations were linear and reflected the fact that lower θ produced greater MDS. The lack of MDS- θ correlations during the rest of the year (data not shown) could be related to less stressful environmental conditions and to the higher θ in PIP (lower θ would have produced greater MDS). The lack of growth in saline treatments (AGP-s throughout the experimentation period and PIP-s by the middle of July) could justify the absence of MDS- θ correlation due to saline stress. No correlations were reported between θ and stem diameter by Fernández and Cuevas (2010) in their review. One of the reasons may be related to the greater θ variability in soil than in a pot, which increases as the stress becomes more pronounced (Russo et al., 1994), reducing the correlation compared with other variables studied (leaf water potential, evapotranspiration [ET], VPD, midday temperature, etc.). However, in young lemon trees grown in pots, Ortuño et al. (2004) found a moderate correlation between θ and MDS at the beginning of deficit irrigation, in agreement with the results of Huguet et al. (1992), which showed that MDS is a versatile indicator of transpiration stream intensity when the soil water content is not strongly depleted. Miralles et al. (2010b) reported in *Myrtus communis* under severe water stress, correlations between maximum and minimum daily diameter vs. ET as well as θ vs. ET, which suggests that this dendrometric indices might also be related with θ . In *Rhamnus alaternus* and *Pittosporum tobira*, Miralles et al. (2010a) described that as water stress increased in summer, MDS first increased and then decreased as stress became more severe, while SGR registered negative values when stress started and tended to zero when it became severe. According to previous studies, *E. japonicus* could be starting to suffer water stress at a θ near or below $0.35 \text{ m}^3 \cdot \text{m}^{-3}$ during summer. The absence of stem growth in AGP-s and later in PIP-s indicated the presence of saline stress, although the MDS did not show a clear response compared with control irrigation treatments. This behavior is related with that reported by Moriana et al. (2003) in olive trees, in which MDS did not respond to water stress. On the other hand, Moriana and Fereres (2002) proposed SGR as a water stress

indicator for olive trees, because a decrease in stem growth is one of the earliest responses to plant water stress, particularly in young trees.

Fig. 6. Correlation between maximum daily shrinkage (MDS) and volume water content (θ) during the period 12 July to 2 August. **Statistically significant at $P < 0.005$, ***Statistically significant at $P < 0.0005$, ns (not significant).

The net photosynthesis (P_n) decreased in AGP-s treatments after the second record taken in 21 June (Fig. 3C), which explained the lower DW and the absence of stem growth in these treatments. Niu et al. (2010a) also registered a 30% lower P_n in *Sophora secundiflora* irrigated with saline water (EC of 3.0 and 6.0 $\text{dS}\cdot\text{m}^{-1}$). Torrecillas et al. (2003) also found a P_n decrease in salt treated plants (irrigated with 70 and 140 mM of NaCl) compared with control plants of *Cistus albidus* and *C. monspeliensis*. So, the PIP system alleviated the negative effect of saline irrigation on P_n .

The chlorophyll fluorescence study showed statistical differences in photosystem II efficiency (ϵPSII) with lower value in salt irrigated plants than in control plants on 21 June (Fig. 3A). The maximum quantum yield of PSII (F_v/F_m) and non-photochemical quenching (NPQ) were the same throughout the period and in all treatments with an average value of

0.79 and 2.71, respectively. Percival (2005) reported that increasing salinity reduced F_v/F_m values in thirty woody perennials subjected to salinity with two exceptions (*Brachyglottis dunedin* hybrids ‘Sunshine’ and *Euonymus fortunei* ‘Silver Queen’), in which F_v/F_m values increased. This later response is associated with improved photosynthetic efficiency in response to excess salinity. So, both *Euonymus japonicus* and *E. fortunei* are species that develop adaptation mechanisms to salinity which do not affect chlorophyll fluorescence in general terms. The lower $\phi PSII$ could be a response to lower P_n , although its subsequent increase could indicate the presence of photorespiration or disorder in electron transport because P_n did not recover (Fryer et al., 1998). On other hand, saline irrigated plants presented sings of leaf tip burn, perhaps because they accumulated salt in the tips, which did not affect the leaf as a whole and would have produced similar records for chlorophyll fluoresce, because when measuring a leaf the burnt part was avoided. Other works reported changes in chlorophyll fluorescence parameters under saline irrigation, such as Niu et al. (2008) in three rose rootstocks in which F_v/F_m decreased with salt and Cha-um and Kirdmanee (2010) in *Eucalyptus camaldulensis*, in which F_v/F_m , $\phi PSII$ and NPQ were reduced in NaCl treated plants.

4. Conclusions

The PIP cultivation system improved *Euonymus japonicus* tolerance to salinity. This produced similar DW in PIP-s plants as in control irrigated plants (both PIP-c and AGP-c) and a higher WUE because of the lower IWET. Stem growth was enhanced by PIP in both saline and control conditions and PIP-s delayed plant growth cessation by about four months compared with AGP-s. The P_n was not affected in PIP-s but was reduced in AGP-s plants, which maintained a similar $\phi PSII$, pointing to the presence of photorespiration. So, the use of PIP with saline water in the production of this species is useful to obtain *E. japonicus* plants without growth loss.

Acknowledgements

The authors thank R. Valdes and P. Cánovas for irrigating and supervising the shrubs while the experiment took place. This research was supported by CICYT projects (CICYT AGL2008-05258-CO2-1-AGR and CICYT AGL2008-05258-CO2-2-AGR), SENECA project

(08669/PI/08) and by the Consejería de Agricultura y Agua de la Región de Murcia, program (UPCT-CEBAS-IMIDA 2008).

References

- Alarcón, J.J., M.F. Ortuño, E. Nicolás, A. Navarro, and A. Torrecillas. 2006. Improving water-use efficiency of young lemon trees by shading with aluminised-plastic nets. *Agric. Water Mgt.* 82:387-398.
- Burnett, S.E., and M.W. van Iersel. 2008. Morphology and irrigation efficiency of *Gaura lindheimeri* grown with capacitance sensor-controlled irrigation. *HortScience* 43:1555-1560.
- Cha-um, S., C. Kirdmanee. 2010. Effects of water stress induced by sodium chloride and mannitol on proline accumulation, photosynthetic abilities and growth characters of eucalyptus (*Eucalyptus camaldulensis* Dehnh.). *New Forests* 40:349-360.
- Dai, Y., Z. Shen, Y. Liu, L. Wang, D. Hannaway, and H. Lu. 2009. Effects of shade treatments on the photosynthetic capacity, chlorophyll fluorescence, and chlorophyll content of *Tetrastigma hemsleyanum* diels et Gilg. *Env. Exp. Bot.* 65:177-182.
- De Rijck, G. and E. Schrevers. 1998. Distribution of nutrients and water in rockwool slabs. *Scientia Hort.* 72:277-285.
- Fernández, J.E. and M.V. Cuevas. 2010. Irrigation scheduling from stem diameter variations: A review. *Agric. Forest Meteorol.* 150:135-151.
- Fryer, M.J., J.R. Andrews, K. Oxborough, D.A. Blowers, and N.R. Baker. 1998. Relationship between CO₂ assimilation, photosynthetic electron transport, and active O₂ metabolism in leaves of maize in the field during periods of low temperature. *Plant Physiol.* 116:571-580.
- Flowers, T.J., R. Ragab, N. Malash, G. Abdel-Gawad, J. Cuartero, and A. Arslan. 2005. Sustainable strategies for irrigation in salt-prone Mediterranean: SALTMED. *Agric. Water Mgt.* 78:3-14.
- Geissler, N., S. Hussin, and H.-W. Koyro. 2009. Interactive effects of NaCl salinity and elevated atmospheric CO₂ concentration on growth, photosynthesis, water relations and chemical composition of the potential cash crop halophyte *Aster tripolium* L. *Env. Exp. Bot.* 65:220-231.

- Huguet, J.G., S. Li, J. Lorendeau, and G. Pelloux. 1992. Specific micromorphometric reactions of fruit trees to water stress and irrigation scheduling automation. *J. Hortic. Sci.* 67:631-640.
- Jaleel, C.A., R. Gopi, B. Sankar, M. Gomathinayagam, and R. Panneerselvam. 2008. Differential responses in water use efficiency in two varieties of *Catharanthus roseus* under drought stress. *C. R. Biologies* 331:42-47.
- Jones, H.G. 2004. Irrigation scheduling: Advantages and pitfalls of plant-based methods. *J. Expt. Bot.* 55:2427-2436.
- Karlberg, L., A. Ben-Gal, P.-E. Jansson, and U. Shani. 2006. Modelling transpiration and growth in salinity-stressed tomato under different climatic conditions. *Ecol. Model.* 190:15-40.
- Kim, J. and M.W. van Iersel. 2009. Daily water use of abutilon and lantana at various substrate water contents. *Proc. SNA Res. Conf.* 54:12-16.
- Lloyd, J. and H. Howie. 1991. Salinity, stomatal responses and whole-tree hydraulic conductivity of orchard 'Washington Navel' orange, *Citrus sinensis* (L.) Osbeck. *Austr. J. Plant Physiol.* 16:169-179.
- 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.
- Miralles, J., M.J. Sánchez-Blanco, A. Navarro, J.J. Martínez-Sánchez, J.A. Franco, and S. Bañón. 2010a. Comparison of stem diameter variations in three small ornamental shrubs under water stress. *Hortscience* 45:1681-1689.
- Miralles, J., M.J. Sánchez-Blanco, J.J. Martínez-Sánchez, and S. Bañón. 2010b. Response of EC-10 capacitance sensor and ecomatik DD dendrometer compared with ET rate in potted *Myrtus communis* under stress. In: Transactions, The third International Symposium on soil water measurement using capacitance, impedance and TDT, Murcia, Spain, April 7-9,2010. Eds. I.C. Paltineanu and J. Vera. Paper 2.11. pp 1-10.
- 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.
- Molz, F. J. and B. Klepper. 1973. On the mechanism of water-stress-induced stem deformation. *Agron. J.* 65:304-306.
- Moriana, A. and E. Fereres. 2002. Plant indicators for scheduling irrigation of young olive trees. *Irrig. Sci.* 21:83-90

- Moriana, A., F. Orgaz, E. Fereres, and M. Pastor. 2003. Yield responses of a mature olive orchard to water deficits. *J. Amer. Soc. Hortic. Sci.* 128:425-431.
- Naasz, R., J.-C. Michel, and S. Charpentier. 2005. Measuring hysteretic hydraulic properties of peat and pine bark using a transient method. *Soil Sci. Soc. Amer. J.* 69:13-22.
- Navarro, A., S. Bañón, 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.
- Naveh, Z., Lieberman, A., 1994. *Landscape Ecology: Theory and Application*. Springer Verlag, New York, p. 360.
- 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. *Scientia 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. *Scientia Hort.* 110:292-297.
- Niu, G. and D.S. Rodriguez. 2006. Relative salt tolerance of five herbaceous perennials. *Hortscience* 41:1493-1497.
- 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.
- Ortuño, M.F., W. Conejero, F. Moreno, A. Moriana, D.S. Intrigliolo, C. Biel, C.D. Mellisho, A. Pérez-Pastor, R. Domingo, M.C. Ruiz-Sánchez, J. Casadesus, J. Bonany, and A. Torrecillas. 2010. Could trunk diameter sensors be used in woody crops for irrigation scheduling? A review of current knowledge and future perspectives. *Agric. Water Manag.* 97:1-11.
- Ortuño, M.F., J.J. Alarcón, E. Nicolás, and A. Torrecillas. 2004. Interpreting trunk diameter changes in young lemon trees under deficit irrigation. *Plant Sci.* 167:275-280.
- Parkerson, C.H. 1990. P & P: A new field-type nursery operation. *Proc. Inter. Plant Prop. Soc.* 40:417-419.
- Percival, G.C. 2005. Identification of foliar salt tolerance of woody perennials using chlorophyll fluorescence. *Hortscience* 40:1892-1897.

- Rodríguez, 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. Agric. Sci.* 128:439-444.
- Rubio, J.S., F. Rubio, V. Martínez, and F. García-Sánchez. 2010. Amelioration of salt stress by irrigation management in pepper plants grown in coconut coir dust. *Agric. Water Manag.* 97:1695-1702
- Russo, D., J. Zaidel, and A. Laufer. 1994. Stochastic analysis of solute transport in partially saturated heterogeneous soil. 1. Numerical experiments. *Water Resour. Res.* 30:769-779.
- Ruter, J.M. 1998. Pot-in-Pot production and cyclic irrigation influence growth and irrigation efficiency of 'Okame' cherries. *J. Environ. Hort.* 16:159-162.
- Snyder, R., R.H. Shaw, and K.T. Dawld. 1986. Vapour pressure deficit and other psychrometric properties of air from temperature and relative humidity. *Agric. Res.* 2:183-192.
- Tenhunen, J.D., H.P. Meister, M.M. Caldwell, and O.L. Lange. 1984. Environmental constraints on productivity of the Mediterranean sclerophyll shrub *Quercus coccifera*. *Options Medit.* 1:33-53.
- 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. *Scientia Hort.* 97:353-368.
- van Iersel, M.W., S. Dove, J.G. Kang, and S.E. Burnett. 2010. Growth and water use of petunia as affected by substrate water content and daily light integral. *Hortscience* 45:277-282
- van Iersel, M.W. and B. Bugbee. 2000. A multiple chamber, semicontinuous, crop carbon dioxide exchange system: desing, calibration, and data interpretation. *J. Amer. Soc. Hort. Sci.* 125:86-92.
- Young, R.E. and G.R. Bachman. 1996. Temperature distribution in large, pot-in-pot nursery containers. *J. Environ. Hort.* 14:170-176.

Chapter 6

Pot-in-pot increases salt tolerance in *Euonymus japonicus*: Maintains aesthetic value and reduces chloride uptake

Authors: J. Miralles¹, R. Valdés¹, J.J. Martínez-Sánchez^{1,2} and S. Bañón^{1,2}

¹Dpto. de Producción Vegetal, ETSIA, Universidad Politécnica de Cartagena (UPCT), Paseo Alfonso XIII, 48. 30203 Cartagena (Murcia), Spain

²Unidad Asociada al CSIC de Horticultura Sostenible en Zonas Áridas (UPCT-CEBAS), Paseo Alfonso XIII, 48. 30203 Cartagena (Murcia), Spain

Under review in HortScience since 13 February 2011.

Abstract

The appropriate management of crop conditions can improve the salt tolerance of ornamental species and produce good quality plants with saline irrigation. The aim of this study was to determine whether the pot-in-pot (PIP) cultivation system can improve the saline irrigation tolerance at $9 \text{ dS}\cdot\text{m}^{-1}$ of *Euonymus japonicus* compared with above ground potting (AGP) in terms of growth and development, aesthetic quality, ion accumulation and leaf water and osmotic potentials. A five month experiment starting on 6 March with four treatments was set up: AGP and PIP with control irrigation at $1.2 \text{ dS}\cdot\text{m}^{-1}$ (AGP-c and PIP-c, respectively); and AGP and PIP with saline water at $9 \text{ dS}\cdot\text{m}^{-1}$ (AGP-s and PIP-s, respectively). A soil moisture sensor-controlled irrigation system was used to irrigate all the treatments when AGP-c reached a volume water content (θ) of 0.33 or $0.35 \text{ m}^3\cdot\text{m}^{-3}$. With saline irrigation PIP reduced the damaged leaf area, increased plant dry weight (DW) and the compactness index to control levels, and kept plant survival high (93% compared with 57% in AGP-s). The substrate temperatures were milder in PIP independently of the irrigation water, and the pore water EC was 36% lowered 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 Ψ_o in PIP. Saline irrigation produced a general accumulation of Cl^- and Na^+ in leaves and decrease Ca^{2+} , Mg^{2+} , K^+/Na^+ ratio, Ψ_o , S:R ratio and height. In general, the PIP increased the salinity tolerance of *Euonymus japonicus*, the main effect being the lower Cl^- ion uptake, which improved its aesthetic value (less damage and greater compactness index and growth).

Keywords: Pot plant, automated irrigation, aesthetic value, ornamental, pore water electrical conductivity, biomass distribution.

1. Introduction

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 due to its high solar radiation. Many ornamental growers try to make the most of the climatic conditions using low quality water. 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), on the level of water salinity, and on 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 sometimes occur (Niu et al., 2010a), negatively affecting plant aesthetic quality, which is of serious concern since in ornamental plants, visual quality is more important than maximum growth (Niu et al., 2008). Growth decrease is related with the osmotic component of salinity which limits the water extracted from the soil (Rodríguez et al., 1997). Leaf damage is related to the Na^+ and Cl^- accumulated in leaves and not compartmentalized in vacuoles, which can be 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 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, since 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); 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).

The salt tolerance of a species can be altered by other factors, and good management can help produce good quality plants. 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. So, any factor which can reduce salt accumulation in the substrate is likely to improve the salt tolerance of a species. In this line, the pot-in-pot (PIP) system compared with above ground potting (AGP) reduces root zone temperature stress (Young and Bachman, 1996), and enhances efficient water use by decreasing container evapotranspiration (Martin et al., 1999). The two properties which make the PIP system susceptible to increasing the salt tolerance of plants is the effect on substrate water content (less evapotranspiration) and on the cation exchange capacity in the root zone (less root stress due to temperature).

The PIP crop system was introduced around 1990 (Parkerson, 1990) in the USA, 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). There are many works related with PIP, although no previous studies comparing PIP and AGP using saline irrigation were found in a literature review.

Euonymus japonicus 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. Percival (2005) determined that two cultivars of the same genus species (*Euonymus fortunei*) were within the first four in order of salinity tolerance within 30 woody perennials species studied. This study focused on leaf chlorophyll fluorescence and leaf damage. However, there is little information available concerning the response of this species in terms of its salt tolerance mechanism.

The aim of this study was to evaluate the potential benefits of the PIP system for increasing the salt tolerance of *Euonymus japonicus*. Plants of this species were grown in PIP and AGP and irrigated with good quality and saline water, and the following points were studied: 1) substrate temperature and leachate electrical conductivity (EC) and pH evolution; 2) growth and development of the plant (biomass production, anatomy, leaf damage) and 3) The effects on pore water EC, leaf water and osmotic potentials and ion concentrations.

2. Material and methods

2.1. Plant material and crop conditions

One year old seedlings of commercial *Euonymus japonicus* var. Marieke were used grown in 96 multi-pot trays measuring 60 cm (length) × 30 cm (width) × 3 cm (height) (Plasnor S.A. Legazpi, Spain). The pots were arranged in a 12 × 8 configuration, had an inverted cube form, and a volume of 56 cm³. The seedlings were transplanted in January 2010 to black PVC pots (cultivation pot) of 2.5 L volume, 16 cm upper external diameter and 15 cm height. The substrate was a mixture of white peat, coconut fiber and perlite (40/40/20, v/v/v), and was amended with 3 g·L⁻¹ of a slow release fertilizer (osmocote plus 14-14-14; 14N-4.2P-11.6K; release time 2-3 months at 21 °C; The Scotts Company LLC, Marysville, OH). A total of 250 seedlings were transplanted to the pots (one seedling per pot). For establishment, the plants were grown in a greenhouse for two months, after which 220 plants were transferred to the experimental plot.

The experiment was conducted in an open air plot of 70 m² at the “Tomás Ferro” Experimental Agro-Food Station of the Polytechnic University of Cartagena (UPCT), (37°35' N, 0°59' W), starting on 6 March 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 temperature were registered.

2.2. Experimental design

The PIP system was the same as described by Miralles et al. (2009). A drip irrigation system was installed with one auto-compensated $2 \text{ L}\cdot\text{h}^{-1}$ dripper (Netafim USA, Fresno, CA) per plant connected to a spaghetti tube which finished in an arrow. Each row of 22 pots received the same quality of water during the experiment, so five rows received saline water ($9 \text{ dS}\cdot\text{m}^{-1}$) and the other five good quality water (about $1\text{-}2 \text{ dS}\cdot\text{m}^{-1}$). This resulted in a total of four treatments with five blocks of 11 plants each: AGP with good water (AGP-c), PIP with good water (PIP-c), AGP with saline water (AGP-s) and PIP with saline water (PIP-s). 7.5 grams per pot of slow release fertilizer (described above) was applied on 1 March and 1 June.

A datalogger 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 S.L., Barcelona, Spain) per treatment and volume water content (θ) with five soil moisture sensors (Decagon Devices, Ltd., Pullman, WA) per treatment. Soil moisture sensors were connected to the CR1000 through one multiplexer. The CR1000 was programmed to collect data every 10 minutes and to calculate average θ and its standard error 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.

Irrigation was applied when the average θ of the AGP-c treatment reached the threshold of $0.33 \text{ m}^3\text{m}^{-3}$ from 6 March to 7 June. After this date, the threshold was increased to $0.35 \text{ m}^3\text{m}^{-3}$ (because of an error in the CR1000 program), until the 24 August. 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 good water and saline water pumps through an Agronic 3000 irrigation controller (Sistemas Electronics Progres, S.A., Lerida, Spain). The saline solution of $9 \text{ dS}\cdot\text{m}^{-1}$ was obtained by adding about $4 \text{ g}\cdot\text{L}^{-1}$ of sodium chloride to the irrigation water in a 1000 L water tank. Each time an irrigation event took place, all the plants were irrigated for the same time (about 15 minutes and 550 ml) which led to average leaching of $\sim 32\%$ ($\sim 180 \text{ mL}$) for AGP-c. Leaching (32%) was the double the fraction recommended for irrigation with good

water in the area, in order to ensure that no substrate salinity would affect the good water irrigation treatments. This irrigation criterion was established because previous studies with the PIP system concluded that it saves water (Miralles et al., 2009), while Navarro et al. (2007) observed a significant increase in θ , as the salinity stress increased, so the highest water needs were associated with the AGP-c treatment.

2.3. 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 an EC meter (Dist® 6, Hanna Instruments S.L., Eibar, Spain). The samples were collected in bottles and plastic bags (irrigation water and leaching, respectively) from May to July. The bottles were placed inside the buried pots with one dripper in the bottle to collect the irrigation volume (three replicates were used for each type of irrigation water). The bags were placed inside the empty chamber in five replicates of the PIP system or inside buried pots below five replicates of the AGP treatments, where pot-grown plants were held in place by a mesh structure.

Measurements of pore and bulk EC were taken with a WET sensor (Delta-T devices Ltd, Cambridge, UK). A total of ten measurements per replicate were taken on 22 July. Each sample was taken near the dripper, because in other places of the pot readings were above 20 $\text{dS}\cdot\text{m}^{-1}$ (its threshold high scale). Substrate pore water EC was measured in five replicates per treatment in the last week of July following the pour-through method (Wright, 1986). In this case, 1) the substrate of all the whole pot was totally saturated with irrigation water; 2) a beaker was placed under the pot to collect the leachate; 3) distilled-deionized water was poured onto medium surface until about 50 mL of leachate was collected in the beaker; 4) finally, the EC was measured with a conductance meter (the same as described above), and the sample was diluted for EC above 20 $\text{dS}\cdot\text{m}^{-1}$.

2.4. Measurements of architecture, anatomy and salt damage

On 30 July 2010, the plant architecture (height and profile area) was determined with a flexometer Blister (3 m long, 1 mm precision, Germans Boada S.A., Rubi, Spain) and a photograph 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 = $\text{profile area} / ((\pi/4) * ((\text{height} + \text{width})/2)^2)$, 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 to unity, the more compact the plants.

Leaf area and dry weight (DW) of roots, stem and leaves (healthy, damaged and fallen) were also determined in the same plants. The leaf area, 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 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 series precision balance (sensitivity of 10 mg and up to 1200 g, Gram Precision SL, Barcelona, Spain).

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

To evaluate salt damage, the fallen leaves, damaged leaves, and the damage leaf area were determine. To determine the damaged leaf area, the percentage of damage from the leaves showing damages was calculated with the image analysis software for plant disease quantification ASSESS 2.0 (University of Manitoba, Winnipeg, Canada).

2.5. Leaf water and osmotic potentials

Midday leaf water potential (Ψ_{hmd}), midday leaf osmotic potential (Ψ_{omd}), midday leaf turgor potential (Ψ_{pmd}), leaf osmotic potential at full turgor (Ψ_{oft}) were measured 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), for which leaves were enclosed in a plastic bag and sealed in the chamber within 20 s of collection and pressurized at a rate of 0.02 MPa s⁻¹ (Turner, 1988). Leaves from the Ψ_{omd} and Ψ_{oft} measurements were frozen in liquid nitrogen. 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 osmotic potential (Ψ_{omd} and Ψ_{oft}) was measured using a Wescor 5520 vapour pressure osmometer (Wescor Inc., Logan, UT, USA) according to Gucci et al. (1991). Estimates of Ψ_{pmd} were based on the difference between Ψ_{hmd} and Ψ_{omd} for each time. Leaves for Ψ_{oft} measurements

were excised in the dark, placed in plastic bags and allowed to reach full turgor by dipping the petioles in distilled water overnight.

2.6. 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 (the rest of the treatments showed no or very little damage). A total of five samples per treatment were collected, each sample consisting of 40 g fresh weight. The samples were dried and the DW was determined. The leaves and roots were chopped to a maximum size of 0.5 mm of diameter. Then, 0.2 g of dry weight was added to 50 mL of distilled water. The solution was mixed for 30 min at 117 rpm and 27 °C using an analogue magnetic stirrer, model ACS-100 C/C" (ITC, SL, Barcelona, Spain). The mixture was filtered using a DP 145 110 filter paper (Albet-Hahnemuehle S.L., Barcelona, Spain) and a funnel, and collected in a round based flask. 10 mL of the filtered dissolution were analysed in the SAIT laboratory of the University using the ion chromatography method in a Metrohm 850 Ion chromatography system (Metrohm AG, Herisau, Switzerland) with 838-861 column.

2.7. Statistical analysis

The design used was a randomized complete block. There were five blocks of 11 plants per treatment which were set in the same row. A total of five replicates (one per block) were used, except in the case of substrate temperature for which there were two. Data were subjected to a two-way ANOVA with a $P < 0.05$ using the software Statgraphics Plus 5.1 software (StatPoint Technologies Inc., Warrenton, VA).

3. Results

3.1. Substrate temperature

Substrate temperature was not affected 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 $\pm$ s.d.)]. Minimum daily

temperatures were greater in PIP (average minimum of 16.74 ± 5.28 in AGP and 20.39 ± 5.86 in PIP). Mean average temperatures were similar in both treatments (average mean of 24.02 ± 6.01 in AGP and 23.58 ± 6.28 in PIP). Environmental mean and maximum temperature were lower, especially in summer (June, July and August). However, minimum environmental temperatures were similar to AGP substrate temperature and lower than PIP (Fig. 1).

Fig. 1. Maximum daily (A), minimum daily (B) and average daily (C) temperatures in substrate of PIP and AGP treatments, and maximum daily (A), minimum daily (B) and mean daily (C) environmental temperature. Error bars are standard errors (n=2).

3.2. Water quality, leaching and substrate

The pH in the different leachates was similar in general terms (an average of ~ 7.5 , Fig. 2A). The irrigation water pH was about 8.05 with little difference between the saline and control water. Leachate EC differed between irrigation water treatments. However, the crop production systems made little difference, the most important characteristic being the lower EC registered in PIP-s with lower standard error at the beginning of the experiment than in AGP-s (Fig. 2B). For saline irrigation (which was maintained about $9 \text{ dS}\cdot\text{m}^{-1}$, Fig. 2C) the leachate EC was about $12.31 \text{ dS}\cdot\text{m}^{-1}$ in both crop production systems at the end of the experiment. Control irrigation leachate was similar in both crop production system at about $2.49 \text{ dS}\cdot\text{m}^{-1}$ for an irrigation water of $1.76 \text{ dS}\cdot\text{m}^{-1}$. Some days previous to the 20 June, a sharp increase in leachate EC in all the treatments (especially in saline irrigate treatments) was registered (about 14.7, 20.5, 5.96 and $7.92 \text{ dS}\cdot\text{m}^{-1}$ in AGP-s, PIP-s, AGP-c and PIP-c, respectively). Which was produced by a heavy rain event (the pH and EC of the rain were 8.4 and $0.68 \text{ dS}\cdot\text{m}^{-1}$, respectively).

Table 1. Level of significance from the two-way anova conducted to determine the effects of salinity (S), crop system (CS) and interaction (S x P) on the studied parameters.

Parameters	Salinity (S)		Crop system (CS)		Statistics		
	Salt	Control	AGP	PIP	S	CS	S x 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	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	8.00	0.13	7.63	0.50	**	*	*
Damage leaves	7.13	0.13	6.25	1.00	****	***	***
Damage leaf area	5.56	0.00	4.97	0.59	****	***	***
Bulk EC WET ($\text{dS}\cdot\text{m}^{-1}$)	7.91	2.45	5.65	4.71	****	ns	ns
Pore EC WET ($\text{dS}\cdot\text{m}^{-1}$)	9.74	3.26	6.09	6.91	****	ns	ns
Pore EC ($\text{dS}\cdot\text{m}^{-1}$)	36.1	11.9	28.6	19.4	****	***	**

*Significant at $P < 0.05$. ** Significant at $P < 0.005$. *** Significant at $P < 0.0005$. **** Significant at $P < 0.00005$ and ns (not significant).

Fig. 2. Weakly leachate pH (A) and leachate electrical conductivity (EC) (B) of saline irrigation (AGP-s and PIP-s) and control irrigation (AGP-c and PIP-c) treatments. C) Irrigation water pH and EC. Error bars are standard errors (n=5 for leachate, n=3 for irrigation).

The pore water EC and the bulk EC registered by the WET sensor (Pore EC WET and Bulk EC WET) only increased with saline irrigation (Table 1). However, the pore water EC calculated with the pour-through method (Pore EC), presented an interactive effect (Table 1 and 2). In this case, the pore EC was similar in both control irrigation systems, but was lower in PIP-s than in AGP-s by about 34%, although it was still 62% higher than in the control irrigated treatments. The absence of differences between the crop systems as determined by the WET sensor, may be related to less accurate readings above 3 dS·m⁻¹ according to Scoggins and van Iersel (2006).

3.3. Growth, development and survival rate

Root and stem DW were not affected 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 inhibited by the PIP system (Table 1 and 2). Similar results were obtained for the leaf area and the number of leaves. However, the individual leaf area was similar in all the treatments. The S:R ratio was lower in the saline. PIP-c also reduced this index compared with AGP-c. The SLA increased in AGP-s, and PIP-s produced similar results to PIP-c. Leaf DW/plant DW was only affected by salinity, which reduced it. The height was reduced by salt irrigation and the AGP system (Picture 1). The compactness index presented an interaction effect similar to leaf DW (AGP-s reduced it). The fallen leaves, damaged leaves and the damaged leaf area, had an interactive effect, in which the PIP system inhibited the damage produced by salinity and the AGP-s produced the highest values in these parameters (Pictures 2 and 3). The percentage of plants with any visual damaged 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.

Table 2. Interactive effects of salinity and crop system on parameters studied.

Parameter	Salinity	Crop system	
		AGP	PIP
Fallen leaves	control	0.00a	0.25a
	salt	*15.3a	0.75b
Damage leaves	control	0.00a	0.25a
	salt	*12.50a	1.75b
Damage leaf area (cm ²)	control	0.00a	0.00a
	salt	*9.94a	1.17b
Pore EC (dS·m ⁻¹)	control	13.7a	10.1a
	salt	*43.5a	*28.7b
Leaf DW (g)	control	6.35a	4.89a
	salt	1.98b*	4.69a
Plant DW (g)	control	10.8a	9.43a
	salt	*5.39b	10.4a
Leaf area (cm ²)	control	613a	555a
	salt	*365b	557a
Number of leaves	control	137a	129a
	salt	*77a	130b
S:R	control	2.67b	2.03a
	salt	*1.41a	*1.63a
SLA (cm ² ·g ⁻¹)	control	103a	117a
	salt	*201a	123b
Compatness index	control	0.96a	0.92a
	salt	0.75a*	0.94b

*Asterisks indicate significant difference by salinity; different letters indicate significant differences by crop system, both according to the least significant difference (LSD) test at a P < 0.05.

Picture 1. From left to right, above ground pot with saline irrigation (AGP-s), pot-in-pot with saline irrigation (PIP-s), above ground pot with control irrigation (AGP-c) and pot-in-pot with control irrigation (PIP-c).

Picture 2. Differences between AGP (left) and PIP (right) with saline irrigation.

Picture 3. Leaf damage by salt in above ground pot and pot-in-pot under saline irrigation (AGP-s up and PIP-s down).

3.4. Leaf potentials and ion concentrations

The Ψ_{hmd} only differed between crop systems, and was slightly lower in PIP. The Ψ_{omd} and Ψ_{pmd} depended on both the irrigation water and the crop system. The Ψ_{omd} decreased with saline irrigation and AGP, while Ψ_{pmd} behaved in the opposite way. Ψ_{oft} was the same for all the treatments.

Table 3. Level of significance from the two-way anova conducted to determine the effects of salinity (S), crop system (CS) and interaction (S x CS) on the leaf potentials parameters.

Parameters	Salinity (S)		Crop system (CS)		Statistics		
	Salt	Control	AGP	PIP	S	CS	S x 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
Ψ_{oft}	-1.65	-1.56	-1.65	-1.55	ns	ns	ns

*Significant at $P < 0.05$, ** Significant at $P < 0.005$, *** Significant at $P < 0.0005$. **** Significant at $P < 0.00005$ and not significant (ns).

The leaf concentration of Cl^- increased with saline irrigation and AGP, but was not affected in roots. The Na^+ concentration increased with saline irrigation in both leaf and roots, the opposite to Ca^{2+} and Mg^{2+} (Table 4). K^+ had an interactive effect in leaf, while in roots it decreased with saline irrigation and AGP. The leaf K^+ concentration decreased in AGP-s (Table 5). However, the PIP-c decreased leaf K^+ (opposite 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^+ . The K^+/Na^+ ratio was not affected (Table 6).

Table 4. Level of significance from the two-way anova conducted to determine the effects of salinity (S), crop system (CS) and interaction (S x CS) on the ions concentration ($\text{mg}\cdot\text{g}^{-1}$ of DW).

Plant part	Ions	Salinity (S)		Crop system (CS)		Statistics		
		Sal	Control	AGP	PIP	S	CS	S x 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	****	****	***

*Significant at $P < 0.05$, ** Significant at $P < 0.005$, *** Significant at $P < 0.0005$. **** Significant at $P < 0.00005$ and not significant (ns).

Table 5. Interactive effects of salinity and crop system on ions concentration ($\text{mg}\cdot\text{g}^{-1}$ of DW).

Plant part	Parameter	Salinity	Crop system	
			AGP	PIP
Leaf	K ⁺	control	16.6a	13.0b
		salt	12.3a*	14.8b
	K ⁺ /Na ⁺	control	6.5b	19.5a
		salt	0.7*	1.0*
Root	K ⁺ /Na ⁺	control	0.82b	1.26a
		salt	0.12*	0.23*

*Asterisks indicate significant difference by salinity; different letters indicate significant differences by pot, both according to the least significant difference (LSD) test at a $P < 0.05$.

Table 6. Ions concentration ($\text{mg}\cdot\text{g}^{-1}$ of DW) in AGP-s of healthy leaves and damaged leaves.

Parameter	AGP-s	
	Healthy leaf	Damaged leaf
Cl ⁻	42.9*	69.71
Na ⁺	17.3*	30.14
Ca ²⁺	1.6*	2.35
Mg ²⁺	1.3*	1.62
K ⁺	12.3*	19.58
K ⁺ /Na ⁺	0.71	0.65

*Asterisks in the same row indicate significant difference at $P < 0.05$.

4. Discussion

Substrate temperature is a crucial factor in root development and function. For example, root growth decreases, water and nutrient uptake is affected, and root morphology may be altered above or below the range 15-27 °C (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 °C and 37.4 °C respectively when the air temperature was 38.8 °C 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 produced greater plant DW. No such effect was observed in control irrigated

Myrtus communis (Miralles et al., 2009). However, many studies have found greater growth in both shoot and root in PIP: for example, Ruter (1998a) in Heritage river birch reported a 20% and 31% increase in shoot DW and root DW, respectively, than in AGP. Miralles et al. (2009) reported an increase in the S:R ratio in AGP, as 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 reduced shoot and root growth, the shoot growth reduction occurring earlier. In *Euonymus japonicus* S:R was also reduced by salinity, mainly due to lower leaf DW (Table 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 due to greater quantities of solutes in 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, as in our results, due to 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 (Table 1, 2 and 6). Kchaou et al. (2010) reported the same behavior in olive trees subject to salinity, which occurred preferentially in old leaves.

The aesthetic properties of *Euonymus japonicus* plants were strongly reduced in AGP-s, because of lower growth, height and compactness index, and especially due to the increase in damaged leaves, because, as reported by Niu et al. (2008) for landscape ornamental plants, visual quality is more important than maximum growth. 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 damages 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 had an inhibitory effect on the negative aspects concerning the compactness index and leaf damage, and increased height. The PIP system increased plant height, as Martin et al. (1999) reported in *Acacia smallii* (by about 80%). 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.

34% lower pore EC in PIP-s compared with AGP-s, together with the milder substrate temperatures of the PIP system would explain the inhibitory effects of saline irrigation. This decrease in pore EC could be explained by the 9% increase in leaching in PIP-s than in AGP-s (data not shown), since a smaller leaching fraction would lead to salt accumulation (Niu et al., 2008). However, the leachate EC did not show differences at the end of the experiment within the same irrigation water treatments (Fig. 2). The absence of differences, despite different leaching fractions, may be related with drip irrigation, in which the water has a tendency to flow downwards than to spread horizontally, as reported by De Rijck and Schrevens (1998) in a rockwool slab. So, salts were washed from the same substrate portion and accumulated in the rest. The proof of this is found near the 20 June, when leachate EC increased substantially due to a heavy precipitation event that irrigated all the substrate of the pot, flushing out the salts accumulated in all the substrate. This phenomenon also explains the high pore EC registered by the pour-through method at the end of the experiment compared with the WET sensor readings near the dripper and with the leachate EC.

The Ψ_{hmd} was not affected by salinity, despite the fact Navarro et al. (2007) in *Arbutus unedo*, and Torrecillas et al. (2003) in *Cistus albidus* and *C. monspeliensis*, found that Ψ_{hmd} decreased with salinity. The PIP system produced slightly more negative values, which could be due to greater control of water transpiration in AGP in summer. Miralles et al. (2009) reported that lower soil matric potential in AGP in *Myrtus communis* 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 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 due to 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 the salt stress imposed, which would have lead to Ψ_{pmd} increasing, as in our results. Torrecillas et al. (2003) and Navarro et al. (2007) also found that Ψ_{oft} was reduced by salinity, an effect which was not significant in our results, although the same tendency was observed (Table 3). 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 (Table 3 and 4). The greater pore EC in saline irrigation and AGP system would explain these results (Table 1 and 2).

Ion accumulation could also be responsible for leaf damage and decreased growth, especially the ion Cl^- which had greater concentration and was significantly reduced by PIP. The greater toxicity of Cl^- was reported by Martinez-Barroso and Alvarez (1997) in strawberry, where the leaf scorch observed was basically due to the increase in the foliar Cl^- concentration. 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 non significant 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 under salinity. However, Cl^- was not retained in roots, which would confirm that lower leaf accumulation was related to lower concentrations in pore water substrate.

5. Conclusion

Euonymus japonicus grown in PIP with good quality water increased its height and reduced its S:R ratio compared with AGP. Even when saline water was used, PIP inhibited many of the negative effects of AGP-s, which reduced its aesthetic value: a) leaf area damage was strongly reduced; b) neither the dry weight nor the compactness index was affected; and c) plant survival was 93% in PIP-s vs. 57% in AGP-s. These benefits are explained by less extreme substrate temperatures and lower pore EC, which allowed better root development and function. So, PIP reduced Cl^- accumulation in leaves due to lower concentration in substrate water because roots did not prevent its transportation to leaves, unlike in the case of Na^+ . The absence of differences in Na^+ , other nutrients (Ca^{2+} and Mg^{2+}) and the K^+/Na^+ ratio compared with AGP suggested that Cl^- ion was mainly responsible for leaf damage. On other hand, saline irrigation had certain other effects compared with control irrigation, such as lower S:R ratio decrease, less height, Ψ_{omd} decrease, Ψ_{pmd} increase, the accumulation of Cl^- and Na^+ in leaves and the lower Ca^{2+} , Mg^{2+} and K^+/Na^+ ratio. So, PIP can be recommended

for the cultivation of this species using bad quality water, since salinity damage will be inhibited for at least four months.

Acknowledgements

The authors thank P. Cánovas for irrigating and supervising the shrubs while the experiment took place. This research was supported by CICYT projects (CICYT AGL2008-05258-CO2-1-AGR and CICYT AGL2008-05258-CO2-2-AGR), SENECA project (08669/PI/08) and by the Consejería de Agricultura y Agua de la Región de Murcia, program (UPCT-CEBAS-IMIDA 2008).

References

- 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.
- Cassaniti, C., C. Leonardi, and T.J. Flowers. 2009. The effects of sodium chloride on ornamental shrubs. *Scientia 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* (1-2):69-79.
- Colmer, T.D., R. Munns, and T.J. Flowers. 2005. Improving salt tolerance of wheat and barley: future prospects. *Aust. J. Exp. Agric.* 45:1425-1443.
- De Rijck, G. and E. Schrevens. 1998. Distribution of nutrients and water in rockwool slabs. *Scientia 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.
- 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. *Scientia Hort.* 124:306-315.

- Heuer, B. and A. Nadler. 1998. Physiological responses of potato plants to soil salinity and water deficit. *Plant Sci.* 137:43-51
- Maathuis, F.J.M. and A. Amtmann. 1999. K^+ nutrition and Na^+ toxicity: the basis of cellular K^+/Na^+ ratios. *Ann. Bot.* 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. *Scientia Hort.* 71:177-188.
- 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. *Scientia Hort.* 108:423-441.
- Navarro, A., S. Bañón, 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. *Scientia 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. *Scientia 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. *Scientia 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.
- Percival, G.C. 2005. Identification of foliar salt tolerance of woody perennials using chlorophyll fluorescence. Hortscience 40:1892-1897.
- 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. Agric. 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. Scientia Hort. 78:5-38.
- Scoggins, H.L., and M.W. Iersel. 2006. In situ probes for measurement of electrical conductivity of soilless substrates: Effects of temperature and substrate moisture content. HortScience 41:210-214.
- 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. Scientia 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.

General conclusions

The pot-in-pot (PIP) crop system offers advantages compared with above ground pot (AGP), mainly because this system moderates extreme substrate temperatures. So, in *Myrtus communis* the soil matric potential was maintained higher and root development was favored, which produced a lower shoot/root ratio, and reduced plant height. This change in plant morphology favored its aesthetic value and its survival in severe water stress situations (PIP survival of 90% vs AGP survival of 62%). In *Euonymus japonicus*, PIP enhanced stem growth, plant height (contrary to *M. communis*), water use efficiency, and reduced the S:R ratio. Furthermore, the PIP system improved *E. japonicus* tolerance to salinity, because it produced a similar dry weight (DW) in PIP cultivated plants submitted to saline irrigation (PIP-s) as control irrigated plants, while in AGP plants submitted to saline irrigations (AGP-s) the DW was reduced. The lower DW in AGP-s was due to greater photorespiration (lower net photosynthesis and similar light adapted photosystem II efficiency rates). The aesthetic value was also maintained by PIP-s, because it inhibited the following damage produced in AGP-s: a) high leaf area damage; b) reduced compactness index; c) reduced leaf area; c) greater chloride concentration in leaves, and d) plant survival of 57% compared with 93% in PIP-s. The PIP-s reduced substrate pore EC because the greater WUE produced 9% extra leaching, which reduced salts accumulation in substrate. The better root temperature and the lower substrate pore EC would explain all these benefits in the plant's aesthetic value and growth.

Euonymus japonicus irrigated with saline water suffered independently of the crop system used: lower S:R ratio, greater substrate pore EC, Ψ_{omd} decrease, Ψ_{pmd} increase, the accumulation of Cl^- and Na^+ in leaves and the lower Ca^{2+} , Mg^{2+} and K^+/Na^+ ratio.

Euonymus japonicus was more susceptible to chloride toxicity due to the absence of differences between PIP and AGP in Na^+ , other nutrients (Ca^{2+} and Mg^{2+}) and the K^+/Na^+ ratio.

PIP can be recommended for the cultivation of both species, especially *E. japonicus* with bad quality water, since salinity damage will be inhibited for at least four months.

The use of dendrometers in low diameter ornamental crops for irrigation scheduling could be achieved using stem growth rate (SGR) and/or maximum daily shrinkage (MDS). However, it is necessary to compare well-irrigated plants with stressed plants and test which one of the two indices is more appropriate. To ensure that the minimum irrigation frequency is at least every other day will also be essential. Furthermore, a moderate stress may result, although this condition may not be a problem for ornamental shrubs, because although it may affect plant growth and quality, it can also be useful for plant hardening and growth control.

The response to water stress of these indices was clearer under severe environmental conditions.

Specifically, the conclusions reached for the species studied were that maximum and minimum daily stem show a good response, although leaf water potential (Ψ_{leaf}) is more sensitive to water stress. The SGR and the MDS in the drought to death period were sensitive to daily plant ET ratios between 130 and 300 mL·day⁻¹, respectively, and to $\Psi_{\text{leaf}} < -3.00$ MPa. *C. citrinus* showed no correlations with MDS- Ψ_{leaf} , and Ψ_{leaf} could not be determined in *P. tobira*; on the other hand *P. tobira* had the best R^2 for the MDS and SGR-daily plant ET correlations. The correlations obtained for the irrigation periods confirmed the different behaviors of the dendrometric indices under water stress. These results suggest that irrigation could be based on the evolution of these indices, especially SGR in *C. citrinus*, MDS in *P. tobira* and both in *R. alaternus*, although a moderate degree of stress may result.

Shading applied to *Rhamnus alaternus* produced morphological changes, which showed the plasticity to different light environments of this species (e.g. increasing leaf area to compensate lower gas exchange rates per unit area).

From a nursery point of view, shading $\geq 84\%$ would produce low quality plants, with a low compactness index and high S/R index. Shading between 32% and 48% applied in spring and summer, respectively would optimize photosynthesis and improve leaf color.

From a forestry point of view, this species is interesting as an understory species in Mediterranean forests, where shade is not drastically reduced, and can be well-adapted to light changes.

The use of time domain transmissometry sensors (TDT sensor) for irrigation scheduling of potted ornamental crops was tested with success. The TDT sensors provide accurate measurements of volume water content (θ) after a substrate-specific calibration and, in combination with the CS3500 irrigation controller, plants were irrigated as needed. So, it can be used for irrigation control of container-grown crops as well as for drought stress research without the need for very specific knowledge (the CS3500 irrigation controller is commercially available and easy to install). However, due to the length of the TDT sensors, its use will be limited to pots of at least 20 cm height, while the measurements of bulk substrate EC made with TDT sensors were not accurate and should not be used to make fertilization decisions, at least in soilless substrate.

Begonias semperflores, submitted to different θ , reduced shoot dry weight, leaf area, leaf photosynthesis and stomatal conductance at the lowest θ threshold ($0.136 \text{ m}^3 \cdot \text{m}^{-3}$), while no differences were found from $0.348 \text{ m}^3 \cdot \text{m}^{-3}$ and above. Overall, shoot dry weight was much

better correlated with the area of the uppermost fully-expanded leaf than with leaf photosynthesis. This suggests that drought effects on plant growth are caused more by reductions in photosynthetically active surface area than by photosynthesis per unit leaf area. The leaf chlorophyll fluorescence study did not show any differences between treatments, which confirms that no damage in the photosynthetic apparatus was produced.