Running title: Response of callistemon submitted to saline and/or water deficit

Title: Comparison of individual and combined effects of salinity and deficit irrigation on physiological, nutritional and ornamental aspects of tolerance in *Callistemon laevis* plants

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Summary

The effect of water deficit, salinity and both applied simultaneously on several physiological and morphological parameters in the ornamental plant *Callistemon laevis* was studied to identify the tolerance mechanisms developed by this species to these sources of stress and to evaluate their adaptability to such conditions. *C. laevis* plants were grown in pots outdoors and subjected to four irrigation treatments lasting ten months: control (0.8 dS m^{-1}, 100% water holding capacity), water deficit (0.8 dS m^{-1}, 50% of the amount of water supplied in control), saline (4.0 dS m^{-1}, same amount of water supplied as control) and saline water deficit (4.0 dS m^{-1}, 50% of the water supplied in the control). Water and saline stress when applied individually led to a reduction of 12 and 39% of total biomass, respectively, while overall plant quality (leaf color and flowering) was unaffected. However, saline water deficit affected leaf color and flowering and induced an excessive decrease of growth (68%) due to leaf tissue dehydration and a high leaf Cl and Na concentration. Biomass partitioning depended no only on the amount of water applied, but also on the electrical conductivity of the water. Water stress induced active osmotic adjustment and decreased leaf tissue elasticity. Although both Na and Cl concentrations in the plant tissues increased with salinity, Cl entry through the roots was more restricted. In plants submitted to salinity individually, Na tended to remain in the roots and stems, and little reached the leaves. However, plants simultaneously submitted to water and saline stress were not able to retain this ion in the woody parts. The decrease in stomatal conductance and photosynthesis was more marked in the plants submitted to both stresses, the effect of which decreased photosynthesis, and this together with membrane damage, could delay plant recovery. The results show that the combination of deficit irrigation and salinity in *C. laevis* is not recommended since it magnifies the adverse effects of either when applied individually.

Keywords: Gas exchange; Ion uptake; Ornamental potted plant; Water relations; Water use efficiency; Elastic modulus.

***Abbreviations*:** C, control; C*, chroma; DW, dry weight; EC, electrical conductivity; g_s, stomatal conductance; h^o, hue angle; J, absorption rate of ions by the root system; L*, lightness; P, significance; P_n, net photosynthesis rate; P-V, pressure-volume; RWC_{tpl}, relative water content at turgor loss point ; S, saline treatment; W, deficit irriation treatment; WUE, water use efficiency of production; W+S, simultaneous saline and water stress treatment.; Ψ_l, leaf water potential; Ψ_s, stem water potential; Ψ_{100s}, leaf osmotic potential at full turgor; Ψ_{tpl}, leaf water potential at turgor loss point. ε; bulk modulus of elasticity.

Introduction

Salinity and drought are the major constraints affecting physiological processes and their effect may have severe consequences for plant growth and survival in semiarid regions (Vilagrosa et al., 2003; Álvarez et al., 2012).Therefore, in these regions it is important to consider the use of salt and drought-tolerant species for gardening or landcaping (Slama et al,. 2007; Razzaghi et al., 2012). Drought and salt tolerance in plants may be explained by functional and structural adaptations, such as growth regulations, osmotic adjustment, regulation of stomatal conductance, changes in cell wall elasticity, mineral nutritional and hormone balance all of which may help alleviate the harmful effects of both stresses (Zheng et al., 2010, Suárez, 2011). However, although salinity and drought stress are physiologically related and some of the tolerance mechanisms overlap, other aspects of plant physiology and metabolism may differ it the plant experiences saline and water individually or both stresses simultaneously (Sucre and Suárez, 2011). In relation to the comparative physiological processes in saline versus drought, both stresses reduce the ability to take up water, but when high Na^+ and Cl^- concentrations are present in the irrigation water, ion toxicities (associated with an excessive Na and Cl uptake and/or transport to aerial parts of the plant) and nutritional deficiencies may arise because of competition between cations or anions, depending upon the composition of the saline solution (Acosta-Motos et al., 2014).

Water stress and salinity often occur simultaneously in arid regions because, as soils dry, the salts become concentrated in the remaining soil solution (Munns, 2002; Chaves et al., 2009). The ability to overcome multiple and simultaneously stresses is of great importance for plant growth and survival in a stressful environment (Slama et al., 2008; Glenn et al., 2012). Numerous studies have investigated drought and salt stress separately but fewer have examined their interactions. Several studies have demonstrated that the molecular and metabolic responses of plants to the combination of drought and salinity are unique and cannot be directly extrapolated from the corresponding response of plants to either when applied individually (Suzuki et al., 2005; Mittler, 2006). The response by plants is more complicated than a simple additive effect of these two stress factors (Brown et al., 2006; Glenn et al., 2012). Drought may magnify the adverse effects of salinity and, when combined with salinity may interfere with nutrient accumulation, contributing to further growth inhibition or even reduce plant survival (Brown et al., 2006; Slama et al., 2008). Moreover, some studies have shown that the interaction of saline and water stress strongly reduces the capacity of plants to recover the water and carbon balance even after stress alleviation compared with plants subjected to a single

source of stress (Onami and Hammes, 2006; Pérez-Pérez et al., 2007). By contrast, other studies have found that the addition of salt to plants subjected to a water-deficit stress ameliorates the negative effects of deficit irrigation, improves the plants' ability to cope with water stress and enhances drought tolerance. For examples, Martinez et al. (2003; 2005), Glenn et al. (2012) and Alla et al. (2011), demonstrated that salinity actually has a protective effect on biomass production in a variety of species deficit-irrigated with saline water, and Sucre and Suárez (2011) reported that the water and carbon balance were enhanced when both stresses were applied simultaneously. Similarly, other studies have shown that the physiology of plants affected by a combination of salinity and drought is less altered than in the case of plants affected by drought only and that plant survival is enhanced (Glenn and Brown, 1998; Pérez-Pérez et al., 2007). While most studies have been conducted in halophytes plants, it is also important to investigate the physiology of salt and drought tolerance in non-halophytes species, to understand the limits and trade-offs between drought and salt tolerance, and the traits that are associated with tolerance to both factors. Indeed, plant salt tolerance differs significantly between species: halophytes are able to complete their life cycle in 200 mM NaCl or more while some non-halophytes can be injured by one tenth these salt concentrations (Cassaniti et al., 2009).

Among Australian ornamental plants, one of the most widely used genera is *Callistemon*, which includes several species with interesting ornamental features (Mitchen, 1993). In Europe, the most widely used *Callistemon* species are *C. citrinus* Skeels and *C. laevis* Anon, both with a great potential for urban landscaping and gardening due to their good tolerance to environmental stresses (Mugnai et al., 2009). In *Callistemon citrinus,* the effect of drought and salinity on physiological and morphological parameters and the mechanism this species uses to confront both sources of stress have been well established by Álvarez and Sánchez-Blanco (2013; 2014), *C. citrinus* being seen particularly salt and drought tolerant. In these studies it has been reported that both salinity and soil drying led to reductions in dry matter accumulation (a similar slight reduction of 16% of total biomass), stomatal conductance and transpiration and improvements in water use efficiency and root system, while overall quality are unaffected. In addition, salinity induced a slight osmotic adjustment and root storage of Na and Cl. It was accompanied by reductions in photosynthesis and intrinsic water use efficiency due to the cumulative effect of irrigating with saline water (Álvarez and Sánchez-Blanco, 2014). However, it has been documented that the degree of response to salt stress may vary considerably within a genus (Sánchez-Blanco et al., 2002; Lippi et al., 2003). Indeed, Vernieri et al. (2006) found that *Callistemon laevis* appeared to be moderately tolerant to water stress, but less resistant to salt stress, at least using irrigation water of 23 dS m^{-1} (200 mM NaCl). It is well known that plant responses to

salt, besides species-dependent, also depend on the length of exposure and the severity of the salt treatment (electrical conductivity (EC) of the saline water used). Both factors must be considered when saline water is used for irrigation water, as the interaction of both parameters will determine the physiological and molecular changes that take place. Since the growing season also seems to affect the response of shrubs to salt (Valdez-Aguilar et al., 2011), the research described in this paper was carried out over a period of 10 months in *C. laevis* using a salt level (4 dS m^{-1}) similar to that of the irrigation water commonly applied in the Mediterranean horticultural sector (nurseries, growers, gardeners; Pedrero et al., 2010; Álvarez and Sánchez-Blanco, 2014). Moreover, no studies have evaluated the effects of both stresses applied simultaneously to *C. laevis* plants and the mechanism involved during a combination of salinity and drought require more study (Martinez et al., 2003; Pérez-Pérez et al., 2007; Slama et al., 2008; Sucre and Suárez, 2011).

Based on the discussion above, it was hypothesized that combined salinity and deficit irrigation may interfere with nutrient uptake and may therefore modify the tolerance mechanisms developed by this species to confront both sources of stress, enhancing or minimizing their drought and salt tolerance compared with plants subjected to a single source of stress. Consequently, the primary aim of our investigation was to quantify the long-term effects on growth, ion uptake, water relations and the parameters obtained by pressure-volume analysis in plants of *C. laevis* exposed to both saline and water stress and to throw light on the mechanisms the plants use to confront the same. Knowledge of the salt and drought response of ornamental plants may help the horticultural sector (growers and gardeners) to select species that are tolerant to salt and/or water stress, while maintaining an acceptable appearance. The results can also be of great interest for planning irrigation strategies in the Mediterranean area, where low quality waters are very often used in deficit irrigation strategies.

Materials and methods

Plant material and experimental conditions

Rooted cuttings of 2-year-old *Callistemon laevis* Anon grown in 14 x 12 cm pots by a specialised nursery were transplanted into 5 L plastic pots (20 x 16 cm) filled with an 8:7:1 (v/v/v) mixture of coconut fibre:black + sphagnum perlite, amended with 2 g L^{-1} of Osmotocote Plus (14:13:13 N, P, K plus microelements). Plants were placed outdoors in a plot at the CEBAS-CSIC experimental station in Santomera, Murcia, Spain (38º06´N, 1º02´W, 110 m a.s.l.). All the plants were watered daily for 4 weeks to field capacity prior to starting the treatments. The micro-climatic conditions, registered with an automatic weather station located about 50 m

from the experimental site were 13.6 °C (mean minimum), 24.9 °C (mean maximum), and 18.7 °C (average) temperature; and 1.02 Kpa (average) vapour pressure deficit. Additional information about evolution of the daily mean values of air temperature and vapour pressure deficit recorded during the experimental period is detailed in Figure S1.

Treatments

Callistemon laevis plants were subjected to four irrigation treatments (40 plants per treatment) lasting 10 months using a computer-controlled drip irrigation system. The irrigation treatments consisted of a control (C) corresponding to 100% water holding capacity (leaching 15 % (v/v) of the applied water), where the electrical conductivity of the water was 0.8 dS m^{-1}, a deficit irrigation treatment (W) 50% of the control level of irrigation water,1 dS m^{-1}, a saline treatment (S) using tap water with salt added to reach 44 mM NaCl (4.0 dS m^{-1}) and plants subjected to saline and water stress simultaneously (W+S). One drip nozzle, delivering 2 L h^{-1} per plant, 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, which depended on the treatment and on climate conditions. All the plants were irrigated daily.

Growth and plant water measurements

At the beginning and at the end of the treatment period ten plants per treatment were separated into leaves, stems and roots and were then oven-dried al 80ºC until they reached a constant weight to measure the respective dry weights (DW). Leaf area was determined in the same plants, using a leaf area meter (Delta-T Devices Ltd., Cambridge, UK). At the beginning and at the end of the experimental period, the ten plants per treatment (separated into leaves, stem and roots) were washed with distilled water and dried at 80ºC, before being stored at room temperature for inorganic solute analyses. The concentration of Cl^- was analysed by chloride analyzer (Chloride Analyser Model 926, Sherwood Scientific Ltd.) in the aqueous extracts obtained when mixing 100 mg of dry vegetable powder with 40 ml of water before shaking for 30 min and filtering. The concentrations of Na^+, K^+ and Ca^{2+} were determined in a digestion extract with HNO_3:$HClO_4$ (2:1, v/v) by Inductively Coupled Plasma optical emission spectrometer (ICP-OES IRIS INTREPID II XDL Thermo, England). The absorption rate of Na^+ and Cl^- ions by the root system (J) was calculated by considering the total salt content of ten plants per treatment at harvest and expressed as mmol Na^+ and Cl^-, and the mean root DW, using the formula described by Pitman (1975).

$$J = \frac{(M_2 - M_1)}{(WR \times t)}$$

where M_1 and M_2 correspond to a concentration in mmol of Na^+ or Cl^- in the total plant at the beginning and at the end of experimental period, respectively, t corresponds to time in days and WR is the logarithmic mean root biomass, calculated as $\frac{WR_2 - WR_1}{Ln\left(\frac{WR_2}{WR_1}\right)}$ with WR_1 and WR_2 are the root DW at the beginning and at the end of experimental period respectively.

Throughout the experiment, plant height and number of inflorescences per plant were measured periodically in 30 plants per treatment. To assess the compactness of the plants, the ratio of leaf area to plant height was calculated in 10 plants per treatment at the end of experimental period by dividing leaf area by the respective plant heights. 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^o) (McGuire, 1992), using three leaves for each plant and seven plants per treatment. 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 with ten replicates per treatment in mature leaves, according to the method described by Lafuente et al. (1991).

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. Estimates of the bulk modulus of elasticity (ε), leaf osmotic potential at full turgor (Ψ_{100s}), leaf water potential at turgor loss point (Ψ_{tlp}) and relative water content at turgor loss point (RWC_{tlp}) were obtained at the end of the different irrigation treatments in three leaves per plant and five plants per treatment, via pressure-volume (P-V) analysis of leaves, as outlined by Wilson et al. (1979).

Statistical analyses of data

In the experiment 40 plants were randomly attributed to each treatment. The data were analyzed by one-way ANOVA using SPSS 17.0 software (SPSS Inc., 2002). 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. Statistical comparisons were considered significant at $P \leq 0.05$.

Results

Plant growth and ornamental parameters

At the end of the experimental period, while leaf growth (leaf DW and leaf area) was the only biomass parameter reduced in plants submitted to water deficit stress alone (W) compared with control plants, all the biomass production parameters (DWs of all organs and leaf area) were significantly reduced in plants submitted to salinity, whether alone or combined with drought stress (S and W+S), the most pronounced effect being observed for the combined water-deficit and saline stress treatment (Table 1). At the end of the experimental period the reductions in total DW were around 12, 39 and 68% for the W, S and W+S treatments, respectively, compared with the control. As regard root to shoot ratio, no differences between the control and S treatment were observed, but higher root to shoot ratios were found in plants grown under water deficit (with or without salt) compared with control plants, especially in plants submitted to water-deficit alone (W), (Table 1). Throughout the experiment, plant height was similar in the control and saline (S) treatment, (Fig. 1a), but began to be inhibited 16 weeks after the beginning of the deficit irrigation. At the end of the experiment the reductions in plant height were around 8 and 16% for W and W+S, respectively, compared with the control. Salinity, with or without water deficit, reduced the leaf area/height ratio compared with control, especially under the combination of salinity and drought, while this ratio was not modified when water stress was applied separately (Table 1). Drought and salinity had opposite effects on water use efficiency (WUE) (Fig 2). When separately applied water deficit led to an increase in WUE in all parts of the plant (leaves, stem and root) and salinity when separately applied led to a decrease in WUE compared with control. In plants simultaneously submitted to both constraints, the decrease in WUE due to salinity was balanced by the increase in stem and root due to water deficit, although the effect of both factors were not additive in leaf WUE. Reductions in this parameter in plants submitted to the combined effects of water deficit stress and salinity were similar to those found in plants submitted to salt stress alone (Fig 2).

Plants simultaneously submitted to water and saline stress (W+S) had a reduced number of inflorescences per plant compared with control, and were the plants with the lowest number of inflorescences per plant throughout the experiment (Fig. 1b). However, this parameter was not affected compared with control when water stress was applied separately. Salinity when separately applied (S) decreased the number of inflorescences per plant (flowering intensity) in the first 28 weeks of treatments, but the same plants had a higher number of inflorescences per plant than control plants at the end of the experimental period (November- December), when the greatest intensity of flowering occurred. Moreover, salt treated plants, with or without water deficit, showed a certain delay in reaching the maximum compared with control. Leaf colour was not affected when salinity and deficit irrigation were individually applied (Table 2). In contrast, when both stresses were applied simultaneously, L* increased and hue angle decreased, i.e. foliage colour in the W+S plants became more yellow and less dark green than control. Membrane damage, assessed by ion leakage was no affected by salinity or water deficit when applied individually (Table 2). However, this parameter increased compared with control when plants were simultaneously submitted to both constraints.

Mineral distributions throughout the plant

Salinity, whether alone or combined with deficit irrigation, increased the rates of both Na^+ and Cl^- absorption by roots (J) compared with the C and W treatments (Fig. 3a). In C and W treatments, the rate of Cl- absorption by roots was almost triple the corresponding rate of Na absorption, while in both saline treatments the rates of absorption of both ions were similar (Fig. 3a). The ability of *C.laevis* to restrict the entry of Na or Cl through the roots was investigated by calculating the slope of the linear regression between the increasing Na and Cl concentrations in the water and their relative absorption rate by the root system (Fig. 3b). In both saline treatments, the absorption rates of Na showed a higher slope than Cl, which means that *C. laevis* plants are able to restrict Cl^- uptake by roots to a greater extent than Na. The Na^+, Cl^-, K^+ and Ca^{2+} concentrations measured in leaves, stems and roots at the end of experimental period are presented in Table 3. While no accumulation of Cl^- and Na^+ was observed in the plants subjected to water stress treatment (W), the concentrations of both ions increased with salinity in all parts of the plants, except the roots of S plants, where only Na was seen to accumulate. Salinity combined with drought increased the Cl concentration in all parts of the plant and the Na concentration in leaves compared to salinity alone (S plants). The K^+ concentration increased in the leaves W+S plants (up to 40 %), whereas a 20% of decrease was observed in the stem of S plants. As regard Ca^{2+}, its concentration increased by 23 % in leaves and roots of W+S plants. In addition, plants of both saline treatments decreased the K^+/Na^+ and Ca^{2+}/Na^+ ratios in all parts of the plants.

The tendency of *C. laevis* plants to accumulate Na and Cl preferentially in a given part of the plant (leaves, stem or roots) was investigated by calculating the slope of the linear regression between the Na^+ and Cl^- concentration in plant tissue and their relative concentrations in the irrigation water (Table 4). As regards Na^+ distribution in plants irrigated with water of low EC, regardless of the amount of water (C and W), the lowest values for Na were found in leaves and the highest in its roots (Table 3). In S plants, the accumulation of Na in the root system and, especially the stem showed a higher slope compared with the leaves, while in the case of W+S plants higher slopes were found for leaves and stem compared with the root system (Table 4). Plants of the W+S treatment increased their leaf Na concentration sharply, reaching a value six-fold higher than in control plants, so that no significant differences in Na concentration between tissues were found in W+S plants (Table 3). In the case of Cl^-, plants irrigated without added salt (C and W), showed similar values in all organs, leaves, stem and root (Table 3), while under saline conditions higher slopes were found for leaves and stem, compared to the root system, especially in W+S plants (Table 4). This means that the transport of Na ion from the roots to the leaves was only restricted in S plants and that the distribution of each toxic ion differed.

Plant water relations and gas exchange

Parameters derived from the pressure–volume curves are shown in Table 5. At the endof experimental period, leaf osmotic potential values at full turgor (Ψ_{100s}) decreased in plants under stress conditions (W, S and S+W), especially in W (when plants were irrigated without salt), which was indicative of the osmotic adjustment that occurred due to the irrigation. The difference between the values obtained in the control and stressed plants were taken as an estimate of this adjustment (1.0 MPa, 0.4 and 0.5 MPa) for W, S and W+S treatments, respectively). In these treatments, the point of zero turgor (Ψ_{tlp}) also occurred at lower values than in the control. The relative water content at the turgor loss point (RWC_{tlp}) was only affected by deficit irrigation applied independently. In this treatment (W) the point of zero turgor (RWC_{tlp}) occurred at a lower relative water content (78.8%) than in the control (90.9%). The bulk modulus of elasticity (ε) increased in the deficit irrigation (W) treatment compared with control and was not affected by salinity.

The seasonal values of the leaf water potential at midday (Ψ_l) showed greater variability than stem water potential (Ψ_s) (Fig. 4a). Indeed, (Ψ_l) fluctuated strongly throughout the experimental period in all plants, and showed minimum values in summer and maximum values in winter, which was closely related with environmental factors. Leaf and stem water potential values were in general higher in the control than in the rest of the treatments, although Ψ_s showed greater significant differences between treatments because the

standard error for Ψ_s was lower than for Ψ_l. Significant differences in Ψ_s levels among treatments were noted from the outset of the experiment, while differences in Ψ_l were observed occasionally. In contrast, Ψ_s decreased significantly in all stress treatments compared with the control, especially when the two constraints were combined in summer. For each treatment, Ψ_s showed less negative values that those found for Ψ_l. The maximum and minimum differences between Ψ_s and Ψ_l measured simultaneously in the same plant coincided with the maximum and minimum g_s values, respectively (Fig. 4b and 5a).

The values of the stomatal conductance (g_s) and photosynthesis net rate (P_n) during the period can be seen in Fig. 5. The seasonal pattern of g_s consisted of a summer decrease with respect to the control in all stressed plants, particularly in the plants submitted to salt in combination with water deficit (Fig 5 a). The value of g_s fell later in the S treatment than in the both water stress treatments. Such reductions with respect to the control plants were also observed in photosynthesis levels in plants subjected to both salt stress treatments, but W treatment did not reduce photosynthesis levels with respect to the control plants during the experiment (Fig. 5b). W+ S plants had lower P_n and g_s values than the rest of the treatments during most of the experiment, except in winter (at the end of the experimental period), when the lowest P_n and g_s values occurred in all plants, regardless of the irrigation treatment, confirming that plants are most sensitive to gas exchange parameters at this time. In general, the plants of the water stress treatments, whether alone or combined with salinity showed higher P_n/g_s ratios (intrinsic water use efficiency) (Fig. 5c) than control plants throughout the experimental period, except at the end of the experiment when these differences between treatments disappeared. Such increases with respect to the control were longer and more marked under W than under W+S. No differences were found between control and S as regards intrinsic water use efficiency during the experimental period, as P_n and g_s were proportionally reduced compared with control.

Discussion

At the end of our experiment biomass accumulation in *Callistemon laevis* plants was more affected by salinity than by deficit irrigation. However, in previous studies in *Callistemon citrinus* with the same duration and level of water and salt stress used in our trial (Álvarez and Sánchez-Blanco, 2013; 2014), both salinity and soil drying similarly led to a small degree of growth reduction, which confirms the differences between species, even within the same genus, and points to the higher relative salt sensitivity of *C. laevis* compared with *C. citrinus*. The response of species to stresses in terms of growth is the ultimate expression of several interacting physiological and biochemical parameters and has often been used to characterise salt or water

deficit tolerance (Sidari et al., 2008; Cassaniti et al., 2009). Furthermore, the effects of the two factors were additive in the case of biomass parameters, as plans submitted to the combined effects of water-deficit stress and salinity showed the lowest DW values (Glenn et al., 2012). The different stresses separately or in combination applied in our experiment induced different growth responses in *C. laevis*, meaning that the kind of stress and their combination must be considered an important aspect when saline water and/or deficit irrigation is used as an irrigation strategy without reducing quality in ornamental species.

However, unlike biomass, saline water irrigation individually had no effect on *C. laevis* plant height during the experiment, while both deficit irrigation treatments led to the smallest plants. In *C. laevis*, height was more sensitive to soil drying than to salinity. The differences between the response of biomass accumulation and plant height to salt and water stress are due to the fact that the plant growth reduction in deficit irrigation conditions is less pronounced than in saline conditions, although it occurs earlier (Álvarez and Sánchez-Blanco, 2014). It might also be explained by differences in the time taken by salt to affect each parameter; one of the earliest effects of osmotic stress due to salt is a reduction in cell expansion in young leaves, a biomass parameter that is affected earlier than others like plant size or height (Munns and Tester, 2008). Salts take time to accumulate inside plants before the concentrations reach toxic levels and affect plant function (Munns and Tester, 2008). Ionic stress affects growth much later than osmotic stress, when salt reaches toxic concentrations in the old leaves. Although by the end of experiment salinity had inhibited growth in *C. laevis* more than deficit irrigation, such a reduction was only noticeable at a long time after the beginning of the treatments, confirming that the duration of the salt stress is also an important factor.

Aesthetically and commercially, an increase in foliage size in relation to plant height gives the plant a compactness and architectural equilibrium that are much appreciated by customers (Álvarez et al., 2013). Deficit irrigation and salinity has the potential to improve crop quality by promoting a more compact habit, as previously reported for other ornamental species (Álvarez et al., 2013; Acosta-Motos et al., 2014). However, in our conditions salinity decreased the relationship between leaf area and plant height, which would lowered the commercial value of plants. This is one the negative aspects of salinity, especially when combined with deficit irrigation.

As regards biomass partitioning, drought exposed plants in our study reduced their aerial more than root growth, resulting in an increased root to shoot ratio, which did not occur in salt stressed plants. The effect of drought stress is usually greater on shoot growth than on root growth (Bacelar et al., 2007; Chyliński et al., 2007; Navarro et al., 2009). This behaviour is considered a criterion of plant adaptation to drought and could

promote a more rapid establishment of ornamental plants in gardening or landscaping (Franco et al., 2006; 2011). Also consistent with our findings, Álvarez et al. (2012) reported that salt stress salt did not modify this ratio in *P. purpurea* plants watered with a NaCl solution with the same EC (4 dS m^{-1}). Nevertheless, this ratio does not always remains unaltered as a result of salt stress and has been seen to increase in other ornamental species grown in similar conditions such as *E. japonica* (Gómez-Bellot et al., 2013) and *C. citrinus* plants (Álvarez and Sánchez-Blanco, 2013). The absence and presence of increased ratios in both species could also be indicative of the higher relative tolerance to saline stress of *C. citrinus* compared to *C. laevis*. The different distribution of biomass induced by both stress situations may be due to the need to maintain a higher root surface area under drought conditions and the need to reduce root volume in plants exposed to salinity, which may be a favourable trait limiting their capacity to accumulate toxic ions in the shoot (Munns, 2002; Alarcón et al., 2006; Munns and Tester, 2008). Slama et al. (2008) also reported that the preferential biomass allocation to roots of plants submitted to deficit irrigation alone is lowered when salt is present in the irrigation water used, which indicates than this ratio does not only depends on the amount of water applied, but also depends on the EC of the water.

The floriculture market appreciates plants with leaves of intense colour, a high root to shoot ratio and a certain relationship between plant height and leaf area. However, the attractiveness and commercial value of *C. laevis* is primarily associated with flowering. Plants subjected to water or saline stress may reduce flowering intensity, bring forward or delay flowering and shorten the same (Fornes et al., 2007; Cuevas et al., 2009; Bernal et al., 2011; Álvarez et al., 2012; Álvarez et al., 2013). In *Callistemon citrinus* plants irrigated with the same level of salinity as used in our trial and grown under similar conditions, no flowering reduction was observed (Álvarez and Sánchez-Blanco, 2014). Katerji et al. (2001) indicated that sensitivity to salinity was maximum during flowering, particularly during bud formation. Hence, the occasional reduction in flowering observed at the beginning of our experiment in *C. laevis* confirms the higher relative tolerance to saline stress of *C. citrinus* compared to *C. laevis*. According to Munns and Tester (2008), even moderate salinity stress affects reproductive development, such as earlier flowering or a reduced number of florets. In our case, the increased number of inflorescences at the end of the experiment in plants subjected to saline stress alone may have been due to earlier blooming rather than a higher intensity of flowering, as seen in many other ornamental species exposed to saline conditions (Zapryanova and Atanassova, 2009). *C. laevis* plants can cope with water shortage or saline irrigation with mo great loss of their ornamental value, as has been cited for other species (Henson et al., 2006; Álvarez et al., 2012). However flowering was markedly

affected by the combined effects of water and salt stress, and this is considered to be a negative aspect. The absence of significant changes in ion leakage in plants subjected to a single source of stress during the experimental period suggests that there was no membrane damage (Álvarez and Sánchez Blanco, 2013). However, membrane damage was observed in plants submitted to drought combined with salinity, when stress became more severe (Álvarez et al., 2011).

Common responses in species exposed to saline or drought stress are an increase in osmotic adjustment and changes in the cell wall elasticity, which result in the turgor loss point being reached at a lower leaf water potential and at a lower relative water content (Navarro et al., 2008; Zheng et al., 2010; Suárez, 2011). In our conditions, callistemon plants submitted to water deficit stress alone (W) showed osmotic adjustment and significant decreases in elasticity as a tolerance mechanism to drought in order to maintain turgor, as demonstrated in several ornamental species (Sánchez-Blanco et al., 2009; Álvarez et al., 2009, Álvarez et al., 2011). Under drought stress, the osmotic adjustment is mainly achieved through the accumulation of organic solutes synthesized by the plant, while in salt stress the accumulated solutes are mainly the inorganic ions (Na^+ and Cl^-) readily available in the soil solution (Parida and Das, 2005). The results of this study show that moderate salinity did not change cell wall elasticity of the shoot tissue in *C. laevis*, suggesting that elastic adjustment did not play a role in the adaptation mechanism, although some changes in the cell wall composition may have occurred, as the thickness and chemical composition of the cell wall, which could contribute to the decrease in growth recorded in this species in response to salt stress (Mustard, 2004; Sassi et al., 2010; Suárez, 2011).

The retention of either Na^+ and/or Cl^- in roots or leaves has been proposed as a trait related to salt tolerance in plants (Pérez-Alfocea et al., 2000). This ability to limit the transport of these ions to the shoots has also been observed in *C. citrinus*, which was able to accumulate both (Na and Cl) in the roots, a trait which could be related to its greater salt tolerance compared to *C. laevis* (Álvarez and Sánchez-Blanco, 2014). In a saline environment, controlling the salt concentration of the aerial parts of plants, restricting entry through the roots and limiting transport to the shoots (retaining these ions in the root and lower stem) is an important mechanism that allows plant survival and growth under salt stress conditions (Colmer et al., 2005). However, in our study such mechanisms did not prevent the over-accumulation of Na^+ and Cl^- in leaves of W+S plants, which probably contributed to the greater decrease in growth observed in these plants. Higher K^+ and Ca^{2+} concentrations were also observed in the leaves of W+S plants, which although did not avoid the excessive growth reduction, could have partially prevented leaf tissue dehydration (Slama et al 2008). In this sense K^+ and Ca^{2+} play an important

role in plant growth and development, but they are also key players in the maintenance of osmotic adjustment and cell turgor. In addition, lower K^+/Na^+ and Ca^{2+}/Na^+ ratios were observed in the plants irrigated with saline water, correlating with their lower biomass production (Acosta Motos et al., 2014).

Leaf water potential values below the value of Ψ_{tlp} were not found for plants at any sampling time during the experiment. The maintenance of turgor permits cell elongation, stomatal opening and other processes dependent on turgor pressure (Munns, 2002; Dichio et al., 2005). Thus, after osmotic adjustment was reached, W plants were able to maintain relatively high values of P_n and g_s, and only showed slight growth reduction (Sucre and Suárez, 2010). In contrast, the higher inhibition of growth under salt stress compared to water stress suggests that it was not associated with turgor loss (below Ψ_{tlp}) but with an inhibition of photosynthesis due to ionic toxicity and/or ion imbalance (Sucre and Suárez, 2011; Rodríguez et al., 2005). As far as plant water status is concerned, plants under saline or deficit irrigation exhibited slight dehydration throughout the experiment, as indicated by the lower water potential, due to less available substrate water and difficulty in taking up water from the substrate (Sánchez-Blanco et al., 2002; Álvarez et al., 2012). The most negative values of the Ψ_l and Ψ_s were found in W+S, as both salt accumulation and passive dehydration contributed to lowering the leaf water potential in these plants (Slama et al., 2008). According to Álvarez and Sánchez-Blanco (2013; 2014) stem water potential measured at midday is a good indicator of the stress resulting from deficit irrigation or salinity in *C. citrinus* plants due to the small variability observed between bagged leaves. The same behaviour was observed in our experiment with *C. laevis*, not only with salinity and water deficit separately but also when combined, as Ψ_s identified differences between treatments earlier than Ψ_l, which did so only when stress became more severe (Choné et al., 2001). Also, the difference between Ψ_s and Ψ_l measured simultaneously in the same plant has been shown to be an indicator of instantaneous shoot transpiration, which showed the maximum value in summer and minimum in winter coinciding with g_s behaviour (Choné et al., 2001; Álvarez and Sánchez-Blanco, 2013).

The decrease in g_s observed in our study suggested an adaptative and efficient control of transpiration by this species and represents a mechanism to cope with water and saline stress, especially during high transpiration periods: in the case of deficit irrigation plants by limiting water loss (Hessini et al., 2008) and in saline plants by reducing the salt load of leaves and helping to increase longevity by maintaining salts at subtoxic levels for longer than would occur if transpiration rates were not diminished (Koyro et al., 2006). The later reduction in g_s in saline plants than in deficit irrigation plants would be due to the salts taking time to accumulate inside plants before the concentrations reach toxic levels and affect plant functioning (Munns

and Tester, 2008). A decrease in P_n due to salinity stress has also been reported in many other plant species, such as *Viburnum tinus*, a salt-sensitive species (Bañón et al., 2012) or in *A. bettzickiana*, a salt-tolerant ornamental plant (Ali et al., 2012). P_n was also reduced in *C. citrinus* plants irrigated with the same salt level in otherwise similar experimental conditions (Álvarez and Sánchez Blanco, 2014), but this reduction occurred later than g_s reductions, which suggests that *C. citrinus* is more tolerant to saline stress than *C. laevis*, as mentioned above.

Differences in g_s between treatments do not seem to be followed by similar changes in P_n. In this sense, *C. laevis* plants submitted to both deficit irrigation treatments were able to increase their intrinsic water use efficiency (P_n/g_s), especially during the highest water demand period. When plants simultaneously experienced water and saline stress, the reduction in P_n and g_s was more pronounced and the observed increases in P_n/g_s were maintained for less time than in plants submitted to a single stress. This underlines the fact that the severe reduction of g_s that takes places in these conditions could have been one of the main causes of photosynthesis decline, although photoinhibition or increases in mesophyll resistance may have played a role later when stress becomes more prolonged and intense (Flexas et al., 2004; Pérez-Pérez et al., 2007; Sucre and Suárez, 2011). The results of this study are consistent with the finding of Álvarez and Sánchez-Blanco (2014) who reported in *C. citrinus* that intrinsic water use efficiency is sharply reduced at stomatal aperture (g_s) values lower than 100 mmol m^{-2} s^{-1}, and that if plants show g_s values below 100 mmol m^{-2} s^{-1} for long periods, non-stomatal limitations to P_n are predominant and could delay plant recovery or even cause permanent damage. As indicated in the results, WUE was higher in *C. laevis* plants growing in deficit irrigation conditions (W), a response that has been observed in numerous ornamental plants (Cameron et al., 2006; Jaleel et al., 2008; Álvarez et al., 2009; Mugnai et al., 2009; Álvarez et al., 2012).

In conclusion, our results indicate that although both deficit irrigation and saline water reduce plant growth in *Callistemon laevis,* the morphological and physiological responses differ significantly between salinity, water stress and their combined application. Deficit irrigation (W) and the use of saline water of moderate conductivity (S) were able to maintain a good overall quality of the ornamental plants and could be regarded as feasible for an irrigation management strategy in *C. laevis*. The tolerance of *C. laevis* to drought was related to its ability to adjust osmotic potential, to enhance leaf tissue rigidity and to modify leaf gas exchange, accompanied by an increased root to shoot ratio and water use efficiency, which are positive aspects of deficit irrigation. The salinity tolerance of *C. laevis* was related to slight osmotic adjustment, limited Cl uptake from the substrate and the higher Na concentration in roots compared to leaves. Though

Callistemon laevis appears to be particularly salt and drought tolerant, salinity combined with deficit irrigation magnifies the adverse effects of either when applied individually. Salinity combined with deficit irrigation is not recommended, since this treatment affected the quality of plants (reducing flowering and compactness and affecting leaf colour) and induced an excessive decrease in plant height and growth due to leaf tissue dehydration and the high concentration of Cl and Na accumulated in leaves.

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Table 1 Growth and biomass traits at the end of the experiment in *C. laevis* subjected to different irrigation treatments. Values are the mean of ten plants.

Parameter	C	W	S	W+S
Leaf DW (g plant^{-1})	44.0 d	35.7 c	25.7 b	12.6 a
Stem DW(g plant^{-1})	51.8 c	49.3 c	36.0 b	18.8 a
Root DW (g plant^{-1})	24.1 c	24.1 c	13.5 b	8.7 a
Leaf area (cm^2)	2868 d	2361 c	1810 b	654 a
Root to shoot ratio	0.54 a	0.72 c	0.52 a	0.67 b
Leaf area/ height	36.92 c	32.53 c	23.56 b	9.72 a

Means within a row without a common letter are significantly different by Duncan $_{0.05}$ test.

Table 2 Ion leakage and leaf color parameters at the end of the experiment in *C. laevis* subjected to different irrigation treatments. Values are the mean of ten (ion leakage) or seven (leaf color) plants.

Parameter	C	W	S	W+S
Lightness	45.4 a	45.1 a	43.9 a	48.2 b
Chroma	21.9 a	22.4 a	20.5 a	19.8 a
Hue angle	116.6 b	116.3 b	115.4 b	110.8 a
Ion leakage (%)	31.3 a	32.7 a	33.2 a	60.3 b

Means within a row without a common letter are significantly different by Duncan $_{0.05}$ test.

Table 3 Concentrations of Na^+, Cl^-, K^+ and Ca^{2+} at the end of experimental period in *C. laevis* subjected to different irrigation treatments. Values are the mean of ten plants.

(mmol kg^{-1} DW)		C	W	S	W+S
Na^+	Leaves	51.5 aA	33.9 aA	162.5 bA	296.7 c
	Stem	86.6 aB	72.9 aB	307.7 bB	328.4 b
	Root	193.2 bC	131.4 aC	352.6 cB	362.6 c
Cl^-	Leaves	200.0 a	173.5 a	294.9 bAB	465.1 c
	Stem	243.4 a	242.9 a	365.7 bB	467.4 c
	Root	218.8 a	213.4 a	231.8 aA	377.1 b
K^+	Leaves	197.9 a	163.2 a	159.1 a	278.9 b
	Stem	328.1 b	310.8 b	261.1 a	325.8 b
	Root	129.0 a	143.7 a	127.8 a	147.0 a
Ca^{2+}	Leaves	337.7 a	348.9 a	372.5 ab	416.7 b
	Stem	251.2 a	271.5 a	238.4 a	269.6 a
	Root	337.5 a	308.6 a	324.4 a	412.2 b

Means within a row without a common lower case letter are significantly different by Duncan $_{0.05}$ test. Means within a column and within an ion without a common capital letter are significantly different by Duncan $_{0.05}$ test.

Table 4 Slopes of the linear regressions between Na^+ and Cl^- concentration in the irrigation water and plant concentration at the end of the experimental period in *C. laevis* plants subjected to saline (S) and water saline treatment (W+S). Values are the mean of ten plants.

Ion	Part of the plant	S	W+S
Na^+	Leaf	3.07 aA	7.21 aB
	Stem	6.50 aC	7.11 aB
	Root	4.45 aB	4.74 aA
Cl^-	Leaf	2.79 aB	7.80 bB
	Stem	3.60 aB	6.59 bB
	Root	0.39 aA	4.66 bA

Means within a row without a common lower case letter are significantly different by Duncan $_{0.05}$ test. Means within a column and within an ion without a common capital letter are significantly different by Duncan $_{0.05}$ test.

Table 5 Parameters derived from the pressure–volume curves at the end of the experiment in *C. laevis* subjected to different irrigation treatments. Values are the mean of five plants.

Parameter	C	W	S	W+S
Ψ_{100s} (MPa)	-2.60 c	-3.55 a	-2.56 c	-3.13 b
Ψ_{tlp} (MPa)	-3.02 c	-4.28 a	-3.18 bc	-3.46 b
RWC $_{tlp}$ (%)	90.90 b	78.80 a	89.05 b	89.44 b
ε (MPa)	26.18 a	31.73 b	24.37 a	27.12 a
Transpiration	3.78 b	1.62 a	5.01 c	2.99 b

Means within a row without a common letter are significantly different by Duncan $_{0.05}$ test.

Figure captions

Fig. 1 Changes in plant height (a) and number of inflorescences per plant (b) in *C. laevis* plants submitted to different irrigation treatments. Values are means ± s.e., n = 30. Symbols represent the different treatments: Control (filled circles), water deficit (open circles), saline (filled triangles) and saline water deficit (open triangles)

Fig. 2 Water use efficiency of production (WUE) at the end of experimental period in *C. laevis* 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

Fig. 3 Absorption rate of Na^+ and Cl^- ions by the root system (J) at the end of the experimental period in *C. laevis* plants subjected to saline (S) and water saline treatment (W+S). Values are the mean of ten plants. Means within an ion without a common letter are significantly different by Duncan $_{0.05}$ test. The slopes of the linear regressions between Na^+ and Cl^- concentration in the irrigation water and absorption rate by the root system are shown in the bottom part of the figure

Fig. 4 Changes in the stem water potential (Ψ_s) and leave water potential (Ψ_l) (a) in *C. laevis* plants submitted to different irrigation treatments. Values are means ± s.e., n = 8. Symbols represent the different treatments: Control (filled circles), water deficit (open circles), saline (filled triangles) and saline water deficit (open triangles). Solid lines represent Ψ_l and dashed lines represent Ψ_s

Fig. 5 Changes in stomatal conductance (g_s, a), net photosynthesis rate (P_n; b) and intrinsic water use efficiency (P_n/g_s, c) in *C. laevis* plants submitted to different irrigation treatments. Values are means ± s.e., n = 8. Symbols represent the different treatments: Control (filled circles), water deficit (open circles), saline (filled triangles) and saline water deficit (open triangles)

Fig. S1 Changes in daily mean values of air temperature (T) and vapor pressure deficit (VPD) recorded during the experimental period

Fig.1

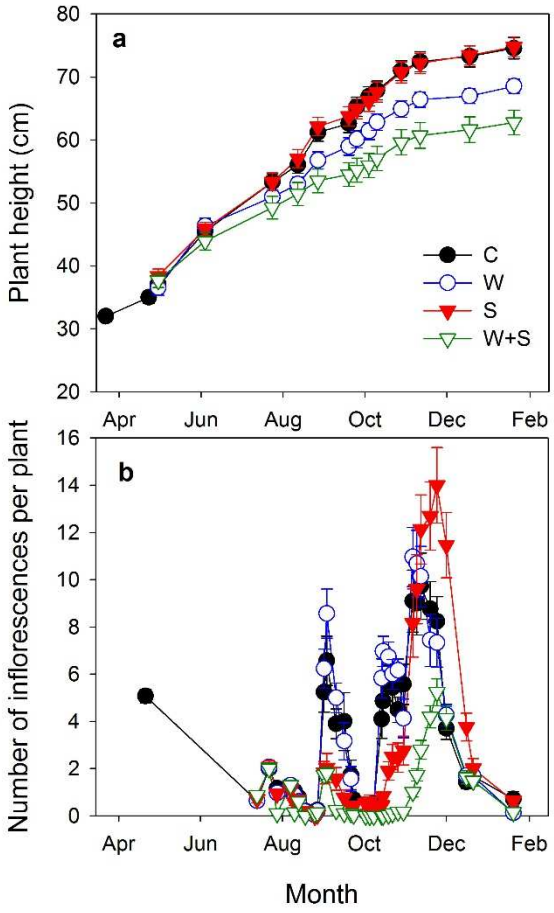

a
b
Plant height (cm)
Number of inflorescences per plant
Month
C
W
S
W+S
Apr
Jun
Aug
Oct
Dec
Feb

Fig.2

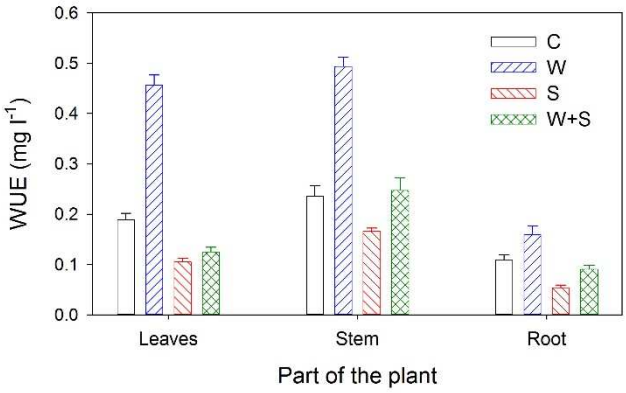
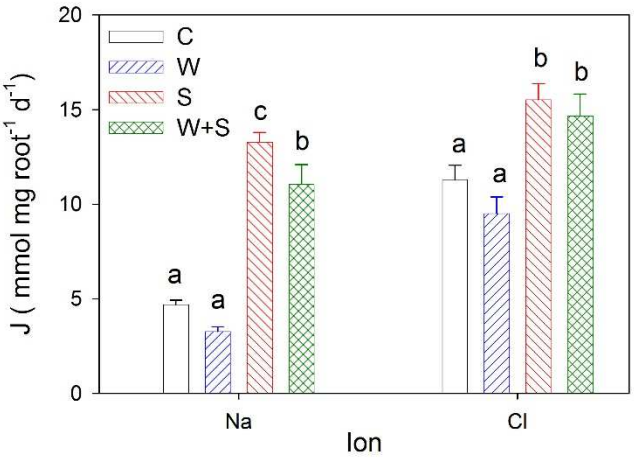

0.6
0.5
0.4
0.3
0.2
0.1
0.0
WUE (mg l-1)
C
W
S
W+S
Leaves
Stem
Root
Part of the plant

Fig.3



	S			W+S		
0.2528	±	0.0115	bA	0.1670	±	0.0313	aA
0.1247	±	0.0103	aB	0.0992	±	0.0167	aB

Fig.4

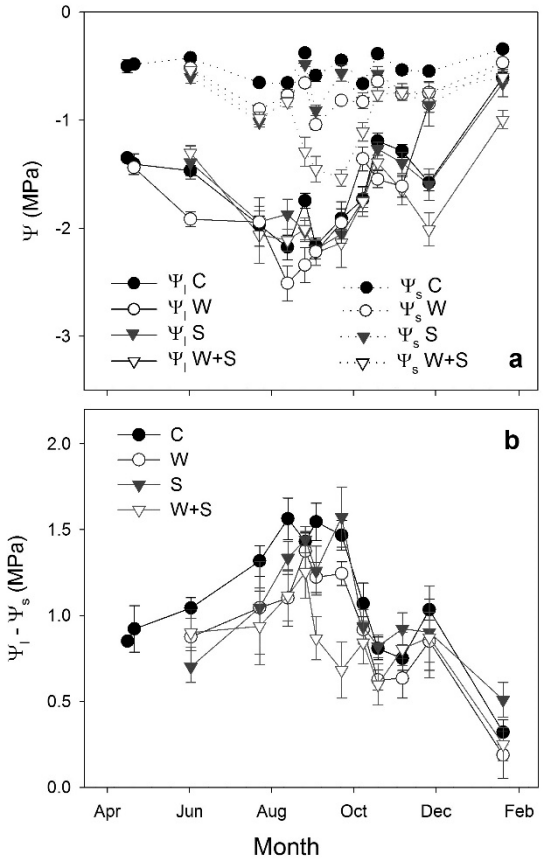

0
-1
-2
-3
Ψ (MPa)
Ψ_l C
Ψ_l W
Ψ_l S
Ψ_l W+S
Ψ_s C
Ψ_s W
Ψ_s S
Ψ_s W+S
a
2.0
1.5
1.0
0.5
0.0
Ψ_l - Ψ_s (MPa)
C
W
S
W+S
b
Apr
Jun
Aug
Oct
Dec
Feb
Month

Fig.5

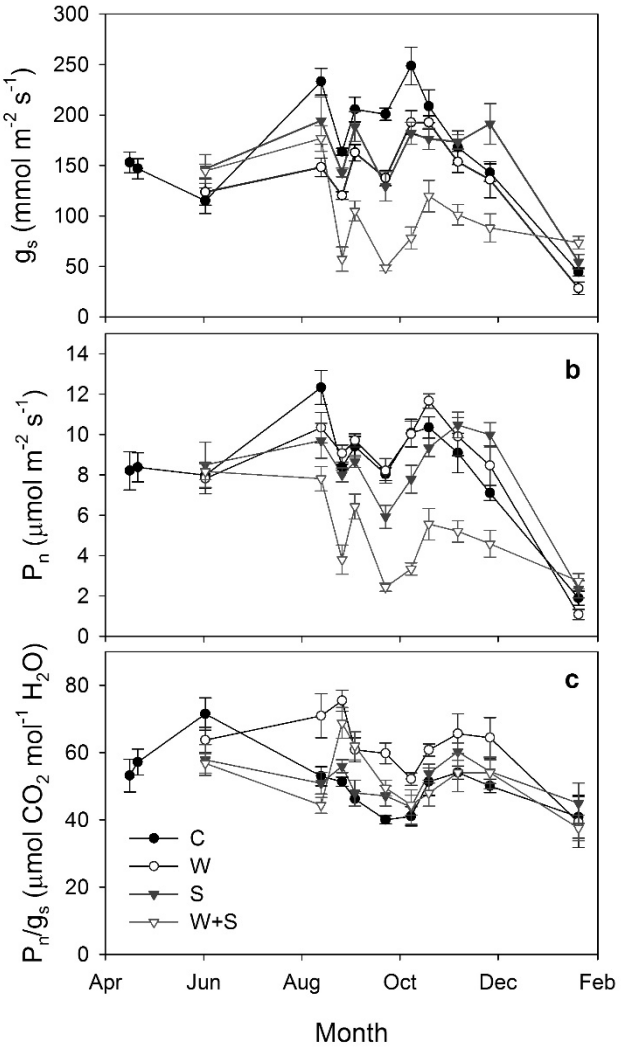
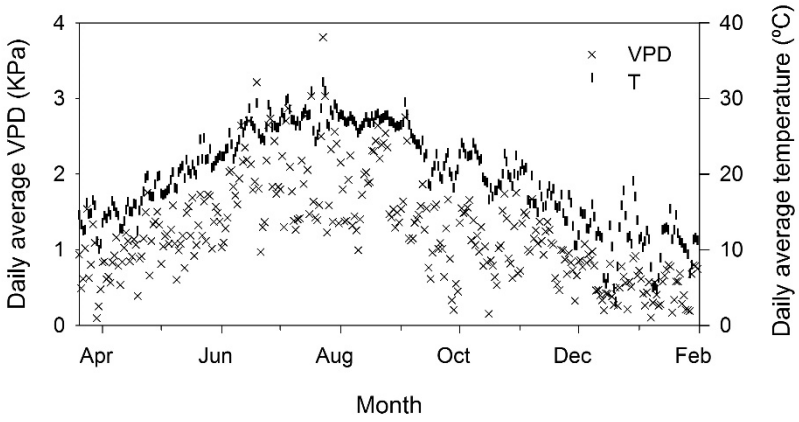

g_s (mmol m^{-2} s^{-1})
P_n (μmol m^{-2} s^{-1})
P_n/g_s (μmol CO_2 mol^{-1} H_2O)
b
c
C
W
S
W+S
Apr
Jun
Aug
Oct
Dec
Feb
Month

Fig. S1


Daily average VPD (KPa)
Daily average temperature (ºC)
VPD
T
Apr
Jun
Aug
Oct
Dec
Feb
Month
0
1
2
3
4
10
20
30
40