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Changes in growth rate, root morphology and water use efficiency of potted *Callistemon citrinus* plants in response to different levels of water deficit

Sara Álvarez, M^a Jesús Sánchez-Blanco*

Departamento de Riego. Centro de Edafología y Biología Aplicada del Segura (CSIC). P.O. Box 164, E-30100 Murcia, Spain

*Corresponding author. Tel.: +34 968396318

E-mail address: quechu@cebas.csic.es (MJ Sánchez-Blanco).

Correspondence to:

Dra. M^a Jesús Sánchez-Blanco

Centro de Edafología y Biología Aplicada del Segura (CSIC)

Departamento de Riego P.O. Box 164

E-30100 Espinardo (Murcia)

SPAIN

Phone: +34968396318

FAX: +34968396211

E-mail: quechu@cebas.csic.es

Abstract

Callistemon is widely used as a flowering shrub in gardening and landscaping in the Mediterranean area. However, prolonged or severe water stress may alter its physiological and morphological behaviour. *Callistemon citrinus* plants were grown in nursery conditions and subjected to three irrigation treatments: a control (watered to container capacity) and two water deficit treatments of 50 and 25% of the amount of water supplied in the control treatment (moderate and severe deficit irrigation, respectively). After 53 weeks, the moderate deficit irrigation plants showed a lower relative growth rate but increased root/shoot ratio, improved the root system and increased water use efficiency, while flowering and leaf colour were unaffected. However, severe deficit irrigation reduced flowering and affected leaf colour. Both deficit irrigation treatments reduced stomatal conductance, suggesting an efficient and adaptive stomatal control in this species. These reductions were marked after longer periods in plants submitted to severe deficit irrigation, which decreased photosynthesis and could delay plant recovery and cause permanent damage. Differences between stem and leaf water potential values have seen to be a good indicator of instantaneous shoot transpiration. Water consumption was influenced by the active periods of growth and inflorescence formation. It is concluded that moderate deficit irrigation can be used successfully in *Callistemon citrinus* plant production to reduce water consumption while maintaining good overall quality.

Key words: Deficit irrigation, Evapotranspiration; Gas exchange; Ornamental potted plant; Root system; Water relations.

Abbreviations: C, control; C*, chroma; DI, deficit irrigation; DW, dry weight; ET, evapotranspiration; g_s , stomatal conductance; h° , hue angle; L^* , lightness; MDI, moderate deficit irrigation; P, significance; PAR, photosynthetic active radiation; P_n , net photosynthesis; RDD, relative chlorophyll content; RGR, relative growth rate; RH, relative humidity; SDI, severe deficit irrigation; SWC, substrate water content; VPD, vapour pressure deficit; WUE, Water use efficiency of production; Ψ_l , leaf water potential; Ψ_s , stem water potential

1. Introduction

An efficient use of limited water resources and better growth under a limited water supply are desirable traits for plants in drought environments (Jaleel et al., 2008). For many crops, it is now possible to accurately schedule irrigation to match the loss of water through evapotranspiration from the crop canopy and the soil, but there has been little quantification of the irrigation requirements of ornamental nursery plants (Mee et al., 2003; Henson et al., 2006). The quantity and frequency of irrigation applied in most nurseries is based on personal experience and is rarely modified to match the requirements of the crop water needs (Grant et al., 2012). However, plant water use varies greatly from day to day, through the course of a season, and with growth. Therefore, regardless of the engineered efficiency of water management, there is a potential to improve the physiological efficiency of water use by the crop. Such physiological approaches, based on the biology of plant water use, provide opportunities to manage both water use and quality in terms of an economic balance (Mahan et al., 2012). Thus, if water productivity is defined as the ratio of yield, measured as biological or economic output to crop evapotranspiration, ornamental crops have high water productivity compared to agronomic crops or even fruit crops.

Deficit irrigation (DI) is the application of water at a rate and volume lower than the evapotranspiration rate and may be used in potted ornamental plants to improve plant quality, by reducing excessive vigour and promoting a more compact habit (Cameron et al., 2006), but the degree and duration of the water stress imposed in each species in field conditions is also critical to reach this purpose (Álvarez et al., 2009). Some shrubs can be maintained with minimal irrigation level if plant selection is appropriate for site conditions (Sachs et al., 1975). Therefore, deficit irrigation requires precise scheduling to minimise the risk of excessive drying of the substrate, even, taking into account that ornamental crops are largely grown in containers with a small water capacity. In this sense, numerous works in ornamental plants have demonstrated that the plant quality decreases as the severity of DI increases (Hansen and Petersen, 2004; Henson et al., 2006; Katsoulas et al., 2006; Chyliński et al., 2007; Silber et al., 2007; De Lucia, 2009; Álvarez et al., 2009; Sánchez-Blanco et al., 2009; Bolla et al., 2010; Andersson, 2011; Bernal et al., 2011).

Adaptation to reduced water supply is achieved primarily through a reduction in stomatal conductance and, over the longer term, a reduction in the new leaves synthesised. The dynamics of root growth under drought conditions might be a key factor to understanding the contribution of roots to drought tolerance. The effect of drought stress is usually greater on shoot growth than it is on root growth, but also other root

characteristics may be changed by drought, aspects that could promote the more rapid establishment of ornamental plants in the garden, particularly in dry regions (Franco et al., 2006). Among Australian ornamental plants, one of the most important genus is *Callistemon*, which includes several species showing interesting ornamental features (Mitchen, 1993). *Callistemon laevis* and *Callistemon citrinus* appeared to be particularly resistant to both water stress and root restriction conditions (Vernieri et al., 2006; Mugnai et al., 2009). Although the general effects of drought on plant growth of *Callistemon* have been studied (Mugnai et al., 2009; Álvarez et al., 2011), further work is required to quantify responses of these plants to different levels of deficit irrigation as regards growth, plant quality and physiological parameters, such knowledge will help optimize water reduction levels. For this reason, the present research aims to investigate the effects of two levels of deficit irrigation on gas exchange parameters, water consumption, quality, growth rates and water use efficiency of *Callistemon citrinus* during the growing season.

2. Materials and methods

2.1. Plant material and experimental conditions

Rooted cuttings of 2 year-old *Callistemon citrinus* (Curtis) Skeels, cv 'Firebrand' (Crimson Bottlebrush) grown in 14 cm x12 cm pots by a specialized nursery were transplanted into 5 L plastic pots (20 cm x16 cm) filled with an 8:7:1 (v/v/v) mixture of coconut fibre : black + sphagnum peat: perlite, amended with 2 g L⁻¹ of Osmocote Plus (14:13:13 N,P,K plus microelements). Plants were placed inside a plastic greenhouse equipped with a cooling system, located at Santomera (Murcia, Spain). The temperature and relative humidity were recorded by a Hoboware Lite Data Logger (Escort Data Loggers, Inc., Buchanan, Virginia, USA). The micro-climatic conditions registered to the total experimental period were 14.8 °C (mean minimum), 25.6 °C (mean maximum), and 19.3 °C (average) temperature; and 1.61 (mean maximum) and 0.80 (average) vapour pressure deficit.

All of the plants were watered daily for three weeks to container capacity prior to starting the treatments.

2.2. Treatments and experimental design

Callistemon citrinus plants were grown in nursery conditions and subjected to three irrigation treatments (25 plants per treatment) using a computer-controlled drip irrigation system from March 2009 to April 2010. The irrigation treatments consisted of a control (C), when substrate moisture was maintained close to container capacity, it was watered so that 15% (v/v) of the applied water was leached, and two deficit irrigation treatments: applying 50% of the amount of water supplied in the control treatment, (moderate deficit irrigation; MDI) and 25% of the control irrigation water, (severe deficit irrigation; SDI). All plants were irrigated daily. The electrical conductivity of the water applied was 0.8 dS m⁻¹. One drip nozzle, delivering 2 L h⁻¹ per pot, was connected to two spaghetti tubes (one on each side of every pot) and the duration of each irrigation episode was used to vary the amount of water applied. The volume of water varied between 200 and 500 ml per pot and irrigation episode for the controls and was determined by noting when the leaching fraction reached 15-20%.

2.3. Growth and plant water measurements

At the beginning and at the end of the experimental period ten plants per treatment were harvested and separated into shoots (i. e. leaves and stems) and roots. These were then oven-dried at 80 °C until they reached a constant weight to measure the respective dry weights (DW). Stem diameter (mm), succulence, leaf number and leaf area (cm²), using a leaf area meter (Delta-T; Devices Ltd., Cambridge, UK), were determined in the same plants. The relative growth rate of biomass accumulation was calculated as the rate of increase of dry weight or leaf area per unit of initial biomass.

The roots were cleaned by low pressure water applied through a flat nozzle. The cleaned root systems were then placed in a metacrylate tray coupled to a double scanner connected to a computer with a root system analyser (Winrhizo LA 1600 Regent Inc., USA). The root systems were put in an oven to dry immediately after the root length and root volume measurements. Roots were classified into three diameter classes: fine (<0.5 mm), medium (0.5–2.0 mm) and coarse (>2 mm). Root density was determined by dividing the dry weight by root volume.

Throughout the experiment, plant height and number of inflorescences per plant were measured in 25 plants per treatment every 1-2 weeks. The relative growth rate was calculated as the rate of increase of height per unit of initial plant height.

Leaf colour was measured at the end of the experimental period with a Minolta CR-10 colorimeter, which provided the colour coordinates lightness (L^*), chroma (C^*) and hue angle (h°) (McGuire, 1992), using three leaves for each plant and ten plants per treatment. The relative chlorophyll content (RCC) was measured using a Minolta SPAD-502 chlorophyll meter (Konica Minolta Sensing Inc., Osaka, Japan) using the same leaves as were used for the colorimetric measurements.

The rate of passive ion leakage from stress-sensitive plant tissue can be used as a measure of alterations of membrane permeability. In our case, ion leakage was estimated at the end of the experiment, according to the method described by Lafuente et al. (1991). Thirty leaf discs, each 2 mm in diameter, from each plant, with ten replicates per treatment, were pooled and incubated in 10 mL 0.3 M mannitol in a 50 mL centrifuge tube. The tubes were shaken at 120 cycles min^{-1} and the conductivity of the solution was measured after 24 h using a Crison Model 524 digital conductivity meter (Crison Instruments S.A., Barcelona, Spain). Tubes containing the solution were weighed and heated to boiling for 10 min. After cooling to room temperature, while still shaking, deionised water was added to restore their initial weight and the total conductivity was measured after an additional 0.5 h of shaking. Ion leakage rates were expressed as a percentage of the total conductivity.

To determine the maximum water holding capacity of the substrate, seven samples were uniformly mixed and packed to a similar bulk density. The pot surfaces were covered with aluminium foil to prevent water evaporation and the lower parts were submerged, to half the pot's height, in a water bath and then were left to equilibrate overnight. The next day, the pots were removed and left to drain freely until drainage became negligible. The fresh weight was then recorded for each individual pot and considered as the weight at field capacity. At the end of the experiment, the substrate was dried in an oven at 105 °C until constant weight in order to obtain the dry weight and calculate the volumetric water content. Later, the difference between the weight at field capacity and oven-dry weight was measured and volumetric water content calculated (65%), which was considered as the substrate field capacity.

Volumetric water content was periodically calculated in seven pots per treatment throughout the experiment, obtaining their weight before and after irrigation, using a balance (Analytical Sartorius, Model 5201; capacity 5.2 kg and accuracy of 0.01 g). Then, the difference between the fresh weight and oven-dry weight was measured, giving the volumetric water content of these monitored pots.

Moreover, one pot of each treatment was placed on a balance with a MITRA programmer that recorded the weight every half an hour, giving the evapotranspiration (ET) throughout the experimental

period. Daily ET was measured using the difference in weights (weight after irrigation and weight before irrigating again) and hourly ET was measured using the difference in weights between two consecutive measurements.

During the experiment, leaf water potential (Ψ_l) and stem water potential (Ψ_s) were measured in eight plants per treatment in mature leaves at midday. Ψ_l 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 placed in the chamber within 20 s of collection and pressurised at a rate of 0.02 MPa s⁻¹ (Turner, 1988). Ψ_s was measured in non-transpiring leaves that had been bagged with both a plastic sheet and aluminium foil for at least 1 h before measurement in order to prevent leaf transpiration; in this way leaf water potential equalled stem water potential (Begg and Turner, 1970).

Leaf stomatal conductance (g_s) and the net photosynthetic rate (P_n) were periodically determined in eight plants at midday using a gas exchange system (LI-6400, LI-COR Inc., Lincoln, NE, USA). While the P_n/g_s ratio was used as an estimation of the intrinsic water use efficiency. Water use efficiency of production (WUE) was calculated at the end of the experiment by dividing the increment in dry weight by the water used.

2.4 Statistical analyses of data

The data were analysed by one-way ANOVA using Statgraphics Plus for Windows 5.1 software. Ratio and percentage data were subjected to an arcsine square-root transformation before statistical analysis to ensure homogeneity of variance. Treatment means were separated with Duncan's Multiple Range Test ($P \leq 0.05$).

3. Results

3.1. Plant growth and ornamental parameters

C. citrinus plants submitted to SDI showed a reduction in the relative growth rate (RGR) for all biomass production parameters, while plants submitted to MDI treatment only decreased the RGR of leaf DW and leaf area compared with control treatment (Table 1). At the end of the experiment deficit

irrigation reduced the number of leaves as the deficit increased, while leaf blade area, stem diameter and succulence were similarly reduced in both deficit irrigation treatments (Table 2). The root to shoot ratio increased proportionally to the imposed drought level (Table 3).

Control plants reached the greatest height, while SDI plants showed a significant reduction from 3 weeks after beginning of treatments, leading to the smallest plants (Fig. 1A). At the beginning of the experiment plant height was similar in both the control and MDI treatment, but was inhibited by the latter 23 weeks after application onwards, (September), (Fig. 1A). At the end of the experiment the reductions were around 11 and 22% for MDI and SDI, respectively, compared with the control. Water deficit also had a significant effect on RGR as a function of plant height (Fig. 1B). Three growth periods were evident during the growing season (first week of May, August and March) in all plants, although the stressed plants showed a certain delay in reaching the maximum compared with the control (Fig. 1B).

Deficit irrigation decreased total root length proportionally to the imposed drought level and a reduction in all root sizes was observed (Table 3). MDI increased the percentage of roots with a diameter between 0.5 and 2 mm and decreased those with a diameter lower than 0.5 mm, compared with the control. All stressed plants showed a reduced root volume, although root dry weight was not modified by MDI, with the result that root density increased in these plants.

C. citrinus plants had flowers during the most of the experiment, although the greatest intensity of flowering occurred at the end of the experiment (Fig. 2). SDI reduced the number of inflorescences per plant compared with control 54 weeks after application of the deficit irrigation. However MDI had no such effect and flowering intensity was even higher than in control plants at some moments. No changes were observed in the leaf colour of MDI plants compared with the control (Table 4). The higher h° and CRC values and lower C^* values recorded in the leaves of the plants submitted to SDI confirmed the darker and less vivid green colour of the foliage compared with control plants (Table 4). Membrane damage, assessed by ion leakage was not affected by the water availability conditions of the substrate.

3.2 Substrate water content (SWC) and evapotranspiration (ET)

The water added to each pot during the whole experimental period was 164 L for the control and 82 and 47 L for MDI and SDI plants, respectively (50 and 25% of the amount of water compared with control treatment). After irrigation, the substrate water content (SWC) in the control plants remained on

average at about $0.55\text{--}0.60\text{ m}^3\text{ m}^{-3}$, above and close to container capacity (Fig. 3A). SWC in MDI was lower than in the control, with average values of around 40% throughout the experiment and slightly lower in summer and at the end of the experiment. In the SDI treatment SWC remained on average around 20%.

The greatest values for water consumption were recorded in summer and the lowest in winter (Fig. 3B), which was closely related to temperature and VPD (Fig. 3C), although daily evapotranspiration (ET) values were also affected by the growth periods and flowering. ET was higher in control plants than in both deficit irrigation plants due to the greater amount of available water in the substrate (Fig. 3B). ET increased during the morning and the highest value was reached at midday in all plants coinciding with the highest VPD, after which, ET decreased (Fig. 4). Despite similar VPD values, ET levels were higher during active growth periods (Fig. 4B) than during non-growth periods (Fig. 4A). Also, even with similar VPD and RGR, ET values were much higher at the end of the experiment (Fig. 4D), (April), coinciding with inflorescences formation and the opening period, than during a period with less inflorescences per plant (Fig. 4C). When plants showed the highest ET values (in growth and flowering periods), the differences between the ET values of the control and the deficit irrigation plants were also the highest (Fig. 4B and D). In contrast, when plants showed the lowest ET values, the water consumption of SDI plants was practically similar to that of control plants, despite the lower levels of water in the substrate in the SDI plants (Fig. 4A and C).

3.3 Water relations and water use efficiency

Leaf water potential at midday (Ψ_l) showed maximum values in December and minimum values in August, when temperature and DPV values were highest (Fig. 5). In general, Ψ_l values were highest in the control plants, although no significant differences in Ψ_l levels were noted between treatments during the first five months, even though the water content of the substrates was clearly different. Stem water potential (Ψ_s) values decreased proportionally to the level deficit imposed. The standard error was much lower for Ψ_s than for Ψ_l , meaning that Ψ_s identified greater significant differences between treatments. The maximum and minimum differences between Ψ_s and Ψ_l measured simultaneously in the same plant coincided with the maximum and minimum ET, respectively (Fig. 5).

Both deficit irrigation treatments produced a reduction in stomatal conductance, which was more marked under SDI (Fig. 6A). The seasonal pattern of g_s consisted of a minor summer depression in all treatments, particularly in SDI, and a major winter depression regardless of the amount of irrigation water applied (Fig. 6A).

In general, the plants of both water deficit treatments showed higher P_n/g_s ratios (intrinsic water use efficiency) than control plants throughout the experimental period (Fig. 6B). The net photosynthetic rates (P_n) increased as stomatal opening (g_s) increased, although this effect was much more evident when g_s was below $150 \text{ mmol m}^{-2} \text{ s}^{-1}$, (Fig. 6C). Furthermore, P_n/g_s increased when g_s decreased from maximum to approximately $100 \text{ mmol m}^{-2} \text{ s}^{-1}$, which indicates a predominantly stomatal control over P_n , but when $g_s < 100 \text{ mmol m}^{-2} \text{ s}^{-1}$, P_n/g_s decreased sharply, suggesting that non-stomatal limitations to P_n were predominant under these conditions (Fig. 6D). All plants had g_s values below $100 \text{ mmol m}^{-2} \text{ s}^{-1}$ during winter, although plants of the SDI treatment also reached g_s values below $100 \text{ mmol m}^{-2} \text{ s}^{-1}$ during some other days of the experiment. At the end of the experiment, plants subjected to deficit irrigation showed that they had increased the water use efficiency of production (WUE) in each part of the plant (leaves, stem and root), regardless of the reduction level applied (Fig. 7).

4. Discussion

The effect of water stress on plant growth and dry matter accumulation has been described in several crops species (Sánchez-Blanco et al., 2002; Rodríguez et al., 2005; Vernieri et al., 2006; Álvarez et al., 2012). According to Mugnai et al. (2009), in *C. citrinus* plants, deficit irrigation reduces plant height, dry weight and leaf area. In our conditions, plant height was significantly inhibited by both water deficit treatments. Also, these plants had fewer and smaller leaves.

The different water stress levels applied in our experiment induced different growth responses in *C. citrinus*, meaning that the severity of the water stress must be considered an important aspect when is used as an irrigation strategy for saving water without reducing quality in ornamental species. The differential provision of water to plants influences their dry matter partitioning between roots and shoots. In our conditions, exposure to deficit irrigation had a less pronounced effect on root mass, indicating that shoots and roots react differently to drought (Bacelar et al., 2007; Franco et al. 2006; Chyliński et al., 2007). This was confirmed by the root/shoot ratio which increased in plants under water deficit conditions

(Sánchez-Blanco et al., 2002; Navarro et al., 2009), probably as a result of the plants needing to maintain the root surface area under drought conditions in order to absorb water from the substrate (Bradford and Hsiao, 1982) and to reduce the evaporative surface area (De Herralde et al., 1998; Alarcón et al., 2006).

The root system is an important factor for successful transplanting and establishment in the field, and root anatomy and structure may be decisive for plant survival (Steudle and Peterson, 1998; Bañón et al., 2004). The shift in root diameters in moderately stressed plants was due to both the greater production of medium sized roots and the lower production of fine roots. Furthermore, the greater root density of these plants suggests greater robustness and, presumably, a higher accumulation of reserves (Cameron et al., 2006; Franco et al., 2006; Álvarez et al., 2011). This may also be interpreted as an accumulation of solutes by the plants in order to maintain the water gradient necessary for absorbing water even when in short supply in the soil. Processes of osmotic adjustment in terms of the roots realised through the active accumulation of organic solutes from the aerial part would alter the physical support, strengthen the roots and lessen the possibility of breaking during transplanting (Bañón et al., 2006). In this sense moderate deficit irrigation in nursery conditions would improve *C. citrinus* plant resistance to water deficit situations when plants grow in field conditions after transplanting (Franco et al., 2001).

Water deficit may reduce flowering intensity, bring forward or delay flowering and shorten the same (Cuevas et al., 2009; Bernal et al., 2011). Moderate deficit irrigation did not reduce the number of flower in bottlebrush plants and no differences in the colour space coordinate values were observed, suggesting that colour is not modified by this level of deficit irrigation and meaning that plants can cope with water shortage without losing their ornamental value (Henson et al., 2006). Plant quality was affected by the severe deficit irrigation treatment (lower number of inflorescences, lower RCC values and colour).

The water consumption of the plants was closely related with environmental factors (Bakker, 1991; Montero et al., 2001; Bañón et al., 2009; Grant et al., 2012). However, the active periods of growth and the inflorescence formation and opening phases clearly affected this parameter, which agrees with previous studies in *Callistemon laevis* and geranium plants under controlled conditions (Álvarez et al., 2011), when climatic conditions were constant throughout the experiment. In general, ornamental species respond to water stress by reducing the daily ET (Jaleel et al., 2008; Lenzi et al., 2009; Bolla et al., 2010) and ET decreases as the stress severity increases (less irrigation water applied or less frequent irrigation) (French et al., 2009; Eiasu et al., 2012) although the intensity of this response depends on the species and variety studied and varies during an experiment (García-Navarro et al., 2004; Lenzi et al., 2009).

Differences between treatments were not constant during the experiment and were greater as the ET values increased. In this sense, Liu et al. (2006) noticed that transpiration and g_s might be partly regulated by VPD, the lower the VPD, the smaller the differences in g_s and T between treatments. In our conditions, water consumption (ET/d) reached the highest values in August and April, leading to the lowest leaf water potential values at midday, and the lowest values in December, when Ψ_l values were the highest. As regards the behaviour of ET along the day, it began to increase at dawn due to stomatal opening, and was the highest at midday, when temperature and VPD were also the highest and the stomata presented their maximum opening. After midday, stomata closing began, producing a decrease in the ET. At night, ET was at its lowest as a result of nocturnal stomata closing and non-evaporation from the substrate (Alarcón et al., 2000).

The stem water potential measured at midday is a good indicator of water stress resulting from the effect of irrigation in *C. citrinus* plants due to the small variability between bagged leaves (Choné et al., 2001). Only when the water deficit became more severe did Ψ_l exhibit significant differences between treatments. Also, the difference between Ψ_s and Ψ_l measured simultaneously in the same plant was shown to be an indicator of instantaneous shoot transpiration, which varied with soil moisture conditions and VPD in the atmosphere (Choné et al., 2001).

The consistent decrease in g_s in deficit irrigation plants suggested an efficient adaptative stomatal control of transpiration (Hessini et al., 2008). Stomatal conductance can be mostly limited by summer drought or by low winter temperatures (Gulías et al., 2009). In *C. citrinus*, gas exchange was more sensitive to winter than summer conditions. The relation between P_n and g_s showed two phases in all treatments: phase I, with high g_s and a wide variability in P_n , and phase II, where P_n was sharply reduced as the stomatal aperture decreased. Similar results were found by Costa França et al. (2000). According to Colom and Vazzana (2003), Bacelar et al. (2007) and Bolla et al. (2010), non-stomatal factors, such as biochemical limitations, would probably contribute to the decrease in P_n following water stress, as was observed at the end of our experimental period. In addition, the greater reduction of g_s compared with P_n (increased P_n/g_s) suggests that stomatal closure was the main cause for P_n depression in deficit irrigation plants in *C. citrinus*, although photoinhibition may have played a role later when stress was more severe or prolonged and when P_n was proportionally more reduced than g_s (decreased P_n/g_s) (Flexas et al., 2004; Gallé et al., 2009; Gulías et al., 2009; Bolla et al., 2010). Previous studies in a variety of ornamental species indicated that P_n/g_s increases under deficit irrigation and CO_2 assimilation remains proportionally

higher than water vapour loss from the stomata as an additional drought acclimatation mechanism (Raviv and Blom, 2001; Jaleel et al., 2008; Shao et al., 2008; Álvarez et al., 2009; Bolla et al., 2010). However, in some ornamental species the opposite effect has been found in response to deficit irrigation (Rasoul Sharifi and Rundel, 1993; Mugnai et al., 2005). The exact response depends on several factors including the species and variety studied, the severity of the stress, the deficit irrigation strategy or the moment of the day when measurements are realized (Costa França et al., 2000; Flexas et al., 2001; Liu et al., 2006; Zollinger et al., 2006; Bacelar et al., 2007; Hessini et al., 2008; Jaleel et al., 2008; Lenzi et al., 2009).

C. citrinus plants submitted to both deficit irrigation treatments increased their intrinsic water use efficiency because stomatal opening was reduced with respect to the control and they had more efficient g_s than the control, and not because of increased CO_2 assimilation at the same stomatal aperture as control.

The parameter P_n/g_s allows the identification of plants that maintain CO_2 assimilation at low stomatal aperture. The most efficient g_s is when values are close to $100 \text{ mmol m}^{-2} \text{ s}^{-1}$, as higher g_s values do not reflect significant increases in P_n and are progressively less efficient, while lower g_s values are much less efficient. Moreover, if plants show a $g_s < 100 \text{ mmol m}^{-2} \text{ s}^{-1}$ for long periods, non-stomatal limitations could be partly responsible for the decrease in P_n , which could delay plant recovery at the onset of the autumn or even cause permanent damages. For these reasons, the most suitable g_s values are those between the 100 and $200 \text{ mmol m}^{-2} \text{ s}^{-1}$, when plants maintain acceptable photosynthetic rates. This was the case with MDI, which presented g_s values in this interval during the greatest part of the season and for longer than plants from the other two treatments.

Also, WUE was higher in *C. citrinus* plants growing in deficit irrigation conditions. This response has been observed in numerous ornamental plants (Cameron et al., 2006; Jaleel et al., 2008; Álvarez et al., 2009; Mugnai et al., 2009). However, according to other authors this parameters is not modified under deficit irrigation conditions (Andersson, 2001), or even decreases (Anyia and Herzog, 2004; Eiasu et al., 2012), depending on the species and variety, genotype, irrigation frequency and stress degree (Anyia and Herzog, 2004; Cameron et al., 2006; Jaleel et al., 2008; Eiasu et al., 2012).

Although plants exposed to deficit irrigation showed lower biomass accumulation, the water use efficiency (P_n/g_s and WUE) was higher in the water stressed plants throughout the experimental period. The advantage in the case of these plants is that controlled drought may lead to an accumulation of carbohydrate reserves in the plants and, together with an increased root to shoot ratio and root density,

which could promote a more rapid establishment of ornamental plants in the garden or landscape (Cameron et al., 2006; Franco et al., 2006).

Ion leakage was unaffected by the irrigation treatment at the end of the experiment. Álvarez et al., (2009) observed membrane damage in younger callistemon plants submitted to deficit irrigation. Drought sensitivity could depend on the age of the plant, younger plants being more sensitive and less tolerant than older plants.

Conclusion

Moderate deficit irrigation (reductions of 50%) decreased the relative growth rate, but flowering and leaf colour were not affected. The root/shoot ratio increased, improved the root system and increased WUE and P_n/g_s . Therefore, this treatment can be used successfully in *Callistemon citrinus* plant production to reduce water consumption while maintaining good overall quality. However, reductions of 75% of the water applied (severe deficit irrigation) are not recommended, since this treatment affected the quality of plants, reducing flowering and affecting leaf colour. In this treatment stomatal conductance was strongly reduced for long periods, which decreased P_n and could delay their recovery and even cause permanent damage.

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Table 1

Relative growth rate (RGR) of biomass accumulation at the end of the experimental period in *C. citrinus* plants subjected to different irrigation treatments. Values are the mean of 10 plants.

Parameters	Treatments			
	C	MDI	SDI	P
Total DW (mg g ⁻¹ d ⁻¹)	45.1 ± 6.3 b	38.0 ± 0.7 b	18.1 ± 1.6 a	**
Leaf DW (mg g ⁻¹ d ⁻¹)	34.7 ± 2.8 c	27.3 ± 1.2 b	13.7 ± 1.8 a	***
Stem DW (mg g ⁻¹ d ⁻¹)	56.0 ± 3.7 b	52.9 ± 2.3 b	33.2 ± 2.7 a	***
Shoot DW (mg g ⁻¹ d ⁻¹)	44.9 ± 3.0 b	39.6 ± 1.3 b	23.0 ± 2.1 a	***
Root DW (mg g ⁻¹ d ⁻¹)	38.3 ± 0.5 b	35.7 ± 0.2 b	11.7 ± 0.5 a	**
Total eaf area (mm ² cm ⁻² d ⁻¹)	1.84 ± 0.1 c	1.37 ± 0.2 b	0.57 ± 0.1 a	***

Means within a row without a common letter are significantly different by Duncan_{0.05} test. (P; probability level, ** P ≤0.01, *** P ≤ 0.001).

Table 2

Growth and biomass traits at the end of the experimental period in *C. citrinus* plants subjected to different irrigation treatments. Values are the mean of 10 plants

Parameters	Treatments			
	C	MDI	SDI	P
Number of leaves	1273.6 ± 63.9 c	1030.6 ± 34.0 b	606.4 ± 54.3 a	***
Leaf blade area (mm ²)	130.68 ± 7.85 b	93.02 ± 6.87 a	82.01 ± 4.60 a	***
Stem diameter (mm)	2.26 ± 0.12 b	1.73 ± 0.07 a	1.61 ± 0.07 a	***
Succulence	2.21 ± 0.03 b	1.91 ± 0.10 a	1.81 ± 0.11 a	*

Means within a row without a common letter are significantly different by Duncan 0.05 test. (P; probability level, *P<0.05, *** P ≤ 0.001).

Table 3

Root morphology in *C. citrinus* plants subjected to different irrigation treatments at the end of the experimental period. Values are the mean of 3 plants

Parameters	Treatments				P
	C	MDI	SDI		
Root/shoot ratio (g g^{-1})	1.98 $\pm$ 0.09 a	2.40 $\pm$ 0.21 b	2.99 $\pm$ 0.14 c		*
Total root length (m)	283.32 $\pm$ 14.53 c	186.90 $\pm$ 11.96 b	132.48 $\pm$ 12.45 a		***
$L_{\phi < 0.5 \text{ mm}}$ (m)	158.62 $\pm$ 5.94 c	97.09 $\pm$ 6.46 b	69.54 $\pm$ 5.79 a		***
$L_{0.5 < \phi < 2.0 \text{ mm}}$ (m)	100.15 $\pm$ 5.78 c	75.03 $\pm$ 2.89 b	51.62 $\pm$ 6.00 a		**
$L_{\phi > 2.0 \text{ mm}}$ (m)	24.55 $\pm$ 2.92 b	14.78 $\pm$ 2.90 a	11.32 $\pm$ 0.88 a		*
$L_{\phi < 0.5 \text{ mm}}$ (%)	56.07 $\pm$ 0.46 b	51.95 $\pm$ 0.40 a	52.62 $\pm$ 0.56 a		***
$L_{0.5 < \phi < 2.0 \text{ mm}}$ (%)	35.32 $\pm$ 0.20 a	40.28 $\pm$ 0.60 b	38.79 $\pm$ 0.71 a		***
$L_{\phi > 2.0 \text{ mm}}$ (%)	8.61 $\pm$ 0.32	7.78 $\pm$ 0.15	8.58 $\pm$ 0.15		ns
Root volume (cm^3)	189.0 $\pm$ 18.9 b	117.8 $\pm$ 7.8 a	95.8 $\pm$ 7.4 a		**
Root dry weight (g)	69.54 $\pm$ 9.30 b	65.23 $\pm$ 3.50 b	31.00 $\pm$ 4.46 a		**
Root density (g cm^{-3})	0.35 $\pm$ 0.02 a	0.52 $\pm$ 0.04 b	0.36 $\pm$ 0.03 a		*

Root length (L). Means within a row without a common letter are significantly different by Duncan_{0.05} test. (P; probability level, ns; non significance, * $P < 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$).

Table 4

Colour, relative chlorophyll content and ion leakage in *C. citrinus* plants subjected to different irrigation treatments at the end of the experimental period. Values are the mean of 10 plants

Parameters	Treatments			
	C	MDI	SDI	P
L*	41.5 ± 2.9	44.5 ± 2.1	43.2 ± 1.4	ns
C*	17.9 ± 1.8 b	15.4 ± 2.5 b	13.7 ± 0.9 a	*
h°	108.9 ± 2.1 a	107.2 ± 2.0 a	118.4 ± 2.9 b	*
RCC	39.51 ± 1.14 a	39.81 ± 0.84 a	43.27 ± 0.52 b	**
Ion leakage (%)	29.44 ± 1.21	30.71 ± 1.85	28.74 ± 0.87	ns

Lightness (L*), chroma (C*), hue angle (h°) and relative chlorophyll content (RCC). Means within a row without a common letter are significantly different by Duncan _{0.05} test. (P; probability level, ns; non significance, *P<0.05, ** P ≤0.01).

Figure captions

Fig. 1. Evolution of plant height (A) and relative growth rate of height (B) in *C. citrinus* plants submitted to different irrigation treatments. Values are means $\pm$ s.e., $n = 25$. Symbols represent the different treatments: Control (filled circles), MDI (open circles) and SDI (open triangles).

Fig. 2. Evolution of number of inflorescences in *C. citrinus* plants submitted to different irrigation treatments. Values are means $\pm$ s.e., $n = 25$. Symbols represent the different treatments: Control (filled circles), MDI (open circles) and SDI (open triangles).

Fig. 3. Evolution of the substrate volumetric water content (SWC) (A), daily evapotranspiration (ET) (B) in *C. citrinus* plants submitted to different irrigation treatments and daily mean values of air temperature (T) and vapour pressure deficit (VPD) (C) recorded inside the greenhouse during the experimental period. Values are means $\pm$ s.e., $n = 7$ in Fig. 3a and each point represents a single plant in Fig. 3b. Symbols represent the different treatments: Control (filled circles), MDI (open circles) and SDI (open triangles).

Fig. 4. Evolution of hourly ET throughout several representative days of the experimental period in *C. citrinus* plants submitted to different irrigation treatments. Each point represents a single plant.

Fig. 5. Evolution of the stem water potential (Ψ_s) and leave water potential (Ψ_l) in *C. citrinus* plants submitted to different irrigation treatments. Values are means $\pm$ s.e., $n = 8$. Symbols represent the different treatments: Control (filled circles), MDI (open circles) and SDI (open triangles). Solid lines represent Ψ_l and dashed lines represent Ψ_s . For each studied day, * indicates significantly different ($P \leq 0.05$, Duncan's, multiple range test) between treatments for Ψ_s and ** indicates significantly different between treatments for Ψ_s and Ψ_l .

Fig. 6. Evolution of stomatal conductance (g_s , A) and intrinsic water use efficiency (P_n/g_s , B). Values are means $\pm$ s.e., $n = 8$. Relationship between P_n and g_s (C) and between P_n/g_s and g_s (d) in *C. citrinus* plants submitted to different irrigation treatments. Each point represents a single plant. Symbols represent the different treatments: Control (filled circles), MDI (open circles) and SDI (open triangles).

Fig. 7. Water use efficiency of production (WUE) at the end of the experimental period in *C. citrinus* plants submitted to different irrigation treatments. Means within a part of the plant without a common letter are significantly different by Duncan $_{0.05}$ test.

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6

Figure 7