Agronomy Research **16**(5), 2097–2109, 2018
https://doi.org/10.15159/AR.18.194

Split water application for a water supply reduction in *Callistemon Citrinus* pot plant

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Abstract. Irrigation management in Greenhouse Nursery Production (GNP) is based on empiric methods based on farmer personal experiences with over-irrigation results. The effects of irrigation volume and daily application were studied in a pot experiment carried out on rooted cuttings in a greenhouse The irrigation volume treatment was performed on Full and reduced Treatment. The treatment of water application was carried out with split supply and unsplit supply. The effects of the treatments were evaluated in terms of biomass accumulation and partitioning, leaf area, photosynthesis and stomatal response, chlorophyll content, and water productivity. Callistemon showed a good adaptation to the different treatments tested during the experiment. A positive relation was found between biomass accumulation and irrigation volume, moreover split water application increased plant Dry Weight.

Therefore, the highest biomass accumulation was registered in full irrigation volume in split application treatment, and this behavior was confirmed by the photosynthetic rate. No statistical differences were found, in terms of Relative Water Content (RWC), between the treatments. Stem water potential and stomatal conductance values suggest in Callistemon an anysohidric water stress response behavior.

Our results evidenced that, in Callistemon potted plants, an irrigation volume reduction is possible when a split application occurs during the daytime. A full irrigation volume amounts to 10.8 L per plant during the trial period of 90 days while the reduced volume amounts to 8.2 L per plant. Therefore, an increased water productivity can be obtained if the daily water requirement is split on two applications during the daytime. Our results highlighted a possible reduction in environmental impact of Callistemon greenhouse pot production, through the 25% reduction of the volume irrigation.

Key words: photosynthesis, drought, anisohydric, WUE, water productivity.

INTRODUCTION

The genus Callistemon is a woody aromatic tree or shrub (ca. 0.5 m to 7 m tall) belonging to the family Myrtaceae which comprises over 30 species. These plants were originally found in the temperate part of Australia and in south America and Asia and show remarkable adaptability to high temperature heat, sun, aridity and wind.

However, they are now found across the globe as flowering shrubs used in gardening and landscaping.

Callistemon leaves are lanceolate and very aromatic. The flower spikes of bottlebrushes form are made up of a number of individual flowers with prominent red stamens. Their petals are of greenish or pale color, tiny, inconspicuous and in some cases deciduous (Oyedeji et al., 2009). For its ornamental values, Callistemon is primarily produced as a potted plant, representing an important product in the Greenhouse Nursery Production (GNP) in the Mediterranean basin.

High environmental impact of ornamental GNP is also due to the high water consumption for plant irrigation. Nowadays, less attention is given to the water requirement of ornamental plants, therefore there is few available information about ornamental species. Consequently, irrigation management in most nurseries is based on farmers' personal experiences. This results over-irrigation and low Water Productivity (WP). WP may carry different meanings with respect to different water-using production sectors e.g. hydrology, irrigation engineering, field crops, etc. (Ali & Talukder 2008). In crop production, WP can be defined as the ratio between yield and water supply. It's also used to quantify Water Use Efficiency (WUE) on different scale, from individual leaf up to a hydrological basin (Fereres et al., 2014). WP is strictly influenced by the relationship of three parameters (Ali & Talukder 2008; Fereres et al., 2014) environment, genotype and water management. The environmental parameters are the result of a complex interaction among soil, temperature, air humidity and light availability. These interactions can represent a limitation for plant adaptations, especially when plants come from different climatic areas (Giovino et al., 2014). Whereas under GNP, environmental parameters can be controlled and considered constant in the short period. The genotype influence on WP performance can vary through an adequate choice (species, cultivars, etc.), depending on its drought tolerance. Finally, water management plays a key-role in the determination of WP. Excess of water in irrigation represents both economic and environmental loss (Ali & Talukder, 2008). Moreover, it can promote the occurrence of phyto-pathological adversities. Contrariwise, a deficit irrigation may be used, in potted ornamental plants, to improve plant quality, by reducing excessive vigor and promoting a more compact habit (Cirillo et al., 2013). Furthermore, it was demonstrated that a deficit irrigation promotes a better nitrogen use by plant reducing nitrogen loss (Mahdavi-Damghani et al., 2010). Drought in pot occur when water supply is scarce or inadequate.

To avoid excessive drying of the substrates, especially, in ornamental crops grown in pots with a small water capacity, deficit irrigation requires a precise scheduling (Álvarez & Sánchez-Blanco 2013). Splitting strategy could be used for irrigation scheduling to improve WP under pot condition. Indeed during the daytime, the evapotranspiration demand varies depending on plant's endogenous and exogenous (environmental) requirements. The split water application allows the water supply improving WP reducing the exogenous factors influence. Split application must allow physiological and biochemical process in plants reducing water loss trough the evaporation from the soil. The application of split water improves the water supply increasing WP and reducing the exogenous factors influence. Split application must allow physiological and biochemical process in plants by reducing water loss trough the evaporation from the soil. An important process for plant survival is related to restoration ability after photo-damage. Photo-damage frequently occurs during photosynthetic processes (Werner et al., 1999), especially in climates, such as the Mediterranean one, where light intensity often exceeds the requirement of the plant. This damage affects the proteins of PSII that are usually restored by the 'PSII repair cycle' (Takahashi & Badger, 2011), under the availability of light and water. However, when there is excess light during photosynthesis, the restoration process is depressed and it does not depend to water availability. PSII repair process is also inhibited by the environmental stresses that induce stomatal closure (Aro et al., 2005). Previous investigations focused on the effect of irrigation water supply in relation to phenological stages under different environmental conditions (Patane et al., 2011; Yihun et al., 2013), whereas not much information is available on the effect of day time split water supply on potted plant.

In the light of the problem stated, this research work aim at evaluating the effect of 25% reduction in water supply on the growth rate of potted Callistemon. Furthermore, the authors seek to test the hypothesis that a daily split water amount application can improve Callistemon WUE and their performances growth.

MATERIALS AND METHODS

Plant materials and experimental conditions

A 90 days experiment was carried out on rooted cuttings of 6 months-old of *Callistemon citrinus* (Curtis) Skeels. The cuttings, grown in 7 x 7 x 7 cm pots, were transplanted into 3L plastic pots filled with a mixture of 30% peat 30% sand and 40% perlite (v:v:v) amended with 2 g L^{-1} of Osmocote Plus Scotts© Australia (14:13:13 N, P, K plus micro element). Pots were placed inside an east-west-oriented greenhouse (540 m^2) with a steel structure and methyl polymethacrylate cover, located in Bagheria (PA), Sicily, Italy (38° 5' 28" N, 13° 31' 18 E; 23 m above sea level). During a 10-day acclimatization period, pots were maintained at field capacity. All the plants were daily irrigated (water electrical conductivity was 0.8 dS m^{-1}) using drip irrigation system.

Treatments and statistical analyses

Irrigation volume treatment was performed on two levels, Full (F) and Reduced (R) corresponding to 100% and 75% of daily effective evapotranspiration (ETe) respectively. Application treatment was performed on two levels, Unsplit (U) and Split (S) which correspond to irrigation volume of 1 and 2 applications per day respectively. ETe was determined, on six plants, by weighing pots on a daily basis. The experiment was laid out in a split-plot design with irrigation volume as main factor and daily application as sub-factor, with three replicates. Each treatment was composed by 36 plants.

The experiment was performed on 12 plants randomly attributed to each treatment and each block. U daily irrigation was performed at 8:00 solar time and S daily irrigations were performed at 8:00 and 18:00 solar time.

Data were analyzed using two-way ANOVA computed using XLStat for Windows systems (XLSTAT-Base, version 10). Treatments means were separated with Tukey Test ($P \leq 0.05$).

Growth and physiological parameters

Growth and physiological parameters were measured at 0, 30, 60 and 90 Days After experiment Start (DAS) on 3 plants per replicate and per treatment.

Plants were separated into stems, leaves and roots recording fresh and dry weight, leaf number and leaf area. Leaf area was measured by a WInDIAS leaf area meter (Image Analysis System, DELTA-T DEVICES LTD, Burwell, Cambridge, England). Dry Weight (DW) was measured after oven-drying the samples at 60 °C until constant weight was achieved (around 48 h).

WUE was calculated as the rate of biomass accumulation and total water supplied. During the trial, plants were supplied with 119 mL day per pot (average of the trial period) for F treatments. The total water supply during the trial was 10.8 L per pot for full treatment. Relative Growth Rate (RGR) was calculated as:

$$RGR = (\ln DW2 - \ln DW1) / (T2 - T1) \qquad (1)$$

where DW1 – initial dry weight; DW2 – final dry weight; T1 – starting time; T2 – final time.

Relative Water Content (RWC) was calculated as:

$$RWC = (FW - DW)/(TW - DW)\ 100 \qquad (2)$$

where FW – fresh weight; TW – turgid weight determined after leaf submersion in distilled water at 6-8 °C in the dark for 24 h; DW – dry weight measured after oven drying at 60 °C for 48 h.

Relative Chlorophyll Content (RCC) was determined by a Minolta SPAD-502 chlorophyll meter (Konica Minolta Sensing Inc., Osaka, Japan) at the midpoint of two mature leaves per plant and three plants per treatment.

Stem Water Potential (WP_s) was measured at midday with pressure Scholander chamber (Soil Moisture Equipment Co., Santa Barbara, CA, USA). WP_s was measured using leaves that had been bagged with both a plastic sheet and aluminum foil for at least 1 h before measurement in order to prevent transpiration from leaves, in this way, leaf water potential equaled stem water potential (Begg & Turner 1970; Valladares & Pearcy, 1997; Navarro et al., 2009). Leaf stomatal conductance (g_s), net photosynthetic rate (P_n) and the Vapor Pressure Deficit (VPD) were determined using a gas exchange system (LI-6400, LI-COR Inc., Lincoln, NE, USA) at 9:00, 13:00 and 17:00 solar time during every sampling on one mature leave per plant and three plants per treatment. The VPD, was calculated by the difference between saturation and real air pressures according to the method reported by (Moura dos Santos et al., 2013).

RESULTS AND DISCUSSION

Growth parameters

At the end of the experiment (90 DAS), biomass accumulation was significantly influenced by the treatments (Table 1). Total, Leaf, Root and Stem DW decreased according to the irrigation volume reduction (107.0 ± 5.1 vs 81.0 ± 4.8; 26.1 ± 0.4 vs 21.3 ± 0.5; 68.2 ± 4.8 vs 48.2 ± 4.4; 12.8 ± 0.3 vs 11.5 ± 0.3 in F and R respectively). A similar pattern was registered in Leaf Area (1165.8 ± 17.0 vs 982.5 ± 22.8 cm^2 in F and R respectively) whereas no significant difference was registered in terms of leaves number. Also root shoot rate was not influenced by the irrigation volume treatment.

Total DW, Leaf DW and Root DW were influenced by the application treatments, higher values were measured on S treatment than on U treatment (107.5 ± 4.91 vs 80.5 ± 4.82 ; 24.8 ± 0.76 vs 22.5 ± 0.82; 70.0 ± 4.13 vs 46.3 ± 4.05 g respectively). The same pattern was registered in terms of Leaf Area and R/S (1,118 vs 1,030 cm^{2}; 1.86 vs 1.34 in S and U respectively). Whereas no significant differences were registered in terms of leaves number and above ground RGR. (Table 1). Roots RGR evidences a statistical interaction between the treatments with the highest value in FS treatment. A positive relation was found in terms of roots RGR in the irrigation volume treatment (0.0465 vs 0.0424 in F and R respectively). Roots RGR shown statistical differences also in the application treatment (0.0469 vs 0.0420 in S and U respectively). In terms of WUE (Table 1) statistical difference was found both in the irrigation volume treatment (146.62 g L^{-1} vs 147.94 in F and R respectively) then in the application treatment (168.85 vs 125.69 in S and U respectively).

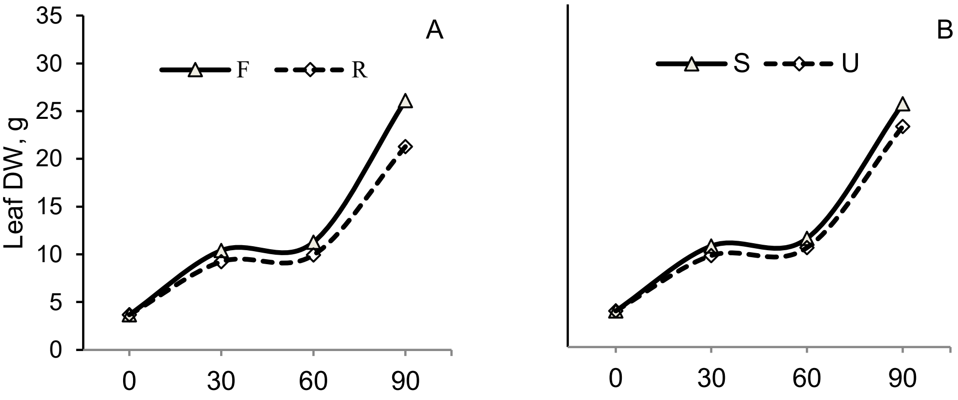
Table 1. Growth parameters in *Callistemon citrinus* pot plants under different irrigation management at 90 DAS

Irrigation Volume (IV)	Full Volume (F)				Reduced Volume (R)				IV	AP	IV*AP
Application (AP)	split (S)		Unsplit (U)		Split (S)		Unsplit (U)		Pr < F	Pr < F	Pr < F
Total dry weight, g	120.3	A	93.7	B	94.7	B	67.3	C	0.0004	0.0003	0.9224
Leaf dry weight, g	27.1	A	25.1	A	22.5	B	20.0	C	< 0.0001	0.0019	0.6117
Root dry weight, g	80.0	A	56.3	BC	60.1	AB	36.3	C	0.0030	0.0012	0.9816
Stem dry weight, g	13.2	A	12.3	AB	12.0	AB	11.1	B	0.0283	0.0807	0.9406
Leaves, n.	311.0		286.0		319.7		271.7		0.8987	0.1381	0.6094
Leaf area, cm^{2}	1,199.5	A	1,132.2	AB	1,037.4	BC	927.6	C	0.0011	0.0304	0.5240
R/S	1.98	A	1.51	AB	1.74	AB	1.17	B	0.0786	0.0091	0.7367
RGR a.g., g day^{-1}	0.0259		0.0210		0.0201		0.0230		0.1886	0.4644	0.1922
RGR roots, g day^{-1}	0.0531	A	0.0400	B	0.0408	B	0.0441	B	0.0388	0.0185	0.0011
WUE, g L^{-1}	164.8	A	128.4	B	172.9	A	122.98	B	0.0011	0.0017	0.2800

DW = dry weight; R/S = root shoot ratio; RGR a.g.: Relative Growth Rate referred to above ground biomass; RGR roots: Relative Growth Rate referred to roots biomass; WUE: Water use efficiency. R – Reduced irrigation volume (75% ETe); F – Full irrigation volume (100% ETe); S – Split irrigation corresponding to two applications per day of the irrigation volume at 8:00 and 18:00; U – Unsplit irrigation corresponding to one application per day of the irrigation volume at 8:00 A.M. Means (n = 3) within a column without a common letter are significantly different by Tukey test.

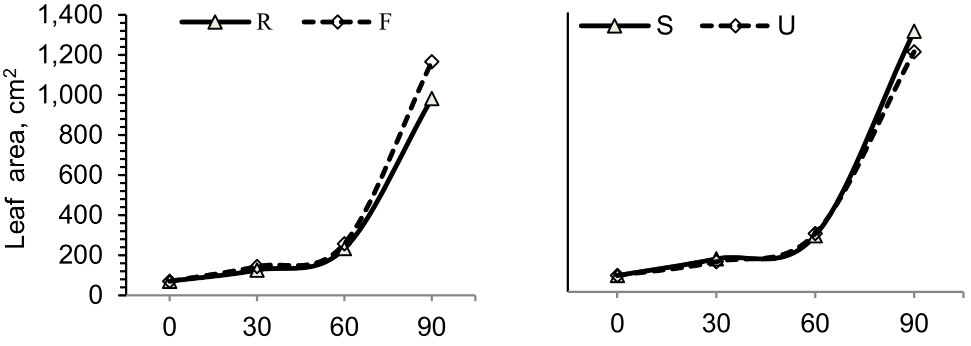
During the experiment, pronounced increases in terms of leaf DW and leaf area (Fig. 1 and Fig. 2) were observed from 60 DAS, when *Callistemon* flowering began (58 ± 3 DAS). No statistical differences among the treatments were observed in terms of flowering time, number of flowers, and flower DW (data not shown).

Statistical interaction was found at 30 DAS in terms of Leaf DW (Fig. 1). FS treatment shown the highest values until the end of the experiment, whereas no differences were found among the other treatments until 60 DAS (Fig. 1). In terms of leaf area, no statistical differences were registered until 60 DAS (Fig. 2).



	30 DAS	**60 DAS**	**90 DAS**
Irrigation Volume (IV)	**0.0246**	0.0625	**<.0001**
Application)AP)	0.2096	0.0924	**0.0019**
IV * AP	**0.0386**	0.4385	0.6117

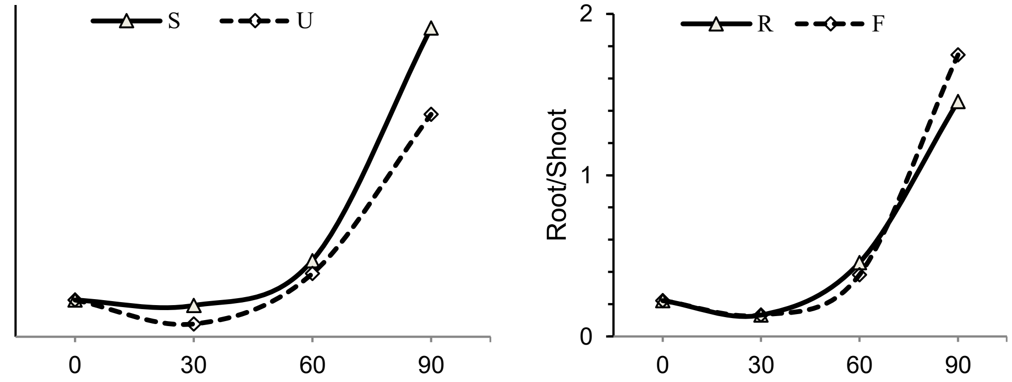
Figure 1. Leaf DW trend during the experiment in *Callistemon citrinus* pot plants under different irrigation management. R – Reduced irrigation volume (75% ETe); F – Full irrigation volume (100% ETe); S – Irrigation volume per day split on two applications at 8:00 and 18:00; U – Irrigation volume on single application per day at 8:00. Means (n = 3) are significantly different by Tukey test.



	30 DAS	60 DAS	90 DAS
Irrigation Volume (IV)	0.1237	0.4094	**0.0011**
Application (AP)	0.2897	0.7585	**0.0304**
IV * AP	0.1733	0.6952	0.524

Figure 2. Leaf Area trend during the experiment in *Callistemon citrinus* pot plants under different irrigation management. Irrigation treatments were carried out in: R – Reduced irrigation volume (75% of ETe) marked with a circle; F – Full irrigation volume (100% ETe) marked with a triangle; S – Irrigation volume per day split on two applications at 8:00 and 18:00 marked with close signs; U – Irrigation volume on single application per day at 8:00 marked with open signs. Means (n = 3) are significantly different by Tukey test.

Root shoots rate evidences a similar trend, during the experiment, among the treatments (Fig. 3) with remarkable difference only at 90 DAS (Table 1).



	30 DAS	60 DAS	90 DAS
irrigation Volume (IV)	0.9816	0.1491	0.0786
Application (AP)	**0.0161**	0.439	**0.0091**
IV * AP	0.3377	0.3478	0.7367

Figure 3. Root/Shoot ratio trend during the experiment in *Callistemon citrinus* pot plant under different irrigation management. R – Reduced irrigation volume (75% of ETe) marked with a circle; F – Full irrigation volume (100% ETe) marked with a triangle; S – Irrigation volume per day split on two applications at 8:00 and 18:00 marked with close signs; U – Irrigation volume on single application per day at 8:00 marked with open signs. Means (n = 3) are significantly different by Tukey test.

Physiological parameters

At the end of the experiment RCC highlights the lowest value in RU treatment (61.9 vs 63.2 respectively) while no statistical differences among the other treatments were registered (Fig. 4).

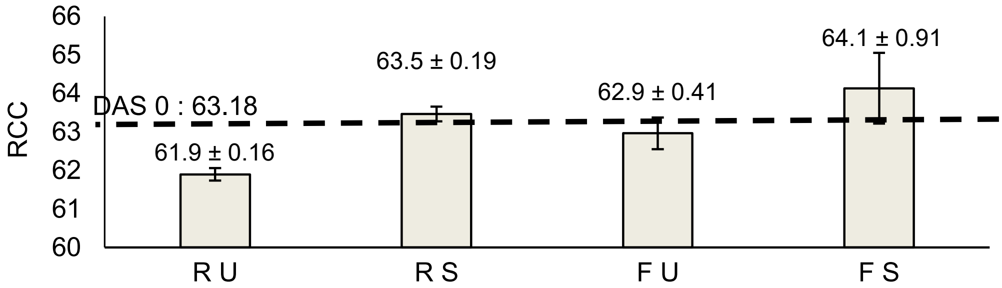


Figure 4. Relative Chlorophyll Content (RCC) in *Callistemon citrinus* pot plant under different irrigation management. R – Reduced irrigation volume (75% of ETe); F – Full irrigation volume (100% ETe); S – Irrigation volume per day split on two applications at 8:00 and 18:00; U – Irrigation volume on single application per day at 8:00. Means (n = 6) represented in columns without a common letter are significantly different by Tukey test, P ≤ 0.05 probability level. Dashed line represent the RCC means value at 0 DAS, columns represent the RCC means value at 90DAS.

During the experiment at 30 and 60 DAS, no statistical differences were registered among the treatments in terms of midday WP_s (Fig. 5); while, at 90 DAS, WP_s was significantly influenced by the application treatment (-2.76 vs -2.37 MPa in U and S respectively); volume irrigation did not affect the data. No statistical differences were registered in terms of RWC between the treatments (data not shown).

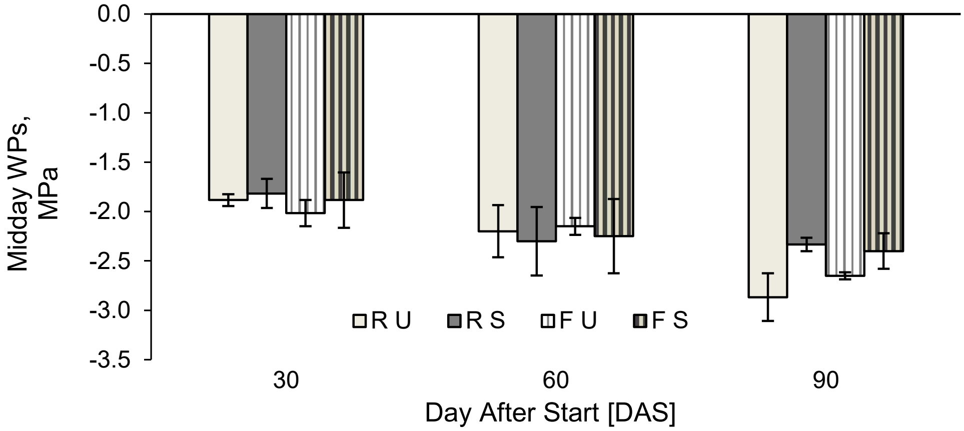


Figure 5. Stem Water Potential (WP_S) in *Callistemon citrinus* pot plant under different irrigation management. R – Reduced irrigation volume (75% of ETe); F – Full irrigation volume (100% ETe); S – Irrigation volume per day split on two applications at 8:00 and 18:00; U – Volume irrigation on single application per day at 8:00. Vertical lines represent standard error (n = 3).

Statistical differences were found in terms of photosynthesis and stomatal conductance (Fig. 6, A, B) between the treatments. Highest values of photosynthesis and P_n/G_s were measured on the split treatments (Fig. 6, A, C). Application treatment shown the higher values of photosynthesis and P_n/G_s, (Fig. 6, C) during the day when split application was adopted. In addition, the highest values of photosynthesis were registered in FS treatment.

A relation between stem water potential and stomatal conductance was determinated (Fig. 7). The regression model highlights a typical anisohydric water potential regulation strategy.

Our results highlighted a different biomass accumulation due to the irrigation volumes applied during the experiment. Indeed, the volume irrigation reduction produced a significant reduction in terms of leaves, root and stem DW.

Generally, in field experiments under drought conditions, an increase in root growth has been reported by many authors (Kashiwagi et al., 2006; Blum, 2009; Hernández et al., 2009; Porcel et al., 2012). In our trial, pot plants responded with a vigorous root growth in full-irrigated treatments. Similar responses were observed in slight water stress conditions in Populus, Erytrina, Eucalyptus and Avocado, (Zollinger et al., 2006; Shao et al., 2008). A significant root reduction, under water scarcity, was reported on *Opuntia* species (Snyman, 2014). The author suggested as limited water availability reduces roots elongation process.

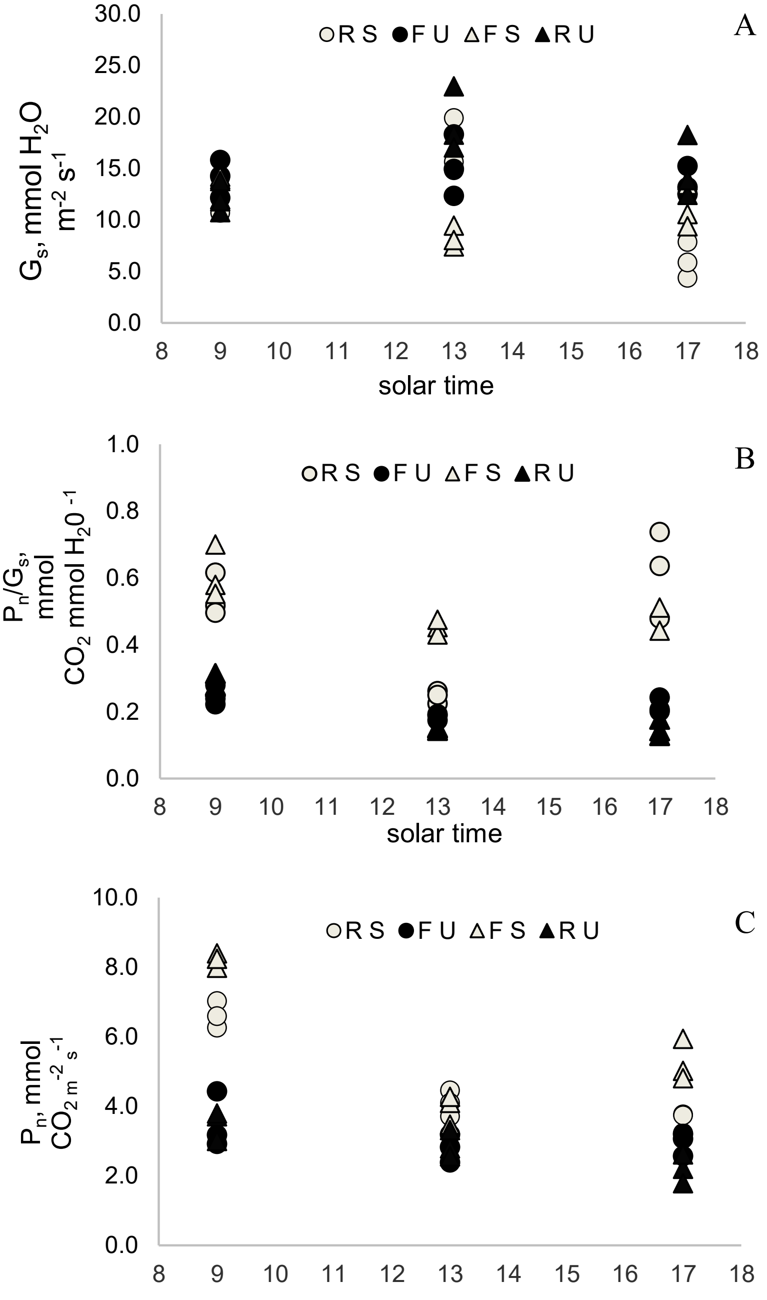


Figure 6. Leaf Gas Exchange parameters measured in *Callistemon citrinus* pot plant under different irrigation management at 90 DAS. R – Reduced irrigation volume (75% of ETe); F – Full irrigation volume (100% ETe); S – Irrigation volume per day split on two applications at (8:00 and 18:00); U – Volume irrigation on single application per day at 8:00. Experimental conditions were PAR: 243.6, 466.3, 232,0; VPD: 4.5, 5.1, 4.7 at 9:00, 13:00 and 17:00 respectively; Air Temperature: 33.1, 35.8, 34.0 at 9:00, 13:00 and 17:00 respectively.

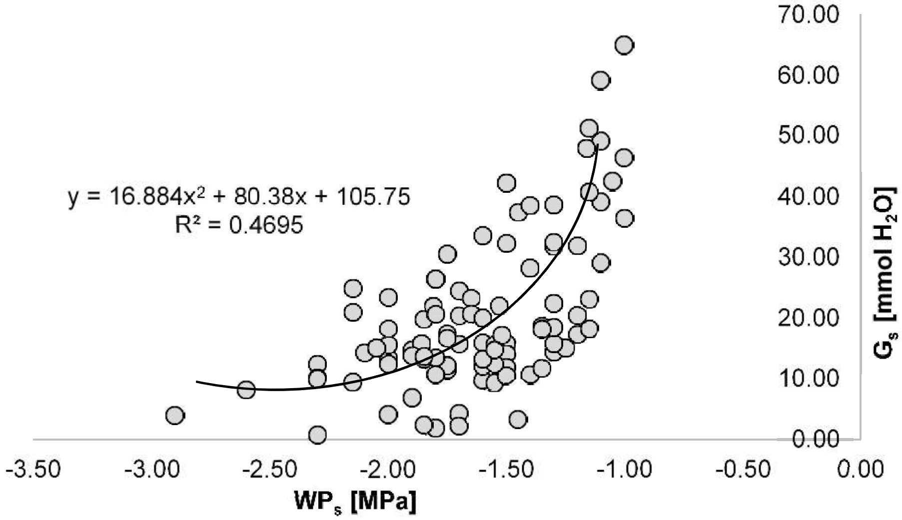


Figure 7. Stomatal conductance (Gs) and Stem water potential (WP_S) relation in in *Callistemon citrinus* pot plant under different irrigation management. R – Reduced irrigation volume (75% of ETe); F – Full irrigation volume (100% ETe); S – Irrigation volume per day split on two applications at 8:00 and 18:00; U – Volume irrigation on single application per day at 8:00.

As for irrigation volume, the application treatment influenced biomass accumulation. Independently to the water amount, split application, increased the plant biomass accumulation. Important information arises from the interaction between volume irrigation and application treatment. Indeed, RS treatment gained a similar value of dry matter than FU treatment. This highlights the possibility to obtain good growth performance on Callistemon potted plant under deficit irrigation by water split application.

Although, no statistical differences were registered in terms of number of leaves among the treatments, some authors report that the effects of a periodic drought stress promote a physiological response in terms of loss of leaves to reduce the evapotranspiration phenomenon (Shao et al., 2008). Especially during the flowering time, when the meristematic activity needs a high water availability, the lower number of leaves and leaf DW would confirm that a water drought condition occurred in the unsplit treated plants.

Water use efficiency is largely influenced by biomass accumulation. Many authors reported that an irrigation reduction involves a growth reduction with a consequently WUE increase as it was seen in herbaceous (*Papaverum somniferum* (Mahdavi-Damghani et al., 2010), *Helianthus annum* (Fereres et al., 2014), *Solanum esculentum* (Savić et al., 2008) and woody plants [*Olea europea* (Fereres et al., 2014), *Pistacia vera* (Iniesta et al., 2008)]. In our experiment, split showed higher WUE than unsplit application, independently by the volume irrigation. This behavior is due to the differences occurred during the experiment in terms of root and leaves dry weight accumulation.

Differently to the irrigation volume treatments, split application treatments affect this rate with higher values than those of the unsplit treatments. Two different Pn/Gs rate behavior were observed between split and unsplit application (Fig. 6). Under unsplit conditions, the lower and constant rate Pn/Gs showed that other factors likely affected the photosynthetic process. Probably, under these conditions, a water deficit condition did not allow for the photo restoration of PSII proteins. This was confirmed by the Pn/Gs higher values present in the split treatments during the last hours of the daytime.

The different relation between photosynthesis and stomatal conductance among the treatments, evidence a different strategy of water deficit avoidance actuated by plants depending on the stress level. This difference can be due to the occurrence of a water deficit adaptation in split irrigation treated plants. Indeed, no differences were measured in terms of RWC (data not shown) among the treatments, but different patterns were registered in terms of WPs and stomatal conductance. Different water potential regulation can be adopted by plants as a survival strategy to drought conditions (McDowell et al., 2008). Similarly to other anisohydric species (Cistus, Myrtus and Olea), also in this case a variation of WP occurs with no other adaptations (Tardieu & Simonneau 1998, McDowell et al., 2008; Quero et al., 2011).

Callistemon showed a typical anisohydric species relation of the ratio Gs/WPs. In these species, transpiration is not tightly regulated by stomatal closure. Under deficit irrigations, the stomatal conductance reduction had a big impact on photosynthetic rate. This is because plants need to drain the exceed of photon energy linked to the reduction of photosynthetic activity. During these stress conditions (Low conductance, low water availability, low photosynthesis) this ‘impact’ is represented by the photo damage of the PSII as consequence to the exceeding and not safely dissipated energy that cause changes in the functional state of the thylakoid membranes of the chloroplasts. The negative effects on photosynthesis due to this morphological adaptation, can be quantified in the leaves, estimating the inhibition or damage in the process of electron transfer in photosystem II (fluorescence) (Moura dos Santos et al., 2013). This characteristic of anisohydric plants explains the low differences in terms of biomass accumulation between the treatments because a very low reduction in terms of water availability occurred. During this low reduction of water amount, the photosynthetic reduction is primarily linked to the stomatal regulation; however this relation decreases when deficit level increases and when another biochemical regulation occurs (down-regulation) Flexas & Medrano (2002).

CONCLUSIONS

Our results evidence that, in Callistemon potted plants, a 25% irrigation volume reduction is possible. A full irrigation volume is 10.8 L per plant during the trial period (90 day) while the reduced volume is 8.2 L per plant.

The water reduction produced a plant biomass reduction, but split application improved performance growth and water use efficiency. Indeed, the plant response to reduced irrigation with split irrigation (RS) was comparable to the response of plant treated with full unsplit (FU) irrigation. Therefore, an increased water productivity can be obtained if the daily water requirement is split on two applications during the daytime. Deficit irrigation strategy for nursery Callistemon pot plants production can be used if water reduction is combined with split irrigation strategies reducing the environmental impact of the production process. A further confirmation of this hypothesis could come from the study of the plant ABA accumulation during the daytime.

ACKNOWLEDGEMENTS. This research was supported by the National Operational Program 'Research and Competitiveness' 2007–2013 PON 'R&C' – Italian Ministery of Education, University and Research. We thank Ignazia Altobello and Domenico Platia for technical assistance.

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