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Interactive Effects of Drought and Saline Aerosol Stress on Morphological and Physiological Characteristics of Two Ornamental Shrub Species

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Abstract: Effects of drought and aerosol stresses were studied in a factorial experiment based on a Randomized Complete Design with triplicates on two ornamental shrubs. Treatments consisted of four levels of water container (40%, 30%, 20%, and 10% of water volumetric content of the substrate) and, after 30 days from experiment onset, three aerosol treatments (distilled water and 50% and 100% salt sea water concentrations). The trial was contextually replicated on two species: *Callistemon citrinus* (Curtis) Skeels and *Viburnum tinus* L. 'Lucidum'. In both species, increasing drought stress negatively affected dry biomass, leaf area, net photosynthesis, chlorophyll *a* fluorescence, and relative water content. The added saline aerosol stress induced a further physiological water deficit in plants of both species, with more emphasis on *Callistemon*. The interaction between the two stress conditions was found to be additive for almost all the physiological parameters, resulting in enhanced damage on plants under stress combination. Total biomass, for effect of combined stresses, ranged from 120.1 to 86.4 g plant^{-1} in *Callistemon* and from 122.3 to 94.6 g plant^{-1} in *Viburnum*. The net photosynthesis in *Callistemon* declined by the 70% after 30 days in WC10% and by the 45% and 53% in WC20% and WC10% respectively after 60 days. In *Viburnum* plants, since the first measurement (7 days), a decrease of net photosynthesis was observed for the more stressed treatments (WC 20% and WC 10%), by 57%. The overall data suggested that *Viburnum* was more tolerant compared the *Callistemon* under the experimental conditions studied.

Keywords: *Callistemon citrinus* (Curtis) Skeels; *Viburnum tinus* L. 'Lucidum'; plant biomass; root/shoot ratio; gas exchange; relative water content

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1. Introduction

The Mediterranean environment is characterized by high summer temperatures often associated with shortage and poor quality of irrigation water. These conditions represent a limit for optimal plant growth and somehow even survival [1]. Along coastal areas, plants in many cases are also affected by salinity stress (soil salinity and spray aerosol) that represents a relevant restriction affecting distribution and survival of native plants [2]. The presence of simultaneous stresses (drought and saline aerosol), which have a cumulative negative effect on plant growth and survival [3]. Álvarez et al. [4] have investigated on drought and saline aerosol stress separately, but only a few studies report the effect of interaction between these stresses. These two abiotic stresses share common physiological, biochemical, and molecular responses in plants. Therefore, plants can undergo synergistic negative effects subjected to both stresses [5].

In coastal areas, the management of landscape and its ornamental value due to the expansion of tourist industry and, as a consequence, the growing of residential uses implies a large interest in green areas realization [6]. Since the end of XX century, tourism has grown by almost 75% in the Mediterranean coasts [7]), and projections, before the COVID-19 pandemic, showed a continuing increase in the number of tourists, until to reach 637 million by 2025 [8]. For these reasons the areas destined for both private gardens and public parks are increasing, due to the importance to have recreational areas near homes and hotels. Due to residential use of coastal area, the consumption of water resources has increased, and the irrigation of these area in the summer months for the metropolitan area of Barcelona (Spain), determines a consumption of approximately of 4.28 litres/m^2/day to watering the gardens; this water quantity represents about the 50% of the total domestic water consumption [9]. These quantities of water not always are available; for this reason the expansion of green areas, especially under suboptimal environmental conditions, has motivated number of studies to identify the most suitable species to be used in gardens [10] and their most suitable cultivation methods [11]. Many ornamental plants adopted in green areas present, at inter and intraspecific levels, relevant differences on response to stress conditions [12]. The interest in ornamental species choice is linked to individuate plants that are able to tolerate environmental stresses. The negative effects of abiotic stresses hamper important physiological functions [4] and damage organographic structure and, consequently, aesthetic features of plants.

In nature, all living organisms included plants are continuously exposed to different abiotic and biotic stresses, quite often overlapping at the same time. Abiotic stresses, like drought, salinity, floods, heat, and frost shock and other environmental extreme events, represent the main reasons of losses in plant growth and crop yield [13]. The combination of abiotic stresses, like drought and salinity, often occurs in climatic areas where warm summers are associated with lack of rain/irrigation water, especially along the coasts, resulting in severe yield losses compared to a single stress [14]. Current knowledge indicate that plants are able to manage with a number of overlapping biotic and abiotic stresses through exhibition of tailored responses, which can hardly be understood from data coming from results of a single independently imposed stress [15–17]. Facing these threats, plant resilience results are especially important when stresses could cause negative effects on plant growth and reproduction [18].

The response of plants to both salt and drought stress differs according to the different potentially usable species [19] and, sometimes, within the cultivars of the same species [20]. Salt stress in coastal areas, can occur both at the root and foliage level. At root level, the presence of high levels of ions in the irrigation water or soil reduce the plants' performance. At foliage level, the direct action of saline water spray is due to salt accumulation on leaves. Plants are generally more susceptible to salt damage when the salt directly arrives on leaves than when the saline water arrives to the soil and roots [21]. The salinity stress can be even worse in presence of sandy banks, since marine aerosol carries salts and dust on leaves. The abrasive action of the dust can enhance the salt damage. Moreover, in coastal environments, marine aerosol contains salts and other pollutants that can affect growth and sometimes even survival of indigenous plant species [22]. These two aspects can show an independent trend [23] or, in other words, there is a positive relationship between tolerance to salt spray and to soil salinity [24]. Plants are usually 3 to 4 times more tolerant to salt in the roots than in the leaves [24].

Effects of saline aerosol strongly depend on the intensity and on the duration of the stress [25]. Effects of marine aerosol and pollutants on coastal vegetation have been studied for several decades in the European Mediterranean regions [26]. Several experiments demonstrated that leaves can absorb sodium and chlorine ions from sea water drops accumulated on the surface [27]. The salt experimentally applied on leaves of sensitive species induces symptoms similar to those observed on coastal or roadside vegetation near the sea [28]. The abrasive action of the wind excoriates leaf surface and creates possible ways for salt absorption [29]. The damage observed in plants near the sea is attributed to the excessive absorption of chlorine and sodium ions, facilitated by the presence of surfactants that are present in polluted sea water [26].

As consequence, the efficiency of photosynthesis and gas exchanges is negatively influenced by saline aerosol [30], although differences can be found among species [31] because of the characteristics of their cuticles and the epithelial cells [32]. The salts that lay on the surface of the leaf after the evaporation of water [33] can negatively modify the water balance and compromise the characteristics of the cuticle or guard cells, resulting in incomplete stomatal closure [34]. Although the combined action of drought and salt stress has been a key issue for ornamental plants quality [35,36]. The response to saline aerosol in plants subjected to differentiated conditions of water availability has been little investigated.

Callistemon citrinus (Curtis) Skeels (Myrtaceae) and *Viburnum tinus* L. 'Lucidum' (Adoxaceae) play an important role in European market as ornamental potted shrubs for the showy flowering of *Callistemon* and brilliant color of foliage in *Viburnum* [37,38]; these two species show from moderate to high tolerance to drought and salt stress, allowing their use also in marginal urban areas [39,40]. Although various investigations have been carried out to study the physiological responses of these species to drought and salt stress [4,40], little information is available on the combined effects of two stresses (drought and saline aerosol), which are very frequent in coastal area in the Mediterranean environments.

In this view, it appears quite relevant to elucidate the morphological and physiological mechanisms of adaptation of potted *C. citrinus* and *V. tinus* to drought and saline aerosol stress in order to discriminate effects of single stress or cumulative actions of the two stresses. Responses of the two species to the different stress factors were focusing on growth parameters, leaf gas exchanges, photosystem efficiency, water relations, and leaf functional traits. The information obtained from this experiment could be used to discriminate the effects of drought and saline aerosol stress and to understand how the combination of the two stressors can modify the responses of plants, with the aim to individuate the most functional strategy adopted by plants to overcome these conditions, which are very common in the Mediterranean coastal areas.

2. Materials and Methods

2.1. Experimental Conditions and Plant Materials

The experiment was carried out during 2019 springtime on two ornamental shrubs, *Callistemon citrinus* (Curtis) Skeels and *Viburnum tinus* L. 'Lucidum', grown in a cold greenhouse located in Catania area, Italy (37°41′ N 15°11′ E 80 m a.s.l.). Two-month-old rooted cuttings of both species were transplanted into 3.4 L pots, filled with sand (75%), silt (18%) and clay (7%), and fertilized with 2 g L^{-1} Osmocote Plus (14/13/13, + microelements). At the beginning of the experiment, the dry biomass of the plants was on average 27.8 ± 2.09 g and 33.6 ± 3.28 g for *Callistemon* and *Viburnum*, respectively.

In the experiment two species (*Callistemon* and *Viburnum*), four water regimes (WC40%, WC30%, WC20%, and WC10%) and three saline aerosol solutions (S0, distilled water; S1, 50% of simulated sea water solution, and S2, 100% of simulated sea water solution) were studied. For the beginning 4 weeks the plants of the two species were subjected to the differentiated water regimes; after this period, three saline aerosol treatments for another 4 weeks were imposed on the same plants differently subject to water stress. To determine the volumetric content of water in the substrate, an automated management system with dielectric sensors EC 5TE (Decagon Devices Inc., Pullman, WA) was used. Two sensors were used for each treatment and for each replicate, and data were recorded using a data acquisition system (data logger) CR1000 (Campbell Scientific Ltd., Loughborough, UK). Sensors were calibrated following the protocol of Starr and Paltineanu [41] and Tribulato et al. [42]. Sensors were calibrated where a series of each measurement is taken in connection with samples of volumetric soil to quantify the relationship between the substrate (measured with the 5TM) and the volumetric water content (WC). The sensors were installed 10 cm below the substrate surface at the center of each sample pot. The use of the probes was preceded by their calibration to determine the real content of water in the substrate samples, which were placed in a thermo-ventilated oven at 70 °C until the constant weight (W cal.) reached ($R^2 = 0.9742$). The irrigation, scheduled at 8:00 a.m. and at 7:00 p.m., was activated when the water content dropped below the pre-set threshold values of WC10% (severe drought stress), WC20% (moderate drought stress), WC30% (light drought stress), and WC40% (control, Capacity Control) of the volume of the substrate. The same threshold values were adopted to determine irrigation interruption. Full composition of saline aerosol solution was as follows: NaCl, Na_2SO_4, $MgCl_2$, $CaCl_2$, and KCl at concentrations of 23.48, 3.92, 4.98, 1.10, and 0.66 g L^{-1} respectively, with a concentration of 401.8 mM NaCl [43] while in the treatment S1 it was reduced to 50%. The treatments were performed by spraying the canopies of plants twice a week.

For each species, triplicates of four plants for 12 treatments (144 plants in total for each species) were adopted. The mean air temperatures and relative humidity levels during the experimental periods were registered on a data logger (CR 1000; Campbell Scientific Ltd., Loughborough, UK). The mean temperature was 25.2 °C, the relative humidity levels were 66% (Figure S1) and mean photosynthetic active radiation (PAR) was 13.7 MJ m^{-2} d^{-1}.

2.2. Biomass and Leaf Area

At the end of the experiment, six plants per treatment were separated into stems, leaves, and roots. The dry biomass was determined by drying weighed fresh samples in a thermo-ventilated oven at 70 °C up to constant weight. Total leaf area was measured with leaf area meter (Delta-T Devices Ltd., Cambridge, UK) and the SPAD index of 25 fully expanded leaves per each replicate was registered using a SPAD-502 chlorophyll meter (Minolta Camera Co., Osaka Japan).

2.3. Leaf Gas Exchanges, Chlorophyll a Fluorescence, and Relative Water Content

At 7, 15, 30, 45, and 60 days from experiment onset, leaf gas exchange was measured using a CO_2/H_2O infrared gas analyzer (LCi, ADC Bioscientific Ltd., Hoddesdon, UK). The measurements were carried out from 09:00 a.m. to 1:00 p.m. For each treatment, net photosynthetic rate (A_N) and stomatal conductance (gs) were measured. The mean irradiance was 224.6, 149.2, 137.0, 170.4, and 290.6 μM m^{-2} s^{-1} of photosynthetically active radiation (PAR) at day 7, 15, 30, 45, and 60 respectively. With a normal concentration of CO_2, the temperature in the measurement chamber was 32.1, 33.3, 32.3, 33.9, and 32.9 °C at day 7, 15, 30, 45, and 60 respectively; the H_2O reference as partial pressure was 20.1, 19.4, 23.6, 20.5, and 22.4 mBar at the day 7, 15, 30, 45, and 60 respectively.

The chlorophyll *a* fluorescence was measured using the modulated chlorophyll fluorimeter OS1-FL (Opti-Sciences Corporation, Tyngsboro, MA, USA). Under abiotic stress conditions, one parameter that is commonly used to identify the presence of photosynthetic plant damage in plants is the measurement of chlorophyll *a* fluorescence. The ratio variable to maximal fluorescence ratio (Fv/Fm) (i.e., the maximum primary photochemical efficiency of the PSII) allows the evaluation of the efficiency of the PSII photosystem, indirectly measuring the physiological state of the plant. Every leaf was dark-adapted using cuvette clips for 15 min. The chlorophyll *a* fluorescence was expressed as the Fv/Fm ratio, which indicates the maximal quantum yield of PSII photochemistry, where Fm = the maximal fluorescence and Fv = the variable fluorescence.

The Relative Water Content (RWC) was measured at the end of the trial between 12:00 a.m. and 2:00 p.m. For the determination of RWC, 30 leaf discs of 10 mm in diameter, per each replicate were collected, and their fresh weights (FW) were registered. Samples were then soaked for 24 h in distilled water in dark conditions and their turgid weight (TW) was determined. The samples later were dried at 75 °C up to constant dry weight (DW). The RWC was measured by using the following formula

$$RWC\% = (FW - DW/TW - DW) \times 100$$

2.4. Statistical Analysis

The experimental design was a completely randomized experiment with three replicates. The CoStat version 6.311 (CoHortSoftware, Monterey, CA, USA) was used for statistical analysis. Data were subjected to three-way ANOVA to compare the effects of drought (D), saline aerosol (A), and species (S). For each species, data were subjected to two-way ANOVA to compare the effects of drought and saline aerosol treatments. The differences between means were conducted by Tukey's test ($p < 0.05$). The data presented in Figures are means ± standard error (SE) (Graphpad 7.0). The Heat map was realized using Graphpad 7.0. The principal component loading plot and scores of PCA were performed using Minitab 16, LLC.

3. Results

Statistical analysis results, main and interaction effects of drought, salinity, and species on morphometric parameters were reported in Table 1. Three-way ANOVA showed that the morphometric characteristics were affected by drought, saline stress, and species (Table 1). The statistical analysis revealed that total biomass data were statistically different for drought and saline aerosol factors and interactions among all three factors were also significant. Epigeous biomass and leaf number data were statistically significant for all factors and interactions. Significant differences were detected in the root/shoot ratio for the three factors, while the interactions were only significant for DxS and AxS. Total leaf area was statistically significant for the three factors and interactions, except AxS. SPAD data were significantly different for saline aerosol and species, while the only significant interaction was the AxS.

Table 1. Summary of the main and interaction effects of drought, saline aerosol, and species treatments on total and epigeous dry biomass, root/shoot ratio, total leaf area, leaf number, and SPAD of potted *Callistemon* and *Viburnum* plants with the corresponding significance of the F-values

	TB	EB	R/S	TLA	LN	SPAD
Main Effects						
Drought (D)	F 41.86	F 45.77	F 3.28	F 32.49	F 5.09	F 2.34
	$p < 0.001$ ***	$p < 0.001$ ***	$p < 0.001$ ***	$p < 0.001$ ***	$p < 0.01$ **	ns
Saline Aerosol (A)	F 24.25	F 34.18	F 5.63	F 46.51	F 3.49	F 4.12
	$p < 0.001$ ***	$p < 0.001$ ***	$p < 0.01$ **	$p < 0.001$ ***	$p < 0.05$ *	$p < 0.05$ *
Species (S)	F 0.85	F 22.91	F 154.95	F 448.94	F 1195.39	F 19.12
	ns	$p < 0.001$ ***	$p < 0.001$ ***	$p < 0.001$ ***	$p < 0.001$ ***	$p < 0.001$ ***
Interaction						
D × A	F 9.40	F 10.34	F 1.35	F 4.29	F 6.07	F 1.30
	$p < 0.001$ ***	$p < 0.001$ ***	ns	$p < 0.01$ **	$p < 0.001$ ***	ns
D × S	F 1.92	F 0.67	F 1.85	F 4.29	F 3.62	F 2.73
	$p < 0.001$ **	$p < 0.01$ **	$p < 0.01$ **	$p < 0.01$ **	$p < 0.05$ *	ns
A × S	F 6.33	F 4.23	F 3.53	F 0.50	F 4.62	F 3.43
	$p < 0.001$ **	$p < 0.05$ *	$p < 0.05$ *	ns	$p < 0.05$ *	$p < 0.05$ *
D × A × S	F 10.77	F 14.11	F 1.80	F 6.20	F 5.00	F 1.13
	$p < 0.001$ ***	$p < 0.001$ ***	ns	$p < 0.001$ ***	$p < 0.001$ ***	ns

TB = Total dry biomass; EB = Epigeous dry biomass; R/S = Root to shoot ratio; TLA = Total leaf area; LN = leaf number. Significance of differences of parameters: ns = not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ with the corresponding significance of the F-values.

To better understand effects of drought and saline aerosol stress, two species were separately analyzed. The individual and the combined effects of drought and saline aerosol stress on plant growth were reported in Tables 2 and 3.

In *Callistemon*, the total dry biomass was affected by drought and saline treatment, but no interaction was detected among the experimental factors. Drought effect on total dry biomass showed a reduction in WC 30%, WC 20% and WC 10% of about 10%, 22%, and 28% respectively. Saline stress also caused a reduction for this parameter by ~10% for S1 and S2 compared with the S0 (Table 2).

In *Callistemon*, the epigeous dry biomass showed a similar trend as the total dry biomass with a reduction by ~10, 24, and 33% WC 30%, WC 20%, and WC 10% respectively as compared with the control (Table 2). Saline stress as well affected a reduction for this parameter by ~9% for S1 and S2 compared with the S0 (Table 2).

The root-to-shoot ratio increased in *Callistemon* plants grown under higher deficit irrigation (WC 10%) as well as for the saline stress conditions in S1 and S2, with an increase by 36% (Table 2, Figure 1a).

The combination of two abiotic stresses resulted in a greater decrease in total leaf area, as shown by the lower values obtained for the WC 10%, S0, S1, and S2 (Table 2, Figure 1b).

Similarly at the total leaf area, the combination of the two stresses resulted in a greater decrease in the leaf number in WC 10% S0, S1, and S2 (Table 2, Figure 1c).

Table 2. Effects of drought and saline aerosol treatments on total (TB) and epigeous (EB) dry biomass, root/shoot ratio (R/S), total leaf area (TLA), and leaf number (LN) of potted *Callistemon* plants

Aerosol/Drought	WC 40%	WC 30%	WC 20%	WC 10%	Mean	A × D
TB (g plant^{-1})						**ns**
S0	130.9 ± 2.9	113.2 ± 2.4	94.2 ± 1.6	94.2 ± 0.9	**108.1 ± 4.7 a ****	
S1	116.1 ± 0.7	102.3 ± 8.5	96.4 ± 0.9	88.1 ± 4.4	**100.7 ± 3.7 b**	
S2	113.4 ± 1.9	107.1 ± 6.9	89.9 ± 2.2	77.0 ± 3.5	**96.9 ± 4.7 b**	
Mean	**120.1 ± 2.9 A *****	**107.6 ± 3.6 B**	**93.5 ± 1.3 C**	**86.4 ± 3.0 C**		
EB (g plant^{-1})						**ns**
S0	113.0 ± 2.4	96.1 ± 0.9	80.2 ± 1.6	76.8 ± 2.0	**91.5 ± 4.4 a ****	
S1	99.3 ± 1.6	89.7 ± 8.6	80.3 ± 1.8	70.6 ± 2.8	**84.9 ± 3.8 b**	
S2	97.4 ± 1.8	91.6 ± 6.6	75.8 ± 1.0	58.9 ± 1.2	**80.9 ± 4.8 b**	
Mean	**103.2 ± 2.7 A*****	**92.5 ± 3.3 B**	**78.8 ± 1.0 C**	**68.8 ± 2.8 D**		
R/S						**$p < 0.01$**
S0	0.16 ± 0.01	0.18 ± 0.02	0.17 ± 0.00	0.20 ± 0.02	**0.18 ± 0.01 ns**	
S1	0.17 ± 0.01	0.14 ± 0.01	0.20 ± 0.02	0.25 ± 0.01	**0.19 ± 0.01**	
S2	0.16 ± 0.00	0.17 ± 0.01	0.19 ± 0.02	0.30 ± 0.01	**0.20 ± 0.02**	
Mean	**0.17 ± 0.01 B *****	**0.16 ± 0.01 B**	**0.19 ± 0.01 B**	**0.25 ± 0.02 A**		
TLA (cm^2)						**$p < 0.05$**
S0	3979.9 ± 139.3	3611.7 ± 14.0	2978.9 ± 116.3	2364.8 ± 60.1	**3233.8 ± 190.3 a *****	
S1	3345.9 ± 123.7	2977.2 ± 61.2	2969.4 ± 122.4	2101.6 ± 90.8	**2932.1 ± 144.7 b**	
S2	3503.9 ± 51.0	3315.5 ± 136.2	2878.1 ± 90.3	2030.8 ± 115.2	**2848.5 ± 176.7 b**	
Mean	**3609.9 ± 110.4 A *****	**3301.5 ± 101.4 B**	**2942.1 ± 57.6 C**	**2165.7 ± 68.4 D**		
LN (n°)						**$p < 0.01$**
S0	1050.2 ± 59.7	928.1 ± 13.3	908.5 ± 57.2	804.5 ± 43.2	**922.7 ± 30.7 a ***	
S1	1073.0 ± 40.0	865.5 ± 118.0	905.6 ± 25.1	651.8 ± 39.9	**871.7 ± 47.3 ab**	
S2	907.8 ± 13.8	931.4 ± 86.1	913.6 ± 160.0	727.7 ± 69.5	**861.3 ± 25.7 b**	
Mean	**1010.3 ± 33.2 A *****	**905.3 ± 43.9 B**	**898.0 ± 10.7 B**	**727.3 ± 34.3 C**		

*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$; ns: $p > 0.05$ Data followed by a different letter were significantly different according to LSD Test. WC40%: control; WC30%: light drought stress; WC20%: moderate drought stress; WC10%: severe drought stress. S0: Distilled water, S1: 50% Synthetic seawater solution, S2: 100% Synthetic seawater solution. Data are means ± standard error (n = 3). Three biological replicates were used for the measurements. TB = Total dry biomass; EB = Epigeous dry biomass; R/S = Root to shoot ratio; TLA = Total leaf area; LN = Leaf number.

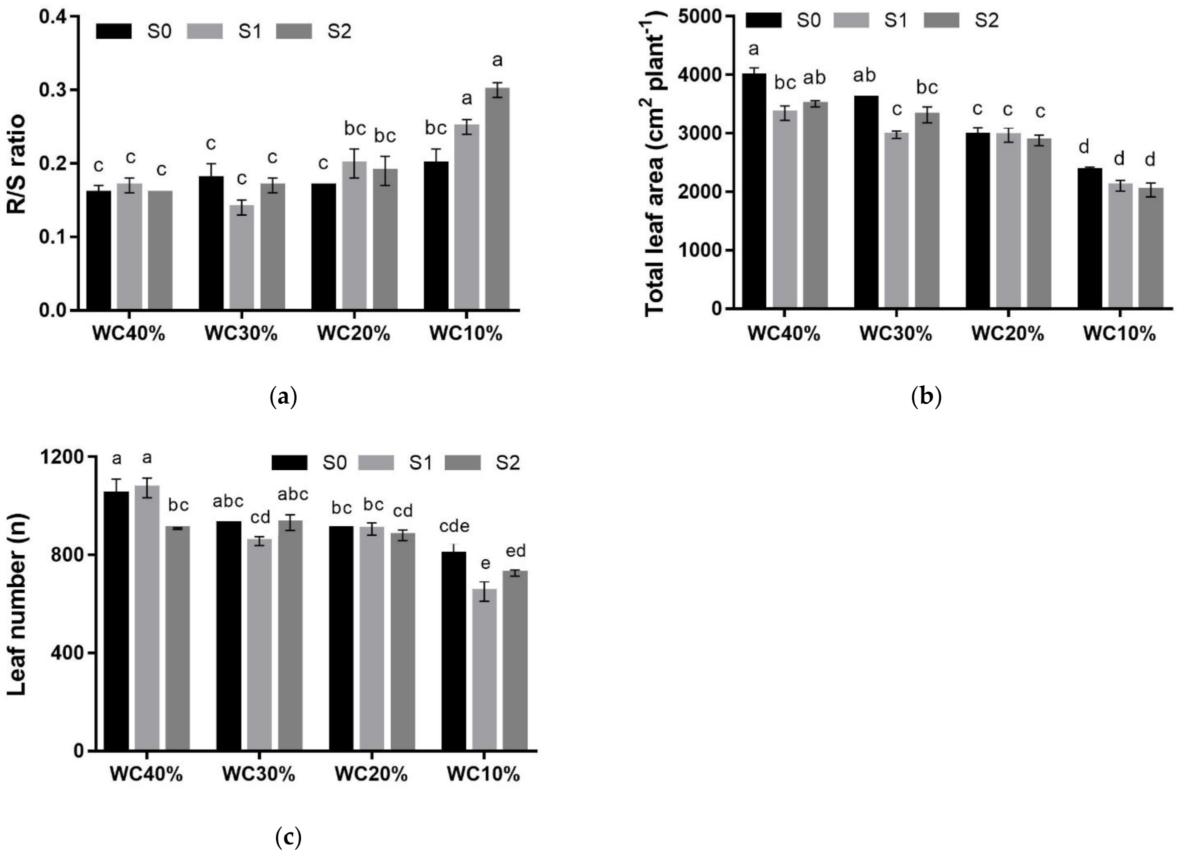


Figure 1. Interaction effect of drought (WC40%: control; WC30%: light drought stress; WC20%: moderate drought stress; WC10%: severe drought stress) × saline aerosol treatment (S0: Distilled water, S1: 50% Synthetic seawater solution, S2: 100% Synthetic seawater solution) for *Callistemon* plants on root to shoot ratio (**a**), total leaf area (**b**), and leaf number (**c**). Data are means ± standard error (n = 3). Three biological replicates were used for the measurements. Data were subjected to two-way ANOVA and differences among means were determined using Tukey's post-test. Different letters indicate statistical differences for $p < 0.05$.

In *Viburnum* plants, the total dry biomass varied with drought, but not with saline aerosol treatment. Drought affected total dry biomass, which showed a reduction in WC 30%, WC 20%, and WC 10% of ~14%, 18%, and 23% respectively, compared with control. The saline stress, instead, did not show any significant reduction (Table 3).

The combined effects of drought and saline aerosol stress showed a significant interaction (Table 3) while the highest decrease was observed from WC10% S2 (~33%) compared with the control plants (Table 3, Figure 2a).

The root-to-shoot ratio varied with saline aerosol stress but not with drought and the interaction among them (Table 3). An increase in *Viburnum* plants grown under saline aerosol stress (S1) was observed with an increase by ~20% compared with the control plants (Table 3).

In *Viburnum* plants, the total leaf area varied with drought and saline treatment, but no interaction was significant. Drought reduced the total leaf area in WC 30%, WC 20%, and WC 10% by ~16%, 19%, and 24% respectively compared with the control plants (Table 3). Saline stress also affected a reduction for this parameter by ~18% for S1 and by 11% for S2 compared with the S0 (Table 3).

At the end of the experiment, with the intensification of water and saline aerosol stress (WC 10% S2), a reduction in leaf number was observed. In particular, leaf number decreased by 33% compared with WC40% and S1 (Figure 2b).

Table 3. Effects of drought and saline aerosol treatments on total (TB) and epigeous (EB) dry biomass, root/shoot ratio (R/S), total leaf area (TLA), and leaf number (LN) of potted *Viburnum* plants

Aerosol/Drought	WC 40%	WC 30%	WC 20%	WC 10%	Mean	A × D
TB (g plant^{-1})						$p > 0.05$
S0	126.3 ± 8.0	105.2 ± 0.7	111.4 ± 2.8	95.4 ± 2.1	**109.6 ± 3.9 ns**	
S1	114.7 ± 1.6	103.8 ± 3.6	95.0 ± 4.4	99.4 ± 6.1	**103.2 ± 2.9**	
S2	126.0 ± 3.9	106.9 ± 2.2	95.6 ± 1.2	88.9 ± 2.2	**104.3 ± 3.5**	
Mean	**122.3 ± 3.2 A *** **	**105.3 ± 1.3 B**	**100.7 ± 3.1 BC**	**94.6 ± 2.8 C**		
EB (g plant^{-1})						$p < 0.05$
S0	103.8 ± 4.9	85.3 ± 1.6	91.6 ± 2.8	76.2 ± 3.3	**89.2 ± 3.3 a ** **	
S1	87.9 ± 4.5	83.4 ± 2.4	71.5 ± 2.3	78.5 ± 4.7	**80.3 ± 2.4 ab**	
S2	99.1 ± 3.8	84.0 ± 2.7	76.4 ± 1.8	69.8 ± 4.2	**82.3 ± 3.6 b**	
Mean	**96.9 ± 3.2 A *** **	**84.3 ± 1.2 B**	**79.8 ± 3.3 BC**	**74.8 ± 2.4 C**		
R/S						**ns**
S0	0.22 ± 0.0	0.23 ± 0.0	0.22 ± 0.0	0.26 ± 0.1	**0.23 ± 0.0 b ***	
S1	0.31 ± 0.1	0.24 ± 0.0	0.33 ± 0.0	0.27 ± 0.0	**0.29 ± 0.0 a**	
S2	0.27 ± 0.0	0.27 ± 0.0	0.25 ± 0.0	0.27 ± 0.0	**0.27 ± 0.1 ab**	
Mean	**0.27 ± 0.0 ns**	**0.25 ± 0.0**	**0.27 ± 0.0**	**0.27 ± 0.0**		
TLA (cm^2)						**ns**
S0	6386.0 ± 240.3	5073.1 ± 9.9	5288.2 ± 436.3	4736.0 ± 111.4	**5370.8 ± 215.9 a *** **	
S1	4890.8 ± 184.6	4584.9 ± 124.1	3963.6 ± 268.0	4202.1 ± 202.5	**4401.4 ± 136.3 c**	
S2	5805.8 ± 251.1	4732.9 ± 308.6	4637.6 ± 145.8	4008.3 ± 319.6	**4796.2 ± 225.3 b**	
Mean	**5694.2 ± 245.5 A *** **	**4784.9 ± 123.0 B**	**4629.8 ± 245.3 BC**	**4315.5 ± 157.5 C**		
LN (n°)						$p < 0.05$
S0	125.0 ± 14.0	113.3 ± 3.2	141.4 ± 8.7	125.0 ± 10.1	**126.2 ± 5.2 ab ***	
S1	157.1 ± 8.9	146.2 ± 11.0	117.5 ± 5.3	127.1 ± 13.5	**137.0 ± 6.4 a**	
S2	137.8 ± 6.6	125.2 ± 10.7	115.5 ± 1.8	106.2 ± 8.2	**121.2 ± 4.8 b**	
Mean	**140.0 ± 7.0 ns**	**128.2 ± 6.6**	**124.8 ± 5.1**	**119.4 ± 6.3**		

*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$; ns: $p > 0.05$ Data followed by a different letter were significantly different according to LSD Test. WC40%: control; WC30%: light drought stress; WC20%: moderate drought stress; WC10%: severe drought stress. S0: 0% Distilled water, S1: 50% Synthetic seawater solution, S2: 100% Synthetic seawater solution. TB = Total dry biomass; EB = Epigeous dry biomass; R/S = Root to shoot ratio; TLA = Total leaf area; LN = Leaf number.

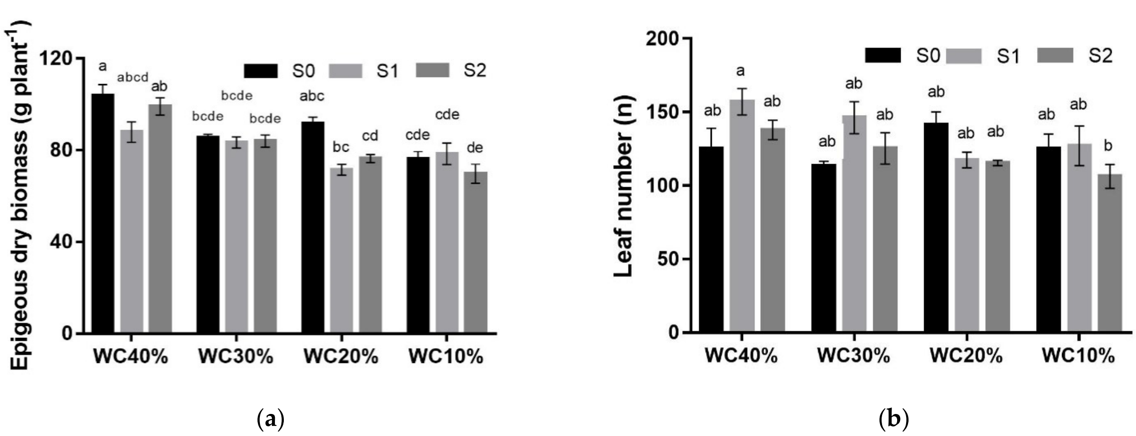
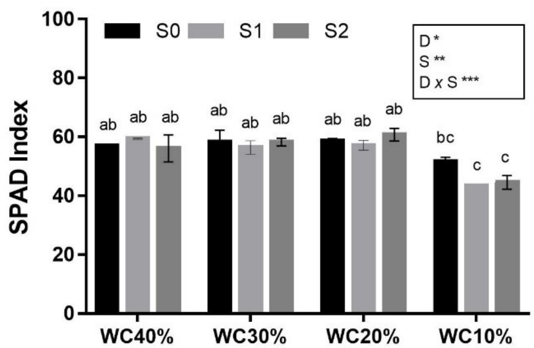


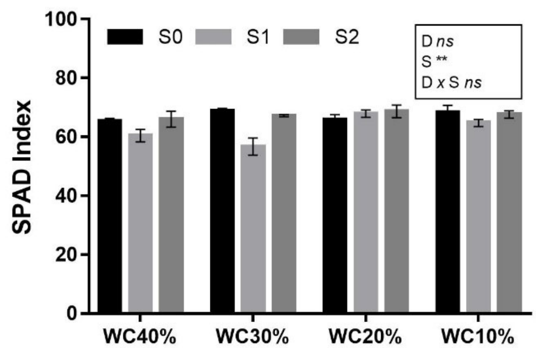
Figure 2. Interaction effect of drought (WC40%: control; WC30%: light drought stress; WC20%: moderate drought stress, WC10% severe drought stress) × saline aerosol treatment (S0: Distilled water, S1: 50% Synthetic seawater solution, S2: 100% Synthetic seawater solution) for *Viburnum* plants on epigeous dry biomass (**a**) and leaf number (**b**). Data are means ± standard error (n = 3). Three biological replicates were used for the measurements. Data were subjected to two-way ANOVA and differences among means were determined using Tukey's post-test. Different letters indicate statistical differences for $p < 0.05$.

In *Callistemon* plants, the combination of the two stresses together resulted in a significant decrease for SPAD index, as shown by the lowest values obtained for the WC 10% in S1 and S2 (Figure 3a). No interaction effects were observed in *Viburnum* plants (Figure 3b).

Leaf damage was observed in both species and in particular in *Callistemon* plants, although the percentage of leaf damaged was always lower than 10% (data not shown).



(**a**)



(**b**)

Figure 3. SPAD index in *Callistemon* (**a**) and *Viburnum* (**b**) potted plants subjected to drought (WC40%: control; WC30%: light drought stress; WC20%: moderate drought stress; WC10% severe drought stress) and saline aerosol treatment (S0: Distilled water, S1: 50% Synthetic seawater solution, S2: 100% Synthetic seawater solution). Data are means ± standard error (n = 3). Three biological replicates were used for the measurements. Data were subjected to two-way ANOVA and differences among means were determined using Tukey's post-test. Different letters indicate statistical differences for $p < 0.05$; *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$; ns: $p > 0.05$.

Gas exchange measurements in *Callistemon* plants were severely affected under water deficit. The A_N was reduced by water deficit and plants exposed to severe water stress (WC10%) for 30 days ($p < 0.0026$ **) showed a reduction by 70% compared to control. At the end of the trial, 60 days, with a reduction by 45% and 53% in WC20% and WC10% plants ($p < 0.0002$ ***). The addition of saline aerosol stress, after 30 days, differences were amplified comparing treatments with control plants, and at the end of the trial, *Callistemon* showed significant differences ($p < 0.0001$ ***) in WC20% S1 and S2 and WC10% S0, S1, and S2 treatments compared with the control plants (Figure 4a, Table 4).

The gs in *Callistemon* plants was reduced in WC10% after 7 days ($p < 0.0068$ **). At 30 days significant differences ($p < 0.0000$ ***) were observed in the more stressed treatments (WC20% and WC10%). No significant differences were observed at the end of the trial ($p > 0.5225$ ^{ns}) (Figure 4b, Table 4).

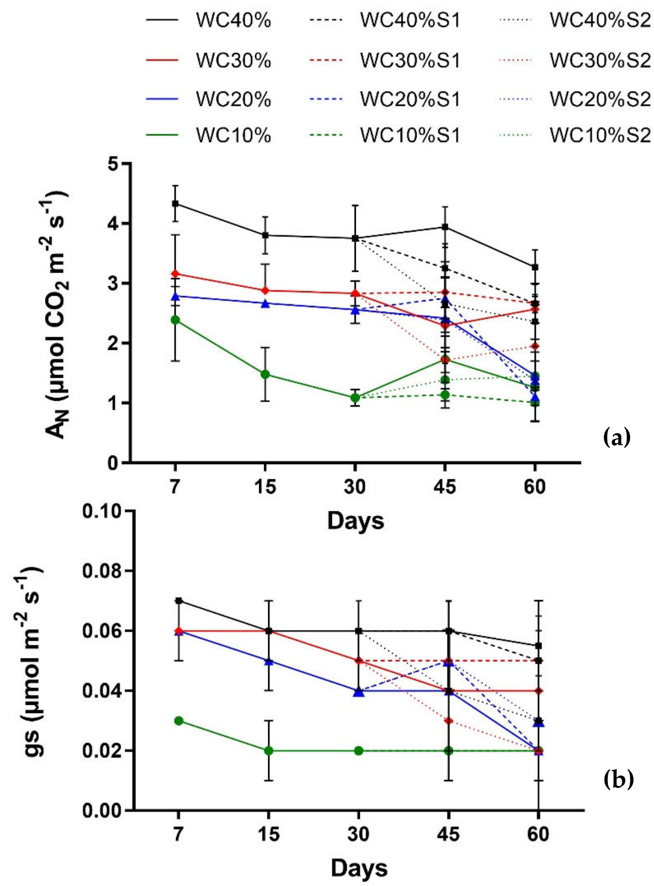


Figure 4. Trend of net photosynthesis (A_N) (**a**) and stomatal conductance (gs) (**b**) in *Callistemon* plants affected by drought (WC40%: control; WC30%: light drought stress; WC20%: moderate drought stress; WC10% severe drought stress) and saline aerosol treatment (S0: Distilled water, S1: 50% Synthetic seawater solution, S2: 100% Synthetic seawater solution). The plants were subjected of drought stress for four weeks; subsequently the plants were also treated with saline aerosol treatment. Data are means ± standard error (n = 3). Three biological replicates were used for the measurements.

Table 4. Summary of the main and interaction effects of days (T) and drought (D) in the first 30 days, and of days (T), drought (D), and saline aerosol (A) for the other 30 days on net photosynthesis (A_N) and stomatal conductance (gs) of potted *Callistemon.*

Factor	A_N	gs
Main effect		
Days (T)	$p > 0.1360$ ns	$p > 0.9479$ ns
Drought (D)	$p < 0.001$ ***	$p < 0.001$ ***
Interaction		
T x D	$p > 0.9195$ ns	$p > 0.3881$ ns
Main effect		
Days (T)	$p < 0.000$ ***	$p > 0.9335$ ns
Drought (D)	$p < 0.000$ ***	$p > 0.7446$ ns
Aerosol Saline (A)	$p < 0.0223$ *	$p > 0.7487$ ns
Interaction		
T x D	$p < 0.02$ *	$p > 0.1859$ ns

T x A	$p > 0.2517$ ns	$p > 0.4371$ ns
D x A	$p < 0.0492$ *	$p > 0.1965$ ns
T x D X A	$p > 0.695$ ns	$p > 0.4665$ ns

Data were subjected to two-way ANOVA to compare the effects of days (T) and drought (D), while data were subjected to three-way ANOVA to compare the effects of days (T), drought (D), saline aerosol (A). Significance of differences of parameters: ns = not significant; * $p < 0.05$; *** $p < 0.001$.

In *Viburnum* plants, as observed for *Callistemon*, since the first measurement (7 days) a decrease of net photosynthesis was observed for the more stressed treatments (WC 20% and WC 10%, by 57%, $p < 0.0068$ **) and remained thereafter significant for the entire experimental period. With the addition of saline aerosol stress after 30 days, also for *Viburnum* plants, the differences with the control plants, at the end of the trial, were more pronounced in WC20% S1 and S2, and WC10% S0, S1, and S2 ($p < 0.0000$ ***) (Figure 5a, Table 5).

The gs in *Viburnum* plants was reduced in WC10% since the first measurement ($p < 0.0068$ **). At 30 days significant differences ($p < 0.003$ **) were observed in the more stressed treatments (WC20% and WC10%). With the addition of saline aerosol stress the differences with the control plants, at the end of the trial, were more pronounced in WC10% S0, S1, and S2 ($p < 0.0009$ ***) (Figure 5b, Table 5).

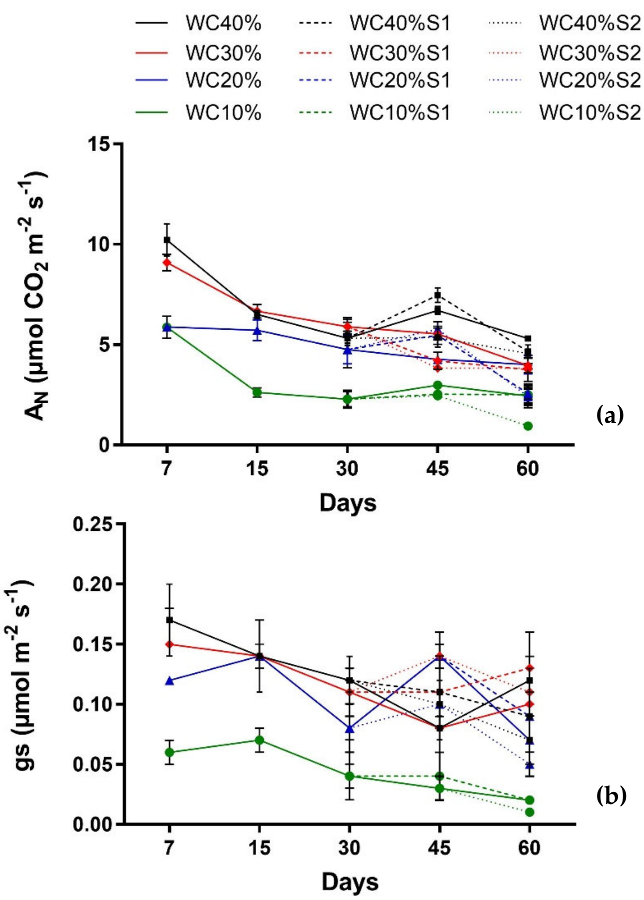


Figure 5. Trend of net photosynthesis (A_N) (**a**) and stomatal conductance (gs) (**b**) in *Viburnum* plants affected by drought (WC40%: control; WC30%: light drought stress; WC20%: moderate drought stress; WC10% severe drought stress) and saline aerosol treatment (S0: Distilled water, S1: 50% Synthetic seawater solution, S2: 100% Synthetic seawater solution). The plants were subjected of drought stress for four weeks; subsequently the plants were also treated with saline aerosol

treatment. Data are means ± standard error (n = 3). Three biological replicates were used for the measurements.

Table 5. Summary of the main and interaction effects of days (T) and drought (D) in the first 30 days, and of days (T), drought (D), and saline aerosol (A) for the other 30 days on (A_N) and stomatal conductance (gs) of potted *Viburnum.*

Factor	A_N	gs
Main effect		
Days (T)	*p* <0.001 ***	*p* <0.0024 **
Drought (D)	*p* <0.001 ***	*p* <0.000 ***
Interaction		
T x D	*p* <0.0117 *	*p*> 0.6831 ns
Main effect		
Days (T)	*p* <0.0000 ***	*p* <0.0123 *
Drought (D)	*p* <0.0000 ***	*p* <0.0000 ***
Aerosol Saline (A)	*p* <0.0001 ***	*p* >0.1899 ns
Interaction		
T x D	*p* <0.0002 ***	*p* <0.0298 *
T x A	*p*> 0.1567 ns	*p* >0.2644 ns
D x A	*p* >0.0677 ns	*p*> 0.1738 ns
T x D X A	*p* <0.0000***	*p* >0.5243 ns

Data were subjected to two-way ANOVA to compare the effects of days (T) and drought (D), while data were subjected to three-way ANOVA to compare the effects of days (T), drought (D), saline aerosol (A). Significance of differences of parameters: ns = not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

Chlorophyll *a* fluorescence is a good non-destructive marker of stress in plants. In our experiment, the maximum quantum efficiency of PSII (Fv/Fm ratio) decreased at the end of the experiment in both species. The Fv/Fm ratio in *Callistemon* showed significant differences ($p < 0.0034$ **) at the end of the experiment in WC20% and WC10% S0, S1, and S2 treatments compared with the control plants (Figure 6a, Table 6). No significant differences for Fv/Fm were observed between WC30% and control plants (Figure 6a). Significant differences was observed in *Viburnum* at the end of the trial in WC20% S0, S1, and S2 and WC10% S0 and S1 ($p < 0.0000$ ***) compared with the control plants (Figure 6b, Table 6).

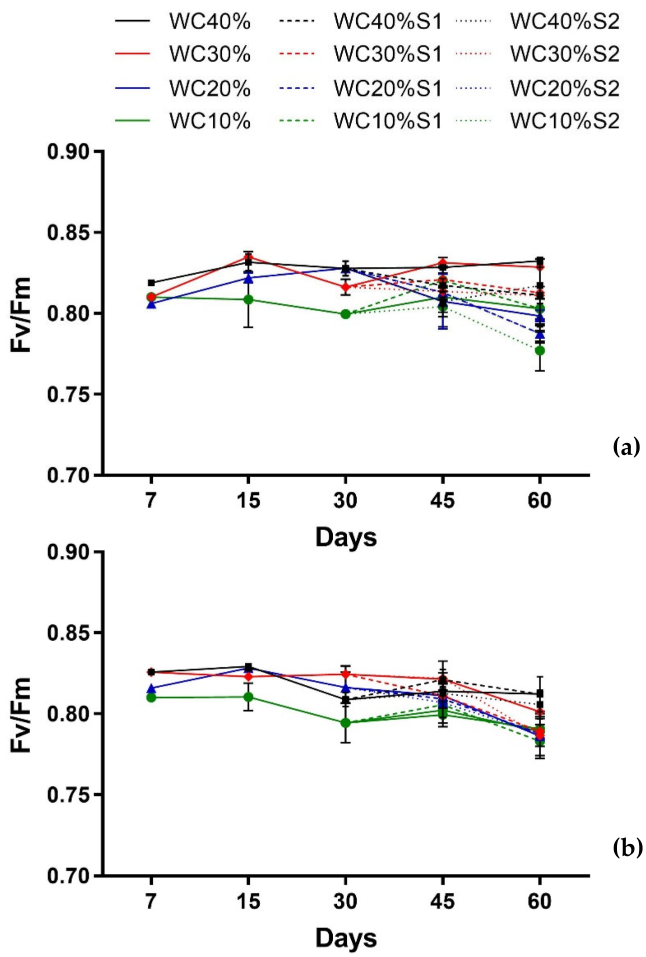


Figure 6. Trend of maximum quantum efficiency of PSII (Fv/Fm) in plants of *Callistemon* (**a**) and *Viburnum* (**b**) subjected to drought (WC40%: control; WC30%: light drought stress; WC20%: moderate drought stress; WC10% severe drought stress) and saline aerosol treatment (S0: Distilled water, S1: 50% Synthetic seawater solution, S2: 100% Synthetic seawater solution). Data are means ± standard error (n = 3). Three biological replicates were used for the measurements.

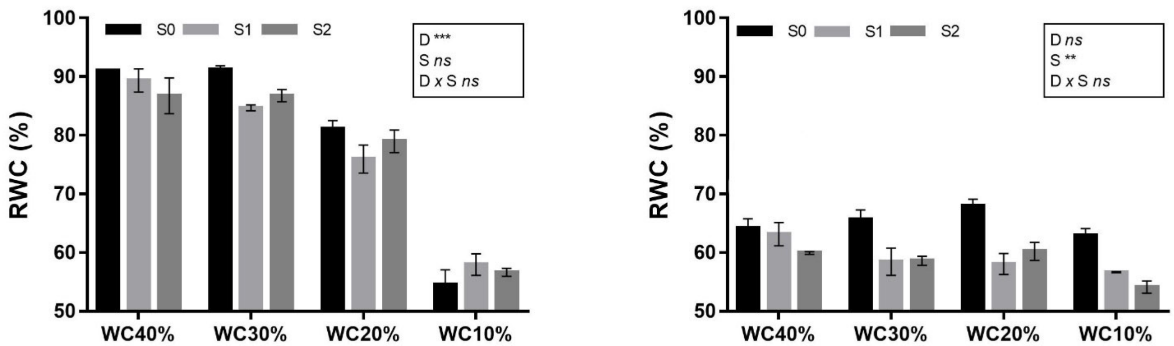
Table 6. Summary of the main and interaction effects of days (T) and drought (D) in the first 30 days, and of days (T), drought (D), and saline aerosol (A) for the other 30 days on of maximum quantum efficiency of PSII (Fv/Fm) of potted *Callisemon* and *Viburnum.*

Factor	Fv/Fm *Callistemon*	Fv/Fm *Viburnum*
Main effect		
Days (T)	*p* <0.0001 ***	*p* <0.0002 ***
Drought (D)	*p* <0.0000 ***	*p* <0.0000 ***
Interaction		
T x D	*p* <0.0018 **	*p* <0.0259 *
Main effect		
Days (T)	*p* >0.4355 ns	*p* <0.0000 ***
Drought (D)	*p* <0.0000 ***	*p* <0.0005 ***
Aerosol Saline (A)	*p* <0.0000 ***	*p* >0.7511 ns
Interaction		

T x D	*p* >0.4146 ns	*p*< 0.0157 *
T x A	*p* <0.0418 *	*p*> 0.1429 ns
D x A	*p* <0.0000 ***	*p* <0.4412 *
T x D X A	*p* <0.0099 **	*p* >0.6064 ns

Data were subjected to two-way ANOVA to compare the effects of days (T) and drought (D), while data were subjected to three-way ANOVA to compare the effects of days (T), drought (D), saline aerosol (A). Significance of differences of parameters: ns = not significant; * P < 0.05; ** P < 0.01; *** P < 0.001

In *Callistemon* the RWC was only influenced by drought treatments and, in the most stressed treatment (WC10%), the decrease from the control (WC40%) was by 37%; in *Viburnum*, instead, the RWC was influenced by saline aerosol stress with a decrease in the more stressed treatments by 8% (Figure 7a,b).



(**a**) (**b**)

Figure 7. Relative water content (RWC) at the end of the experiment in plants of *Callistemon* (**a**) and *Viburnum* (**b**) subjected of drought (D: WC40%: control; WC30%: light drought stress; WC20%: moderate drought stress; WC10% severe drought stress) and saline aerosol treatment (S: S0: Distilled water, S1: 50% Synthetic seawater solution, S2: 100% Synthetic seawater solution). Data are means ± standard error (n = 3). Three biological replicates were used for the measurements. Data were subjected to two-way ANOVA and differences among means were determined using Tukey's post-test. Significance of differences of parameters: ns = not significant; ** P < 0.01; *** P < 0.001.

To visualize the effects of drought and saline aerosol stress on the relationships among the measured parameters, a correlation-based heat map was displayed (Figure 8). The heat map clearly revealed a considerable variation among the lines in their responses to progressive drought stress and interaction with saline aerosol stress. Cumulative effects of increasing the drought quantity and salinity levels have resulted in higher decreases in vegetative growth. Under severe treatments, the total and epigeous dry biomass decreased in all lines, while the R/S ratio increased in both species. The reduction of the total leaf area and the total leaf number was specifically observed in *Callistemon*. In *Callistemon* the combination of the two stresses together resulted in a significant decrease in SPAD values while effects were observed in *Viburnum*. Irrigation and saline aerosol treatments noticeably affected the leaf relative water content (RWC), chlorophyll a fluorescence (Fv/Fm) in the most stressed *Callistemon* plants; the differences in net photosynthetic activity (A_N) and stomatal conductance (gs) were more prominently in *Viburnum* plants.

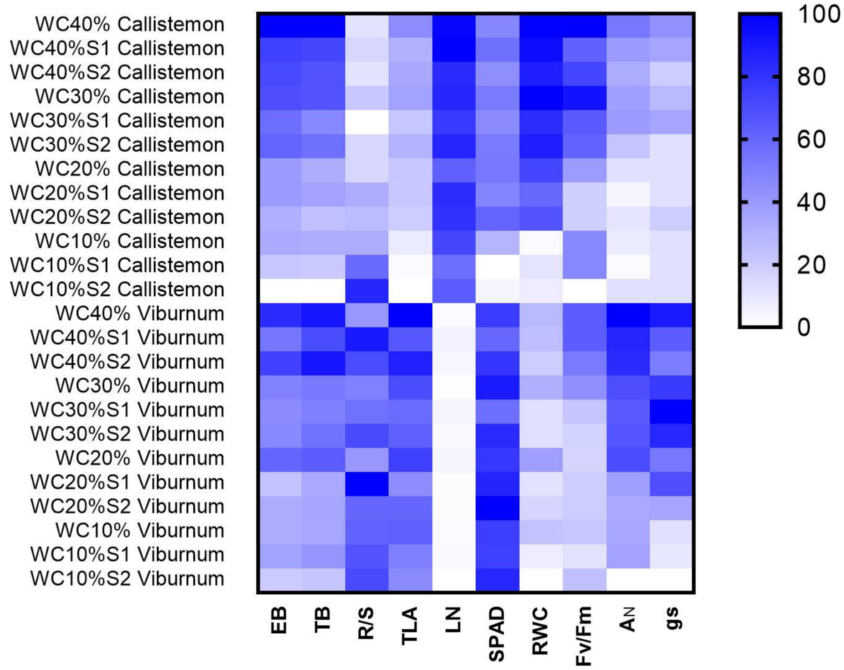
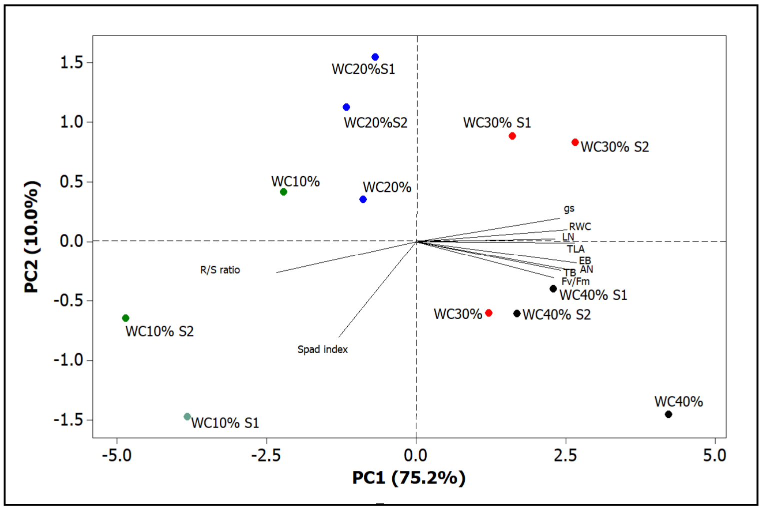


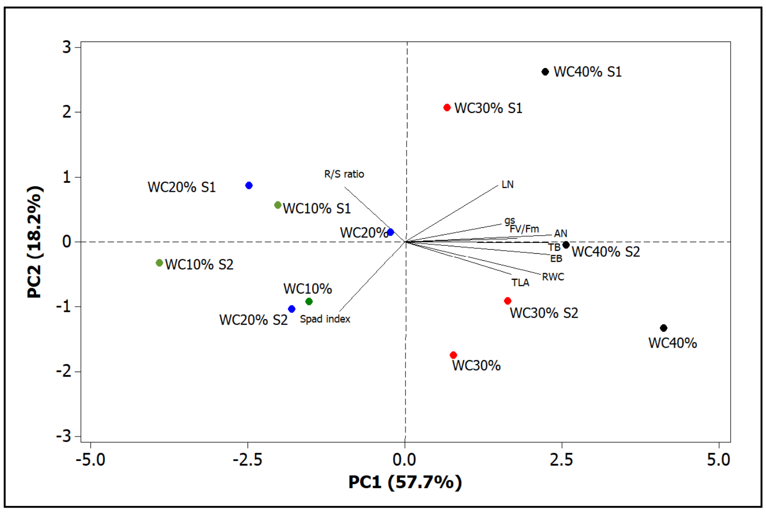
Figure 8. Heat map analysis summarizing the morphological and physiological changes of potted *Callistemon* and *Viburnum* plants responses to drought (WC40%: control; WC30%: light drought stress; WC20%: moderate drought stress; WC10% severe drought stress) and saline aerosol treatment (S0: Distilled water, S1: 50% Synthetic seawater solution, S2: 100% Synthetic seawater solution). Blue color indicates higher and white color indicates lower value. The mean values were normalized. TB = total dry biomass; EB = epigeous dry biomass; R/S = root to shoot ratio; TLA = total leaf area; LN = leaf number; SPAD = SPAD index; RWC = relative water content; Fv/Fm = maximum quantum efficiency of PSII; A_N = net photosynthesis; gs = stomatal conductance.

From the PCA analysis, effects were summary score plot, similar response of the two species to drought and saline aerosol stress treatments were observed (Figure 9a,b). In *Callistemon*, the first two PCs were related with eigen values >1 and explained more than 80% of the total variance, with PC1 and PC2 accounting for 75.2% and 10.0%; in *Viburnum* the values were 57.7% and 18.2% respectively for PC1 and PC2. The PCA showed that the most morphological and physiological parameters were associated in *Callistemon* with the WC40% and WC30% and related saline treatments, whereas the Spad index and R/S ratio, were associated with the WC10% S1 and WC10% S2 (Figure 9a).

In *Viburnum*, the most important morphological and physiological parameters were also associated with the WC40% and WC30% and saline treatments, the SPAD index was associated with WC20% S2, WC10%, and WC10% S2, whereas the R/S ratio with WC20%, WC20% S1, and WC10% S1 (Figure 9b).



(**a**)



(**b**)

Figure 9. Principal component loading plot and scores of PCA epigeous and total dry biomass, R/S ratio, total leaf area, leaf number, Spad index, gas exchange, chlorophyll a fluorescence, and RWC for *Callistemon* (**a**) and *Viburnum* (**b**) as drought and saline aerosol stress treatments. TB = total dry biomass; EB = epigeous dry biomass; R/S = root to shoot ratio; TLA = total leaf area; LN = leaf number.

4. Discussion

The selection of ornamental plants tolerant/resistant to number of stresses is a priority to increasing the private or public gardens, in urban and peri-urban coastal areas, but information in literature to discriminate the response of plant species to abiotic stress and hence help the plant species choice is lacking [2]. In our study, two ornamental shrubs exposed to drought and subsequently saline aerosol for 4 weeks showed different responses. The analysis of physiological and morphological traits presented in this study may help to clarify the different strategies adopted by these species. In our study, the interaction between drought and saline aerosol stress was responsible for plant growth parameters in both shrubs. Dry biomass in *Callistemon* plants decreased under drought and saline aerosol treatments without interaction effects. In *Viburnum*, the effects of the two stresses appeared to be more evident in epigeous dry biomass; in fact, the differences due to the drought and saline aerosol stresses were more pronounced than in total biomass. Plants subjected to drought stress did not show the same amount of total biomass compared to plants grown under optimal irrigation, and these reductions also increased when saline aerosol stress was imposed. Significant interactions were observed between the water regime and saline aerosol treatment: plants, under the WC10% treatment, were the most stressed due to the lack of water and were the most affected by the adoption of the saline solution. Species' response to stresses in terms of growth is the final manifestation of several interacting physiological and biochemical parameters and has been often used to characterize salinity or drought tolerance [44].

When plants are subjected to drought and/or saline stresses, the reduction of leaf area is considered an avoidance mechanism leading to the reduction of water losses by regulating the stomata closure, which is the most common defense strategy of many species under osmotic stress [45,46]. In our trial, the total leaf area of *Callistemon* showed interaction effects with differences due to saline aerosol in the control and light drought stress, while in *Viburnum* plants no interaction effects were observed. Previous attempts to study environmental stresses, such as drought, reported that plants can shift biomass allocation and change root/shoot ratios to cope with various environmental conditions [47]. It is proved that increasing R/S ratio is one of the avoidance mechanisms that enable plants to optimize water uptake under drought condition [48]. In presence of drought stress, plants need a wider root surface, while in saline aerosol stress conditions in some cases this surface can be even reduced to minimize toxic ions uptake and their consequent accumulation in the shoots, inducing in both situations a different distribution of the roots [49–51]. Our results reported that the plants of *Callistemon* in presence of severe drought stress and saline aerosol (10%WC S1 and S2), showed a higher R/S ratio thus optimizing water uptake.

Drought and salinity stresses induce the generic response of creating a physiological water deficit in plants. So far, interaction between drought and saline aerosol stress was reported to be detrimental for a number of physiological parameters, with the result of an increase of damage in plants under combined stress [52]. Both drought and salinity stresses induce a very complex photosynthetic response in plants, which occur in different leaf cell sites during the different steps of plants growth and development. The strength, length, and rate of development of the stress affect plant reactions to water shortage and salinity, because these issues dictate whether mitigation processes occur or not [53]. As a result, both single and overlapped water shortage and saline aerosol stress imposed to plants lead to drastic inhibition of net photosynthetic rate, stomatal conductance, and increased oxidative damage [52].

In our study, the photosynthetic rate and the stomatal conductance in both shrubs significantly decreased under both drought and saline aerosol treatments. As reported by Toscano et al., [54] in a study regarding two ornamental shrubs subjected by drought stress, a severe water deficit also had a negative effect on the photosynthetic rate and stomatal conductance of both species; however, these parameters were more affected, particularly in early summer, when these plants had very low gs values. Other studies reported that both stresses can adversely affect the photosynthetic activity in plants [55–58]. Good photosynthesis activity, even under salinity aerosol stress, can contribute to the biosynthesis of different related primary metabolism as an antioxidant defense mechanism, which help plants in detoxifying free radicals induced by stress conditions [43].

In this work, as expected, drought and saline aerosol stress resulted in reduced photosynthesis as a consequence of reduced CO_2 assimilation following stomatal closure. Achou et al. [59] reported the same results in a study on tomato plants. Moreover, stomatal closure minimizes water loss by transpiration, which leads to a decrease in photosynthetic carbon assimilation [60–62] and affects osmotic regulation [63,64]. Beside the gas exchange analyses, the stress conditions can be also monitored using chlorophyll *a* fluorescence that is a non-destructive method, largely used in studying plant response and adaptation to stressful environments [43]. The reduction of Fv/Fm can help to assess the tolerance of different species to the different stresses.

The combination of drought stress and saline aerosol treatments determined significant reduction in the values of parameter Fv/Fm at the end of the experiment in both species in plants treated with severe water deficit (20%WC and 10%WC). This indicates that no photo-damage of PSII reaction centers or relaxing, developing slowly quenching excitation energy, occurred [65]. Instead, in the combination of the two stresses, the lower Fv/Fm values indicated that PSII had been damaged. In a study conducted by Umar et al. [66] the maximum quantum yield of PSII (Fv/Fm) decreased in all sunflower cultivars when exposed to combined stress as compared with control while the Fv/Fm was not much affected under salt and drought stress alone. A study on the effect of marine aerosol on ornamental plants showed that *Callistemon citrinus* has been considered as intermediate saline spray tolerant species [6]. The tolerance of the species was evaluated using chlorophyll *a* fluorescence and relative derived indexes, classifying this species as medium tolerant [6].

RWC is a strong parameter to detect water status in plant tissues and is more reliable than cell water potential since, through a direct connection with cell volume, it better demonstrates the balance between the plant water content and transpiration rate. In general, by increasing drought stress intensity, the reduction in RWC can be due to a decrease in water potential of leaves [67]. RWC is an indicator of the water status of plant tissues during drought stress. It decreases with the water deficit increase, although this reduction is genotype specific [68]. In particular, in *Callistemon* the reduction for effect of drought stress was of about 37%; in *Viburnum* differences were observed only in correspondence of saline aerosol. In our study, the lowest RWC was obtained at 10%WC treatment in *Callistemon* and at 10%WC, S1 and S2 in *Viburnum*.

Gas exchanges are closely related to the status of leaf water, which could be also considered indicators of stress under drought and saline conditions. In different studies it was reported that gas exchanges had a close relationship to leaf water status, in fact, the net photosynthesis of the plants decreased with the relative water content and leaf water potential [69–71]. Similar results were found in our experiment, where the more stressing treatments, showed a major reduction of RWC and gas exchanges.

To better understand the tolerance mechanisms to environmental stresses it is interesting to study and interpret the morphological and physiological parameters collected from plants grown under stressed and not-stressed conditions. Correlation analysis, PCA, and clustering give useful indication for evaluating the relationships between the parameters and their principal components for stress tolerance [72,73]. In our study, heat map and PCA showed that the differences in stress tolerance between *Callistemon* and *Viburnum* were largely linked to variations in physiological parameters, especially in *Callistemon* plants. The PCA analysis confirmed that the drought and saline aerosol treatments with higher water capacity did not differ between both shrubs analyzed, demonstrating that the level of stress did not influence the plant morphology and physiology. To overcome stressful conditions, plants implement a different change such as the increase in the R/S ratio and the increase in the SPAD index. A heat map is a visual method that can be useful to show the intensity of variation between multiple parameters measured from different treatments. After drought stress, the saline aerosol amplified the negative effects, especially in plants previously damaged by water shortage.

From the visual appearance and ornamental quality point of view, *Viburnum* showed lower changes compared with *Callistemon* at severe stress conditions. In mild stress conditions, *Callistemon* can also be considered as ornamental plants for garden and urban green areas.

5. Conclusions

The experiment demonstrates the combined effects of the two stresses investigated—drought and saline aerosol—on morphological and physiological parameters of two ornamental shrubs largely adopted in Mediterranean green areas near the sea. Both species showed a tolerance to saline aerosol, probably for the sclerophyll leaf characteristics, as demonstrated by small presence of leaf necrosis that can be associated to higher tolerance. Plants exposed to severe drought stress were more sensitive to saline aerosol, as demonstrated by marked reductions in plant morphological and physiological parameters. Results of this experiment can be useful for the utilization of these two species in coastal seaside green areas with or without irrigation systems and subjected to different degrees of salinity stress. The overall data analyses at morphological and physiological levels suggest that *Viburnum tinus* was the more tolerant species compared to *Callistemon citrinus*.

Supplementary Materials: The following are available online at www.mdpi.com/article/10.3390/horticulturae7120517/s1, Figure S1: Mean air temperature (°C) and relative humidity (%) during the trial.

Author Contributions: Conceptualization, S.T. and D.R.; Methodology, S.T.; Software, S.T.; Validation, D.R., A.T., and A.F.; Formal analysis, S.T.; Investigation, S.T.; Resources, D.R.; Data curation, S.T. and A.F.; Writing—original draft preparation, S.T., A.T., and D.R.; Writing—review and editing, S.T., D.R., A.F., and A.T.; Visualization, A.F.; Supervision, D.R. and A.T.; Funding acquisition, D.R. All authors have read and agreed to the published version of the manuscript.

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